Biology of Fishes

Third Edition

Quentin Bone and Richard H. Moore

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To Susan and Robin

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Preface

In the compass of this small book it is impossible to cover all aspects of fish biology. Even at a fairly low level, such an herculean task, would require a very much larger book. Instead, we have chosen to look in more detail at topics that are most interesting to us, some of which are not too well treated in other texts. Our aim is to explain such topics as oxygen acquisition or feeding mechanisms in sufficient detail that the reader will be able to understand research papers on these topics. We have tried to give a sufficiently wide-ranging bibliography that the reader can begin to grasp those topics not covered, and to enjoy making further forays into those that are.

A variety of replies may be given to people who ask why study fish? One kind of reply might mention that commercial and sport fisheries are of great economic importance, often of political interest where stocks have to be shared and hunting limited, and fish farming continues to become increasingly important. These are cogent reasons for studying fish, and, as they are, after all, mainly protein, fish should play a significant role in feeding protein-poor populations.

Quite a different reply might be given by physiologists, who are always interested in special cases that may assist understanding of a general process. The classic example of such a special case is the giant axon of the squid, (at 1–1.5 mm, the largest diameter nerve fiber known) with which axon potential generation was first understood. This book, chiefly about fish as remarkably efficient machines for coping with the many problems that life in water entails, looks at many such special cases. It is immensely fascinating to see how life in habitats ranging from transient puddles to the abyssal depths of the sea has produced unique adaptations to deal with particular problems such as the acquisition of oxygen, locomotion, and sensory awareness. For instance, some fish have eyes that are adapted for vision in air, others have eyes adapted to see at wavelengths invisible to other fish around them. Again, it is obvious that some fish live in water almost of distilled purity, others in the sea or saline lakes, yet they both have to obtain oxygen at the gills where the blood must be very close to such different fluids.

This is the third edition of a book originally published in 1982 and revised in 1995. Prof. Richard Moore joins Dr Quentin Bone for this new edition, which is much altered from the previous editions to make it easier to use. A new chapter on immunology has been added, and in all chapters a serious attempt has been made to take account of the enormous increase in scientific studies of fish each year and to give up-to-date references.

Quentin Bone
Richard H. Moore

The Diversity of Fishes

1.1 Introduction

Biologists interested in fishes are very fortunate today not only in having a very wide range of living fish to study, from hagfish to lungfish, but also in being able to examine fishes adapted to every kind of aquatic habitat (and even some that spend most of their lives out of water). In consequence, we can examine fascinatingly different designs for special modes of life, as well as ways used by different fish for solving problems common to them all. Thus, for example, we can look at the remarkable ways in which fish eyes are adapted for seeing in different environments, or the different merits of methods of internal or external fertilization. These kinds of comparisons are often illuminating, and permit insights into the compromises fishes have to make to satisfy the conflicting demands of their lives. To understand these, we need first to have at least a basic idea of what kinds of fishes live today, their structural features, how they are related to each other, and how they arose.

There are sizeable numbers of species in four of the vertebrate classes: Amphibia 4300; Reptilia 6000; Aves 9000; Mammalia 8000. These are all round numbers, but, even so, they are almost certainly unlikely to be added to significantly by the discovery of new species. More likely, extinction (often our fault by habitat reduction) will reduce them. But the fish class "Pisces" is different to the others, on two counts. First, there are more kinds of fish than all other vertebrates added together. So far, over 25 000 different species of fish are known, and around 100 more are described each year, so that the final total may well exceed 30 000. The great majority are bony teleosts, the cartilaginous elasmobranchs coming a very poor second with just over 800 species. All other fish groups have insignificant numbers of species, although they may be of great zoological interest, and sometimes, as are the sturgeons, of considerable gastronomic and economic importance.

Second, within each class, all the different species should share a common basic morphology. In the class Aves, for example, we see at once that, however modified, ostriches and hummingbirds have a similar basic structure. On structural diversity, the class Aves deserves only to rank as an order of the class Reptilia; separating birds from reptiles is simply a matter of convenience rather than strict taxonomic logic.

In the class Pisces, it does not at first sight seem obvious that, say, sharks and lampreys might reasonably be in the same class. Much emphasis has

always been placed (correctly) on the acquisition of jaws as a very significant step in vertebrate evolution, and although it would probably be more logical to put the agnatha in a class of its own, the current "Pisces" is too deeply ingrained to abandon. Indeed, a recent (1997) text suggests that the class Pisces is *not* monophyletic, that is, derived from a common ancestor, but rather, a mere convenience lumping various unrelated fish groups together! Yet certain features found in all kinds of living fish make it certain that they are all ultimately derived from a common ancestor.

One of the strongest clues to common ancestry lies in the hind brain. Here, in lampreys, in different kinds of bony fish, lungfish, and in some embryo sharks, there are paired large Mauthner neurons found in relation to nerve VIII. They send their large axons down the opposite side of the spinal cord to the cell body, linked to segmental motoneurons, and in many teleosts, command the rapid tail flip escape startle response (Chapter 11, p. 372). Mauthner neurons are not found in all fish, for such a response is not suited to all, and they disappear in adult sharks. They are absent in hagfish, but, although not all would agree, the most recent molecular evidence seems to make it clear that hagfish and lampreys (Figure 1.2) are more closely related to each other than to gnathostomes (e.g. Cotton and Page, 2002; Takezaki *et al.*, 2003; Delsuc *et al.*, 2006).

Another clue comes from the pattern of innervation of the myotomal muscles along the body. With the exception of higher teleosts (which have "invented" a new pattern), in all living fishes the rapidly contracting myotomal muscle fibers are innervated at their ends, a pattern shared with urodele amphibia (Chapter 3, p. 71). Another clue from the nervous system comes from the rapidly contracting myotomal muscle fibers in the myotomes, a pattern shared by all fish groups (and urodele amphibia) with the exception of higher teleosts.

With morphological clues of this kind, backed up by an increasing flood of molecular data, we can be confident that all living members of the class Pisces ultimately share a common ancestor.

What was this common ancestor like, and when was it around?

To be recognizable as a chordate, it must be able to show serial muscle blocks or myotomes along its body, and to have the recognizable remains of an axial notochord. Experiments with the little living acraniate amphioxus (*Branchiostoma*; Figure 1.1), have shown that in animals experimentally "fossilized" (Kear, 1993), the myotomes and notochord can survive for long periods when the animals decay in anoxic conditions, and, indeed, some fossils show both. Fossils of these soft-bodied chordates will certainly be very exceptional; yet there are a few. Among the bizarre fossils of the middle Cambrian Burgess shales in the Rockies, the small *Pikaia* is an amphioxus-like fossil with V-shaped myotomes, a notochord and, apparently, a bifid head. *Pikaia* remained on its own for almost a century (and now seems off the main chordate line), but recent finds from the remarkable early Cambrian fossil lägerstetten fields of Chengjiang in China (Shu *et al.*, 1999; Hou *et al.*, 2002) have included various small fish-like chordates (*Cathaymyrus*, and *Myllokunmingia*), and more are likely to turn up. They have V-shaped myotomes, with rather more complex gills than amphioxus, and (like amphioxus) lack a bony skeleton. These early fish-like chordates were swimming around some 530 million years ago (mya), and, as a group, their ancestors perhaps arose in the Ediacaran before that. Unsurprisingly,

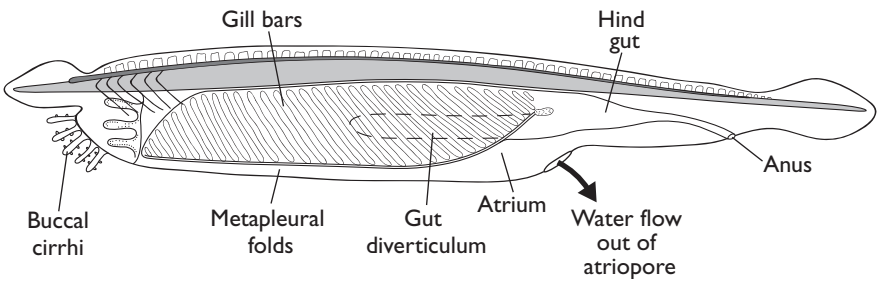

Figure 1.1 The little (5 cm) amphioxus (*Branchiostoma lanceolatum*) which lives buried in coarse sand, filtering its food on a mucus filter using the current from its ciliated gill bars.

the chordate notochord and myotomal muscle design for oscillatory swimming seems to have been part of the makeup of a number of these early fossils, and not enough of other features can be discerned to enable them to be fitted into even a tentative classification. A recent molecular attempt to dethrone amphioxus from its position as the living prototype ancestor is amusingly discussed (with approval) by Holland (2006)

Not very much more is known of a rather different group of early chordates living from the pre-Cambrian to the late Triassic, called the conodonts. Most conodont remains are tooth-like little fossils, built from calcium phosphate, which are known worldwide and are abundant enough to be useful in stratigraphy. All conodonts used to be lumped together, and still may be in some texts, but the earliest, or protoconodonts, probably have nothing in common with the later conodonts. They seem likely to be related to arrow worms or chaetognaths, and there is no evidence for any chordate affinity.

However, the later conodonts long remained one of the most intriguing puzzles in paleontology, for, until recently, only scattered isolated teeth had ever been found. In 1983 the first body outline associated with conodont teeth was discovered, and, subsequently, other better preserved specimens have been found. Astonishingly, these were flattened impressions of early chordate animals! They were rather fish-like, with notochord, V-shaped myotomes, caudal fins, bone-like material in the "teeth," and anterior large eyes. Some Carboniferous conodonts had a curious and complex three-dimensional skeletal pattern in the gullet, rather as if the animal had swallowed the wooden parts of a mixed set of folding deckchairs. This conodont apparatus is nothing like anything in any living animal and just how it operated is (unsurprisingly!) arguable. The relationship of conodonts to living forms has yet to be agreed (some texts suggest a relationship with hagfish, on rather tenuous grounds). What these remarkable fossils show us is that there seem to have been several kinds of fish-like animals with notochords, sense organs, and serial myotomes swimming around in the earliest Cambrian and later seas, before clearly recognizable fishes appeared. From one group of these chordates, all living fish are descended.

This *not* to say that all early kinds of fossil fishes can be fitted into a monophyletic scheme with living fishes. Fragments of fossil fishes with a bony skeleton first appeared in the mid-Cambrian some 600–500 mya, and by 500 mya in the Ordovician a variety of these jawless fishes has been found. Naturally enough, systematists have tidily felt the need to link, for example, fossil

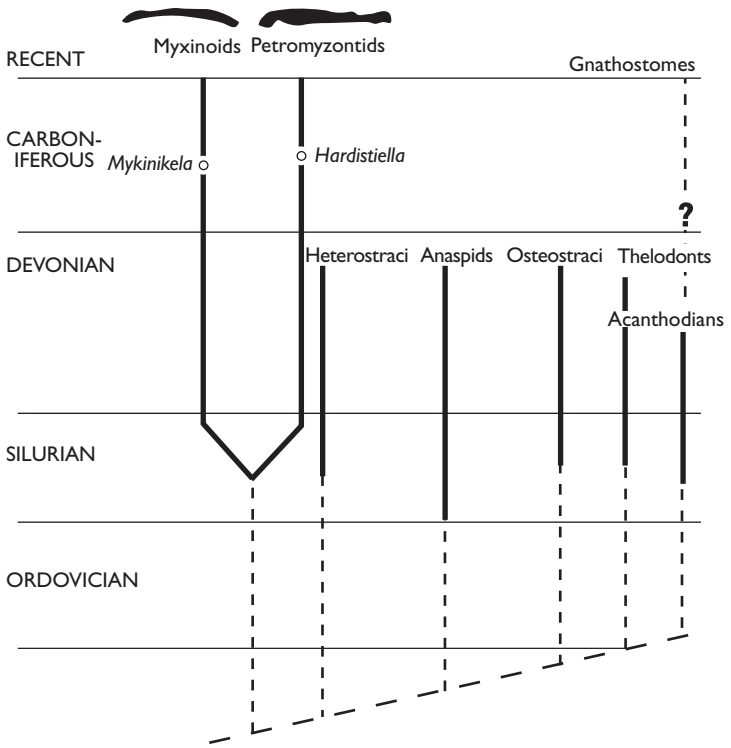

Figure 1.2A One possible arrangement of fossil and recent agnathans, and the origin of the gnathostomes. Modified from Forey and Janvier (1993).

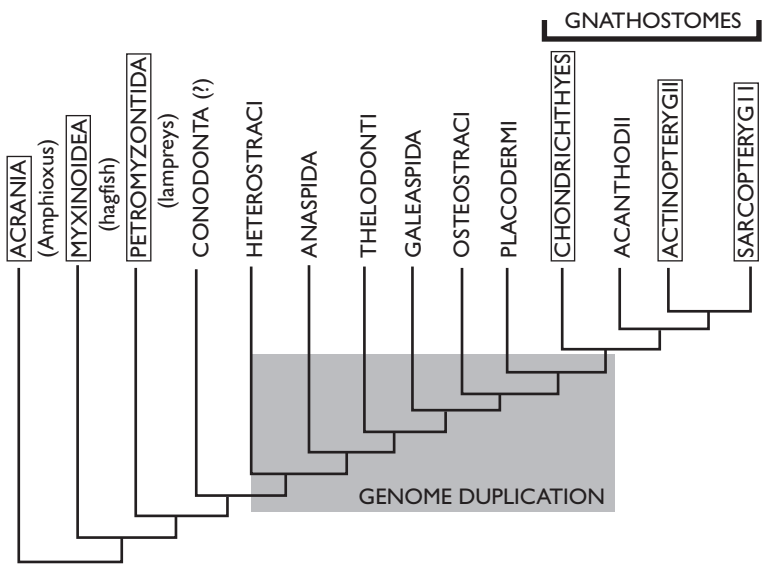

Figure 1.2B Another (more recent) view of relationships between living (boxed) and extinct (unboxed) groups, from tunicates to gnathostomes. Somewhere during the shaded area, gnathostome gene duplication event(s) took place. From Donoghue and Purnell (2006).

agnathan fishes with living hagfish and lampreys (Figure 1.2) and some of these fossils may indeed be close to the ancestry of living forms, but, at present, without further information, schemes such as Figure 1.2A are arguable. A recent proposal by two paleontologists is shown in Figure 1.2B. Note that, in this scheme, hagfish and lampreys are not closely linked but are sister groups, and the relationships of fossil groups are cautiously vague!

1.2 Fish Classification

What can now be called the classical view of the relationships of the major surviving groups of fishes is shown in Figure 1.3. It is based almost entirely on structural features, and was first proposed in the middle of the past century. In its broad outlines, most ichthyologists would have accepted it, although at time of writing there is still disagreement about the correct position of hagfish, and a more modern cladogram would be presented as Figure 1.4.

The names for the different groupings, such as elasmobranchii (plate gills) or coelacanths (hollow spines), may seem rather arcane, but the student should realize that they define the main characters of each. It is well worth looking up these and other names to find out what they mean, as it is easier to remember names that one understands and which contain some information about the fishes, instead of learning them by rote. Although the names seen in Figures 1.2–1.7 are not found in every text; some of the least fashionable have been retained since the reader may come across them elsewhere. Readers of a less advanced age than the senior author are not, on the whole, recommended to follow the example of the distinguished American ichthyologist David Starr Jordan (President of Stanford University) who (sensibly) refused to learn the names of his students on the grounds that if he did so he would forget the names of fishes.

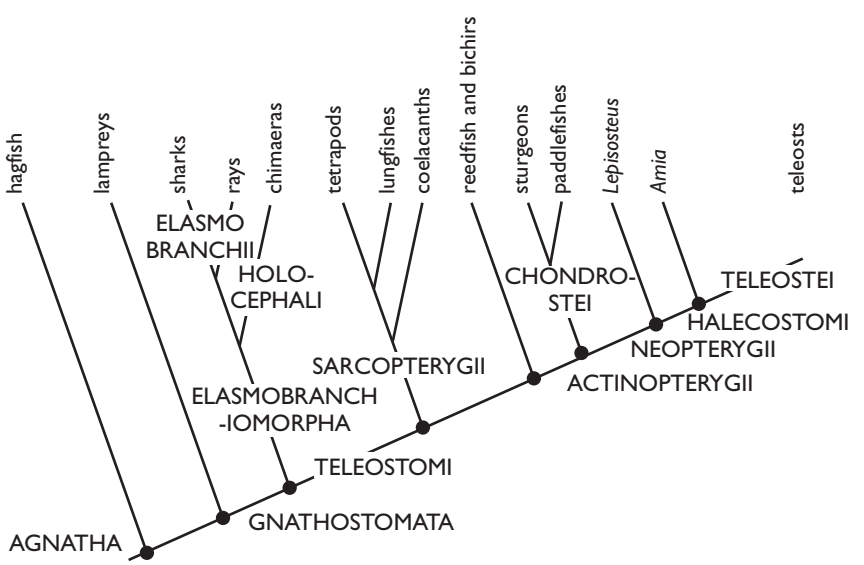

Figure 1.3A The relationships of living fish groups. Mainly after Nelson (2006).

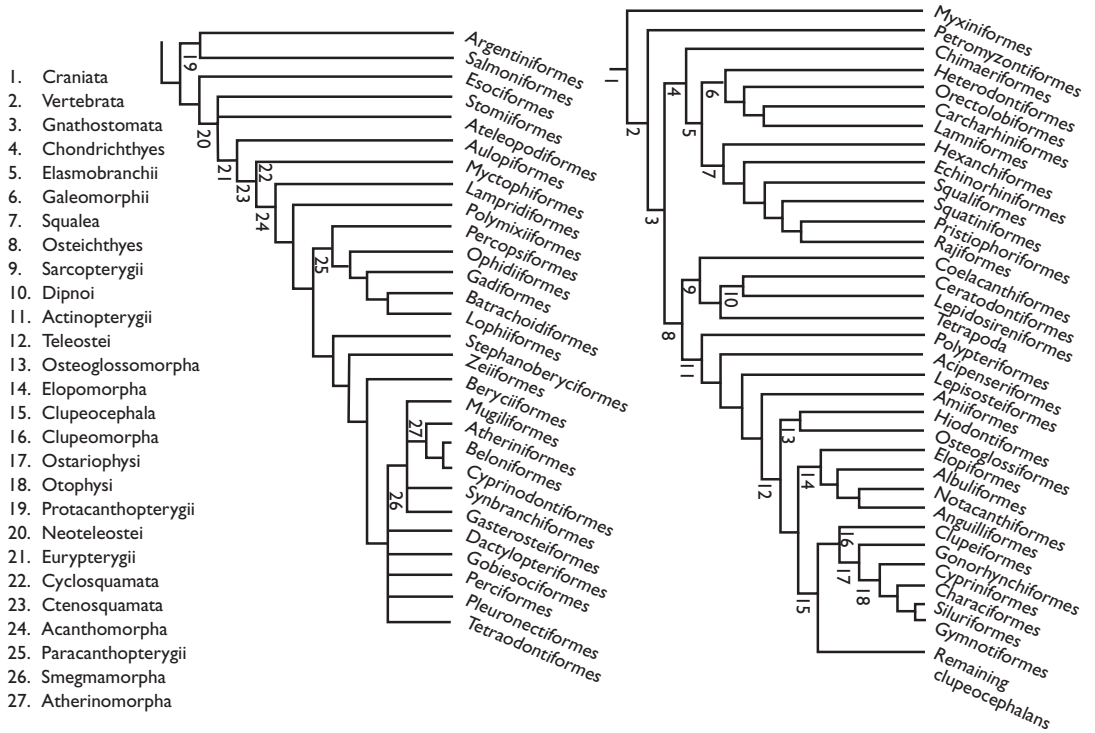

Figure 1.3B Cladogram showing relationships between living fish orders. The numbers refer to higher groupings (i.e. supraordinal taxa). From Stiassny *et al.* (1997).

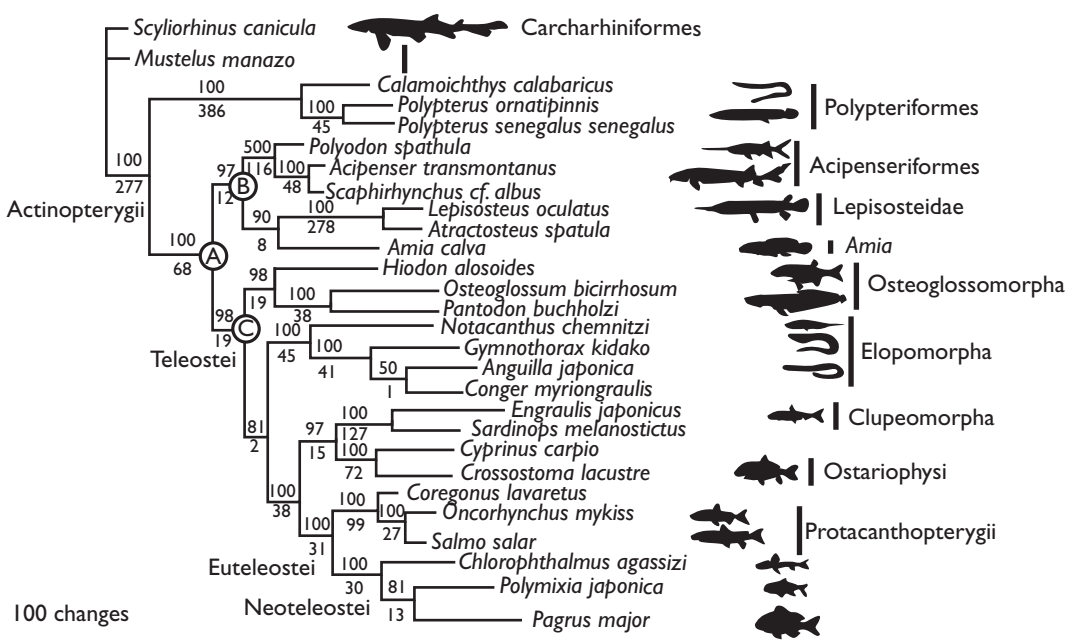

Figure 1.4 Single most-parsimonious tree for basal actinopterygian relationships obtained from unweighted analysis of genomic data (34 genes). From Inoue *et al.* (2003).

However, the relationships of the larger groups or clades have fairly recently been challenged by some iconoclastic molecular information, which has suggested a rather different arrangement. Molecular (mtDNA) data suggested that elasmobranchs are not basal gnathostomes, but instead lie firmly among bony fishes (Rasmussen and Arnason, 1999; Arnason *et al.*, 2001).

What are we to make of this suggested upheaval in fish classification? Most other molecular biologists interested in fish classification (e.g. Robinson-Rechavi *et al.*, 2001) and Inoue *et al.* (2003) have not found the evidence provided for the new position of the elasmobranchs compelling, and have rejected it. Perhaps the best way of looking at such views is to keep a wary eye open for further reports from the molecular front, and, if answering examination questions, ensure that references are provided!

The traditional basal position of Chondrichthyes or elasmobranchs is supported by conventional morphology and palaeontology (e.g. Janvier, 1996), but traces of bone remain in living sharks (p. 22), and there are reasons to suppose that the cartilaginous condition of elasmobranchs is secondary, so that their suggested revised position is not so surprising as it may seem. The recent molecular data suggest that the view of the greatest expert on fossil fish of this century, Erik Stensiö, that the bony placoderms gave rise to the chondrichthyans (Stensiö, 1927, 1969) may yet be vindicated. Unfortunately, space precludes a description and discussion of the various early fossil fishes and their relationships; good figures and a sensible discussion of relationships are given by Miles in his revision of Moy-Thomas, 1971 (a new revision by Forey should appear before too long).

Between protochordates, such as the tunicate *Ciona* and present-day chordates, there was an important large increase in genome size. There is argument about how this may have come about, one view being that early jawless fishes (and later gnathostomes) may have been octoploid (Furlong and Holland, 2002), but, whatever the mechanism, the extra genes permitted an increase in physiological complexity (see p. 10). Figure 1.5 is a recent suggestion for the timing of genomic duplications.

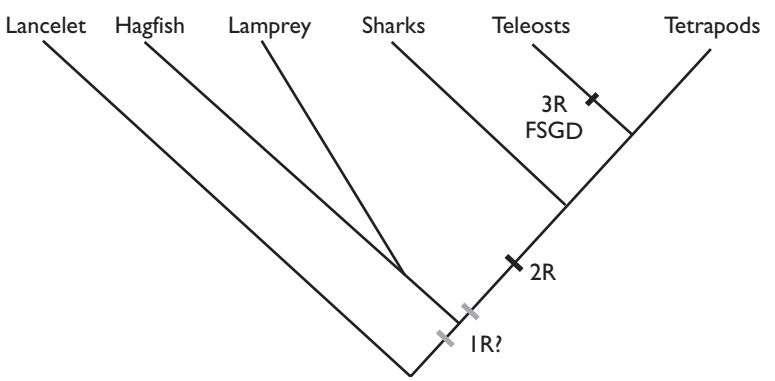

Figure 1.5 Duplications in fish genome and their proposed timing. Gray rectangles depict the possible position of the first genome duplication (1R); the black ones show the second genome duplication (2R), and fish-specific genome duplication (FSGD or 3R). Steinke *et al.* (2006).

Cladistics

We have already glanced at trees or cladograms showing the relationships between various kinds of fish; how do systematists set about using structural information or molecular information to produce these diagrams? Today the great majority use an approach called cladistics, from the branching trees it produces (Greek κλαδε = clade = branch). The cladistic method was first used by the German entomologist Willi Hennig in 1950 and, after his monograph was later translated into English in 1965, became widely popular. Certainly, others, long prior to Hennig (e.g. Müller, see West, 2003), had conceived branching trees of organisms resulting from common descent, but he formalized the way in which such trees were to be formed.

To some zoologists and physiologists (and, in particular, to most students) systematics may perhaps seem essential, but a dry, rather legalistic, and boring branch of science. They could not be more wrong! In recent decades, systematics has become a fascinating and important battleground between rival workers holding different views of the relationship between evolutionary theory and classification. The story told by Hull (1988) of the gradual victory of cladists over numerical systematists in the pages of *Systematic Zoology* reads like a brutally violent detective story, replete with remarkable examples of academic skullduggery! Most recently, a controversial new system of biological nomenclature, the Phylocode, has been proposed, and has already generated much discussion (and, as to be expected from taxonomists, not a little heat). Those interested can check it out on the web at www.ohio.edu/PhyloCode (Ohio University, 2000), but should also read Forey's comments (2001, 2002).

Hennig's phylogenetic method has two components: first, cladistic analysis of characters with rules for discerning cladistic relations, and showing them in cladograms (branching diagrams), and, second, the use of the cladograms to construct classifications with the least number of possible branch points, that is, by maximum parsimony. The cladograms are built from a series of dichotomies, where a parent species gives rise to two daughter species and itself disappears. The two daughter species and all their descendants have equivalent rank in the classification and are called sister groups (in French, curiously, usually groupes frères: brother groups). In Figure 1.3B, for example, tetrapods and lungfish are sister groups as are chimaeras and elasmobranchs. Gradual modification along a single line (anagenesis) has no place in cladistic schemes, for they are based entirely on the recognition of branching points where novel characters arise and new sister groups are born. These novel "advanced" or derived characters of a group are called *apomorphies*, contrasting with the primitive *plesiomorphies* that are shared by all its members, and the latter are ignored. Shared derived characters, or *synapomorphies* define clades, which are monophyletic groups that contain an ancestral form and all its descendants. Figure 1.4 illustrates a cladogram constructed on this basis. Other kinds of character have been given names ending in "*morphy*" (which we may at present neglect) and unkind systematists who are not entirely convinced by the cladistic approach have suggested that the approach is a conspiracy launched by an Irish O'Morphy family.

The critical reader will immediately perceive that the crux of the cladistic approach is the decisions that have to be taken to identify which characters are to be primitive, and which derived. One way of trying to solve this dilemma is to use an out-group in the cladogram as a proxy for the unavailable ancestral form,

and this is chosen to be close to what is expected to be ancestral on other grounds (i.e. the sister group of the taxon being examined), so that the choice of outgroup is not entirely free from subjectivity. This problem is particularly acute in discussions of the relationships of early fossil fishes such as pteraspids and anaspids, where some authorities consider a character primitive, others derived, and so (unsurprisingly!) reach rather different conclusions. There are also problems which may arise when weighting characters objectively, as there were also with the numerical or phenetic approach to classification. Note that however cladograms are set up, they give no information about the distances between branch points. Further, relying only on recognizably derived or apomorphic characters, neglects any information that may lie in the primitive or plesiomorphic characters. The cladograms resulting from Hennig's methods were regarded by him as phylogenetic trees, but an influential group of taxonomists, tranformed cladists as they are called, doubt that cladograms have an evolutionary basis. In consequence they have sometimes argued that fossils are not helpful in classifying living forms, although they use the cladistic *method* for classifying fossils.

Evolutionists or phylogenetic taxonomists accept that there is a systematic relation between a classification and phylogeny, and they use both advanced and primitive characters to recognize natural groups, so deviating from Hennig's strict cladistic rules. On the whole, cladistics allied to a modicum of flexibility and common sense would seem the most sensible systematic approach! A sympathetic account of the philosophical bases of these different approaches is given by Scott-Ram (1990), and in the journals *Systematic Zoology* and *Cladistics* there are many examples of the cladistic method and discussions of contentious aspects. Finally, it is important to recognize that emphasizing different aspects of a group may result in cladistic analyses giving different relationships for the group. An interesting example of discord is provided by the varying positions (Figure 1.6) that the living bichir (*Polypterus*) occupied in different recent classifications based on morphology alone, see also Bagrosky *et al.* (2003).

Finally, how should one think of congruence, or sometimes the lack of it, between the trees provided by cladistic methods, and those from molecular data? For instance, the different trees shown for the relation of teleosts with basal fish groups (Figure 1.6). Of course, in the end, molecular data treated by various methods to determine relationships produce *gene* or *genomic* trees, while trees based on morphology (i.e. the organism carrying the genes) may not necessarily be congruent with them. A further snag is that molecular trees based on 18S RDNA may not accord well with those based on mtRDNA. Yet another interesting aspect is in the treatment of data to infer evolutionary relationships: which method of analysis to choose? Kolaczkowski and Thornton (2004) consider the way that traditional maximum parsimony methods have recently been overtaken in popularity by a Bayesian probabilistic approach, and discuss the difficulties with both. Perhaps the best course is to look through papers discussing these questions, many of which, for example Slowinski and Page (1999) or Posada and Crandall (2001), appeared in *Systematic Biology*. Colin Patterson's (1987) sensible book was an early consideration of these problems, more recently Stepien and Kocher (1997) consider the relationships between molecular and morphological studies of fish evolution. There are also helpful internet sites dealing with the practical and theoretical aspects of cladistics.

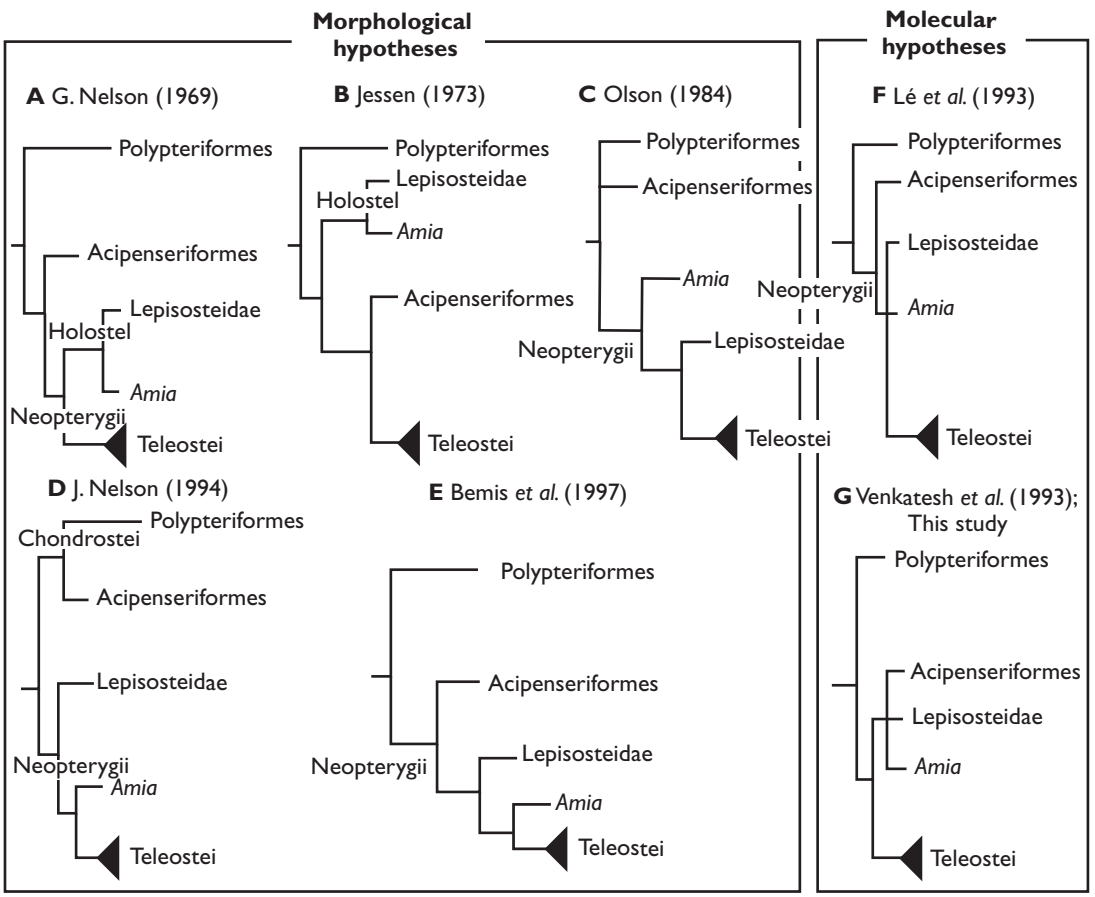

Figure 1.6 Alternative views about the relationships of the "ancient" fishes. From: Inoue *et al.* (2003, Figure1).

Gene and genome duplication

An interesting aspect of molecular phylogenetic studies has been the discussion of the so-called "two tetraploidies" model suggested by Ohno (1970), which was based on estimates of genome size, and on numbers of isozymes. Ohno suggested that there had been two large-scale gene duplications in the chordate line: one before amphioxus and the agnathans/gnathostomes had diverged; and one in the "fish" line. There is now general agreement that there has been gene duplication early in chordate evolution (many gene families are only single genes in amphioxus and ascidians yet there are two or more in fish and higher vertebrates). What is not unanimous is when these duplications occurred, in how many phases, and by what mechanism (e.g. Robinson-Rechavi *et al.*, 2001; Taylor *et al.*, 2003; Postlethwaite *et al.*, 2004; Dehal and Boore, 2005; Steinke *et al.*, 2006). For instance, recent work on hox clusters by Mulley *et al.* (2006) suggests that whole-genome duplication in teleosts took place after separation from the line leading to *Amia* and *Polypterus*. The importance of gene duplications is that they permit genomic change: if the ancestral gene gave rise to two copies, one could become modified to take up

a different function. We shall see in Chapter 9 how new endocrine gene families are thought to have arisen in this way.

Homeobox diversity

Homeobox genes consist of a 180-nucleotide sequence that encodes a 60-amino acid homeodomain. Many homeobox genes are involved in regulating gene expression during development. Holland and Takahashi (2005) give a clear account of their view of the complex evolution of homeobox genes, which has included duplications early in animal evolution as well as during chordate origins.

1.3 Teleost Classification

In view of their vast numbers and great diversity, teleosts have afforded fish systematists, adhering to all persuasions, considerable scope to modify the classifications of their predecessors, a scope of which full advantage has certainly been taken. Somewhat astonishingly, the first generally agreed division of living teleosts (Figure 1.7) into four major monophyletic groups was not made until 1966, when a number of museum taxonomists joined to produce a paper that immediately became influential (Greenwood *et al.*, 1966). Some

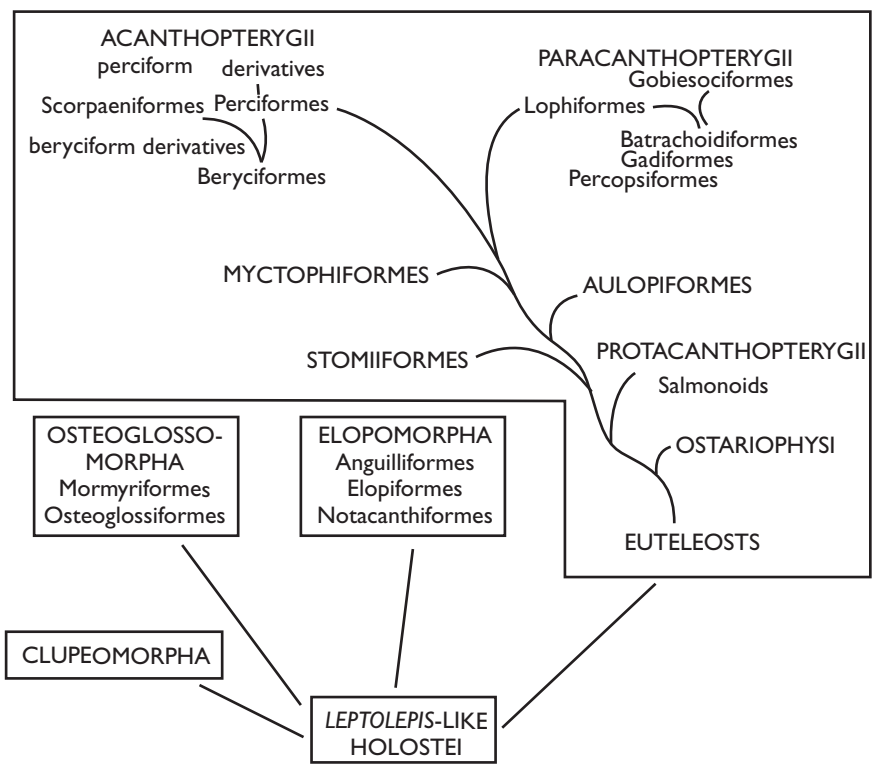

Figure 1.7 The first generally agreed arrangement of the four main radiations or divisions of teleost fish, based on morphological characters. After Greenwood *et al.* (1966).

subsequent minor modifications within the groups have been made in the light of more recent knowledge, but the four major groups seem secure from revision. We have deliberately avoided classing these groups or radiations as clades or grades, or even as super-orders, for there is presently disagreement about their status, as readers will soon see if they care to consult recent taxonomic works on teleosts.

The progressive changes leading to the larger and more derived group, the Euteleosts, from early teleost ancestors, took place more or less steadily over a long period. The beginnings of these changes, that is the beginnings of ray-finned fishes, have very recently been backdated by some 40 myr (million years) to the Paleozoic (Hurley *et al.*, 2007). They suggest earlier fossil evidence is still to be discovered.

From paleoniscid fishes with thick bony scales, similar to *Polypterus* or *Lepisosteus*, the modern Euteleosts have developed thinner scales, and thinner lightened skull bones braced by a fenestrated scaffolding structure, and only three bones in the lower jaw.

The freshwater Osteoglossomorpha (bony tongues) contains some 200 species, in six families, including the electro-locating mormyrids and gymnotids, as well as the largest freshwater fish such as *Osteoglossum*, *Scleropages*, and *Arapaima*. Osteoglossomorpha are regarded as the most primitive of the four teleost radiations.

The Elopomorpha has some 800 species, which include tarpons (*Elops*), many kinds of eels in families ranging from anguillids to congers, and several deep-sea fish such as swallowers (*Saccopharynx*), and gulpers (*Eurypharynx*). All share the thin leaf-like leptocephalus larval stage, sometimes, as in notacanths, over 1 m long.

The third of the three more primitive groups is the Clupeomorpha: herrings, anchovies, sprats, and their relatives. There are over 300 species in this group, they all share an interesting link of the swimbladder to the inner ear (see p. 297).

Last, and most challenging for taxonomists, is the Euteleostei. This is not surprising, since it contains some 17 000 species in 25 orders and no less than 375 families. In the face of this diversity, systematists have not found it easy to offer defining characters for euteleosts, but most have a dorsal ray-less adipose fin in front of the tail, and breeding tubercles. The first attempt to sort out what became known as the Euteleost or acanthomorph "bush" topping the fish tree was made by Rosen (1973). The lower part of Figure 3B shows a possible phylogenetic arrangement of the Euteleostean groups from cladistic analyses, using such characters as branchial artery asymmetry, cranial muscle morphology, and neurocranial structure. Such analyses continue and are being further refined in each group of euteleosts. A good summary of euteleost diversity and current classification (with pictures of different fish) can be found at the Laurentian University of Canada's website by using a search engine to find euteleosts. To take recent examples of progress in euteleost classification at random, Near *et al.* (2004) have looked at the phylogeny of the dominant perciform group in the Antarctic, the Notothenioidei, and Fu *et al.* (2005) have securely placed the curious little neotenous salangid fishes within the Osmeridae. In their excellent paper, they demonstrate the hazards of spellcheckers by referring to W. A. Gosline (a distinguished systematist and functional morphologist who worked on several groups of teleost fish) as W. Gasoline! Such studies have not been confined to the euteleosts, but continue

throughout all kinds of fish, one of the most interesting being Inoue *et al.* (2004) demonstrating the monophyly of the Elopomorpha.

To some extent then, the rather dauntingly formidable (even to ichthyologists) classification of living teleosts is provisional, and will certainly be modified as new morphological and molecular data become available. As Johnson (1993) remarked in a symposium on perciform fishes: "No other vertebrate group, and perhaps no other group of animals, has seen classificatory modifications over the past 25 years equivalent in scope to those in teleost fishes." The contributions in Stiassny *et al.* (1997) abundantly demonstrate this. There is general agreement that there are seven groups in the Neoteleostei, and, again, their status is not yet fully agreed, and need not trouble us here. To those of a tidy mind, it may seem disappointing that a stable classification has yet to be achieved (particularly for the higher teleosts) and cannot easily be found in all textbooks. The senior author of this book, who enjoys a singularly untidy mind, rather regards this as a most encouraging sign of ongoing activity, and looks forward to some interesting surprises; so also should the reader. This view is supported by Dettai and Lecointre (2005) who conclude by saying: "The monophyly of many previously-described groups remains to be assessed with a wider sampling, and the surprises brought by the recent molecular studies are probably far from coming to an end, promising years of exciting research on acanthomorph relationships." The reader may (and probably does) find other research topics more exciting, but there is no doubt that much heat and at least some refulgent light remains to be generated by teleost taxonomy.

1.4 Basic Structural Features of Fishes

Body shape, scales, and fins

The ancient fish ancestors were presumably small and fusiform, looking somewhat like amphioxus (*Branchiostoma*), and many modern fish have retained this streamlined kind of shape, built around an axial notochord, more or less reduced in most by the development of vertebral elements around it. But every other kind of body shape has evolved: globular (puffer fish and some angler fish); elongate (eels, pipefish, ribbon fish, agnathans); compressed dorsoventrally (rays); and laterally (sunfish, flatfish, many coral reef fish). Perhaps the most extreme body shapes are those of tropical seahorses, such as the leafy sea dragon (*Phycodurus*) which looks like animated seaweed, and the many curiously shaped larvae which are quite different to the adults. Living fishes vary so much that they are hard to categorize and define briefly. Indeed, special geometric methods (thin plate spines) are being developed to compare fish body shapes (Parsons *et al.*, 2003). This extraordinarily wide range of body shape is perhaps best appreciated when diving off coral reefs, where Gerstner (1999) showed how body and fin variations affected maneuverability and behavior, but similar diversity is seen in the deep sea and in large tropical rivers. Whatever shape they are, most fish have a series of unpaired fins, and paired pectorals and pelvics. Paired fins are absent in lampreys and hagfish, and were thought to have arisen in gnathostome ancestry by the division of a continuous lateral fin-fold. This famous hypothesis, published by the distinguished Cambridge embryologist F. M. Balfour (1878) the year before he perished climbing in the Alps, held sway for over a century. But it is now realized that the outgrowths from the lateral plate and body wall giving rise to the

unpaired fins are determined by HOX gene expression boundaries, and pectoral (the earliest) and pelvic fins are not parts of an extensive fin-fold either in ontogeny or phylogeny (see Coates and Cohn, 1998, 1999).

Paired fins confer the possibility for delicate maneuverability, and, like body shape, vary greatly in shape and structure (Figure 1.8). Shark fins are stiffened at their bases by cartilaginous plates bearing radial cartilages, and from the radial cartilages bristle-like collagenous rays (ceratotrichia) pass to the edges of the fins. Unfortunately, when boiled, this kind of fin provides a gelatinous soup held by some to be a delicacy, and the demand for shark fins has led to increased fishing effort, and serious decreases in shark populations. This kind of fin cannot be furled, but muscles attached to the basal cartilages can tilt it. The radials of the pectorals and pelvics are much elongated in rays, so that the (rather complicated) muscles inserting on them flex the pectorals up and down as the ray wings its way along (see Bone, 1999).

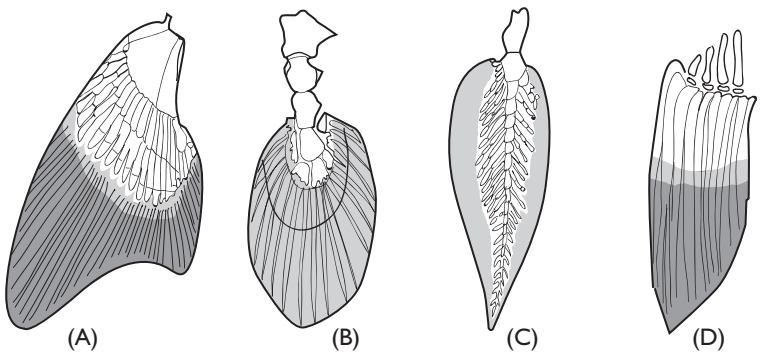

Figure 1.8 Skeletal structure of pectoral fins in different fish groups. (A) Elasmobranch (*Squalus*); (B) *Latimeria*; (C) *Neoceratodus*; (D) teleost. After Goodrich (1909).

But it is teleosts that have much the most flexible and versatile fin musculature. Flexible jointed rays (lepidotrichia) have basal muscles which enable these superbly designed fins to be folded and make the most delicate propulsive movements. The precision of such movements is astonishing, best seen perhaps in seahorses and knifefishes. Sometimes, as in sea robins and gurnards (Triglidae), free pectoral fin rays are used to sample and "taste" the bottom as the fish walks forwards. However, not all teleosts have flexible fins. In the pectoral fins of marlins (*Makaira*), the fin rays are ossified together to form a very stiff foil for maneuvering, and the foil needs to be stiff and strongly built because marlins are capable of high speeds and the stresses in maneuvering are correspondingly high. If not in use, marlin pectorals (unlike those of sharks) can be folded flat against the body.

The body is usually covered with more or less conspicuous overlapping scales or denticles, overlain by an outer epithelium. Sometimes, as in eels, the scales are buried under thick epithelial layers and even when the body is armored by thick scales (*Latimeria*), or tough denticles (sharks and many acanthopterygians), the scales are still covered by an epithelial layer. In herring, and probably in other fish, this outer layer is impermeable, and, if damaged, osmotic forces

cause the scale to swell. Scale shape, ornamentation, and structure vary in different fish groups (Figure 1.9), and is important in the classification of fossil fishes, as Agassiz first showed. On shape, structure, and composition, scales are now classed as they were by Agassiz: placoid, ganoid, cosmoid, ctenoid, and cycloid, although the last two really have basically the same structure.

A difficulty with the first three scale types, is that structure is not always well known, and it is not always clear whether the different variations in, say, "enamel" or enameloid tissue are significant. Studies of scale structure have undergone something of a renaissance recently, and, although scarcely a hot topic, it is worth checking older texts against recent work. Smith and Hall (1990) have reviewed the origins in phylogeny and ontogeny of different skeletal and scale materials, concluding that dentine was the most primitive and that both the cranial endoskeleton and the exoskeleton were derived from neural crest material in the early Ordovician fishes.

Placoid scales characterize elasmobranchs (they are often termed dermal denticles) and have a vascular core of dentine capped by a thin acellular layer of "enamel," rather similar to our own teeth (Figure 1.9A). This is the only type of scale that does not increase in size as the fish grows, instead other scales are

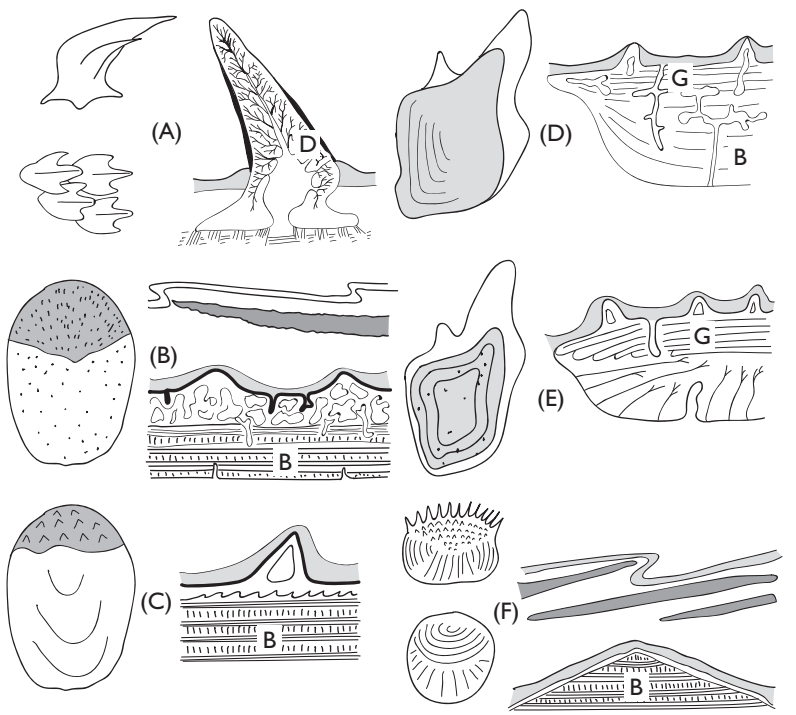

Figure 1.9 Scales of different fish groups. For each, intact scale on left and section through the scale on right. (A) Elasmobranch; (B) *Neoceratodus* (Dipnoi); (C) *Latimeria* (Coelacanth); (D) *Polypterus* (Brachioterygii); (E) *Lepisosteus* (Holostei); (F) Teleost (ctenoid scale above, cycloid below). Light stipple = outer layer of dermis; black layer = enamel or enameloid; G = ganoin; D = dentine; B = bone. B: original, others from Bertin (1958); Kerr (1952,1955); Goodrich (1909); Gnadeberg (1926) and Millot and Anthony (1978).

intercalated. Cosmoid scales of fossil lungfish and crossopterygians are only seen much modified in living fish, for present-day lungfish and *Latimeria* have lost the original outer cosmine dentine-like layer and are built of two bony layers with a thin covering of "enamel". Ganoid scales, characteristic of *Polypterus*, sturgeons, and paddlefish (*Polyodon*) are made up of an outer acellular ganoin layer, above layers of bone (Figure 1.9D). Although the scales on gars (*Lepisosteus*) look externally rather like those of *Polypterus*, the basal bony plate has a different canal arrangement. Teleosts usually have either cycloid or ctenoid scales (Figure 1.9C), the latter perhaps having a hydrodynamic function, as may also have shark denticle grooving (p. 93). The scales of *Polypterus* have an unusual role in breathing (p. 27).

Apart from the elasmobranch placoid scales or denticles, all other kinds grow as the fish does, and so can be used to age the fish, especially in temperate waters, where growth is seasonal. Elasmobranchs are not easy to age, but growth rings (none too easy to calibrate in years *post partum*) can usually be seen in vertebral centra stained in various ways, or in fin spines.

Internal features

In all fish, the body is built around an axial notochord, still present in adult agnathans, sturgeons, and *Latimeria*, but more or less reduced in most adult fish by the development of vertebral elements around it. This provides the incompressible strut flexed by the serial myotomal muscle blocks, and protects the spinal cord, while anteriorly, some kind of cranium protects the brain, in all basically similar in structure. All fishes also share gills for gas exchange, a two-chambered heart, and a circulatory system of essentially the same plan, and a similar complement of viscera.

There are, however, striking differences between the internal structure of hagfish and lampreys, and all other fishes. For instance, in the two agnathans, the branchial skeleton is attached to the cranium and lies *outside* the gill pouches (in jawed fishes it is free from the cranium and supports the gills directly); there is only a single nasal opening leading to a nasohypophyseal sac; "lymphatic" vessels are lacking, and all nerve fibers are non-myelinated.

But of course, much the most significant difference is the development of hinged jaws in the gnathostome fish, accounting for their overwhelming success compared with the very few living survivors of the agnathan radiations between the Ordovician and Devonian. Once jaws had appeared, a whole series of changes from the agnathan level of organization became possible. The appearance of jaws came together with a more streamlined body and paired fins to permit the accurate control of the movements needed to snap up larger and faster prey, and a larger brain to control these movements. New gnathostome fishes of several kinds soon became dominant.

The two main gnathostome groups, the sharks, rays, and chimaeras (elasmobranchiomorphs), and the different kinds of bony fishes, are less different than agnathans and gnathostomes but have a very distinct internal structure and biochemistry. Most obviously, the elasmobranchiomorph skeleton is almost entirely cartilaginous. The adult skull remains as a cartilaginous neurocranium to which are fused capsules for olfactory organs and the ear. This results in a curious-looking braincase (especially in Holocephali), to which the palatoquadrate and Meckel's cartilages of the upper and lower jaw are attached (see

p. 197). The vertebral column is rather simple; circular biconcave centra are separated by intervertebral discs and bear neural arches dorsally (Figure 1.10). Ventrally, there are either short ribs or (in the tail region) hemal arches.

Such a relatively straightforward axial skeleton contrasts with the more complex skull and vertebral column of bony fishes. Although the skull begins in development as a cartilaginous neurocranium similar to that in sharks, it is soon altered by endochondrial bones ossifying in this cartilaginous framework, and by the addition of many dermal (membrane) bones, supposedly derived from a scale layer in the skin. The end result is a complicated composite structure and that the homologies of the different bones are by no means easy to sort out. Thus, for example, the large paired dermal bones above the teleost orbit, which overlie the pineal, were long known as frontals but are now accepted as the equivalent of tetrapod parietals. Gregory (1959) gives beautiful illustrations of teleost skulls, but watch for nomenclatural problems.

In adult bony fish, the jaws are also a mix of endochondrial and membrane bones: separate ossifications in the palatoquadrate cartilage produce the palatine, pterygoid, mesopterygoid, and quadrate (some with dermal contributions), and these are covered at the edges of the upper jaw by the dermal membrane premaxilla, maxilla, and jugal. The dermal dentary makes up most of the lower jaw, for Meckel's cartilage only ossifies at its hinder end to form the articular. The vertebral column is normally similar to that in elasmobranchiomorpha, with biconcave centra (although with more complex ribs).

The major differences in the viscera are the presence of a gas-filled swimbladder in most bony fishes, although lost in some, and in others, such as myctophids and *Latimeria*, filled with lipid (p. 103), and in the reproductive system. Elasmobranchiomorphs always have internal fertilization, sometimes accompanied by bizarre ways of nourishing embryos in the uterus (p. 235), this is known in some bony fish but is uncommon. The spiral valve of the elasmobranchiomorph gut is only found in a few bony fish (*Amia, Polypterus*, and osteoglossids) where it is less complex. Finally, bony fish lack the elasmobranchiomorph salt-secreting rectal gland (p. 180) and high urea content but, curiously, *Latimeria* has both.

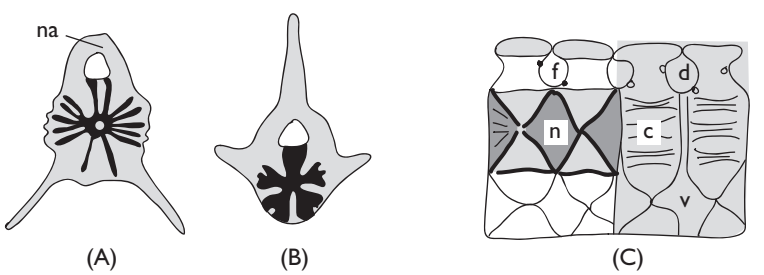

Figure 1.10 The simple structure of the elasmobranch vertebral column. Left, transverse sections through the middle of the centrum of trunk vertebrae in the shark *Lamna* (A) and the ray *Raja* (B) showing radial calcifications (black). Right, lateral view of trunk vertebral column of *Lamna* (in sagittal section on left to show the notochord (n)). (C) Centrum; f: foramina for dorsal and ventral root nerves; d: interdorsal; v: interventral; na: neural arch. After Goodrich (1909).

1.5 Distribution and Morphology

Myxinoids

Until fairly recently, myxinoids and lampreys were regarded as being separately derived from fossil agnathan groups, but some workers provided morphological evidence for their closer relationship (e.g. Yalden, 1985), and the advent of molecular taxonomy has supported this, so they now lie together for most ichthyologists as a sister group to all other craniates (see Figure 1.3). A single fossil hagfish (*Myxinikela*) is known from the upper Carboniferous (330 mya), and looks much like the living forms, if fatter (Figure 1.3). Hagfish are naked and eel-like (Figures 1.11; 1.13), scavenge on dead and dying fishes, and invertebrates with a rasping tongue (Figure 1.12). In several features they are different from other vertebrates. First of all, they have no vertebrae! There is only a single semi-circular canal, the blood is isosmotic with seawater, and vast quantities of tenacious slime can be produced from special thread cells opening along the body. This unique mechanism (a single hagfish can solidify a bucket of water with its slime) acts both defensively and prevents others

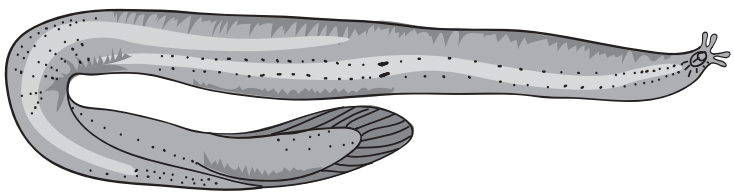

Figure 1.11 Hagfish (*Myxine glutinosa*). Note rows of slime gland openings.

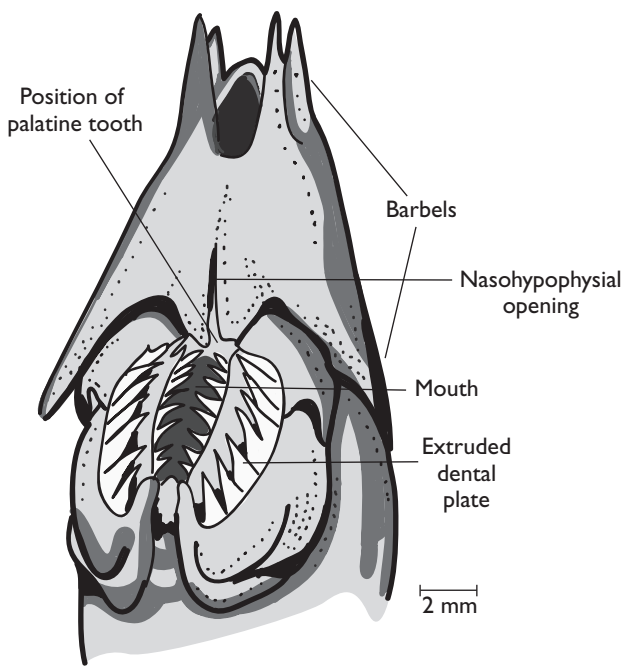

Figure 1.12 The rasping apparatus of the hagfish. Adapted from Dawson (1963).

from sharing hagfish food. It is complemented by the ability of hagfish to tie themselves into a knot which is slid along the body to wipe off the slime. Knotting (Figure 1.14) is also used to escape capture, and (as with the more complex knots made by Moray eels) to tear off food. There are up to 16 pairs of external gill openings and a single olfactory capsule with an exhalent duct to the pharynx. The eyes lack eye muscles and are buried below the skin. Hagfish lay large yolky eggs, and, in contrast to lampreys, development is direct without a larval stage. Around 50 living species of hagfish (all marine) live in temperate seas, down to 2000 m (Figure 1.15A).

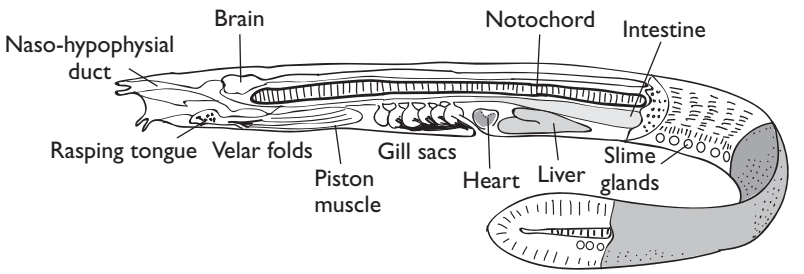

Figure 1.13 General organization of hagfish. After Marinelli and Strenger (1956).

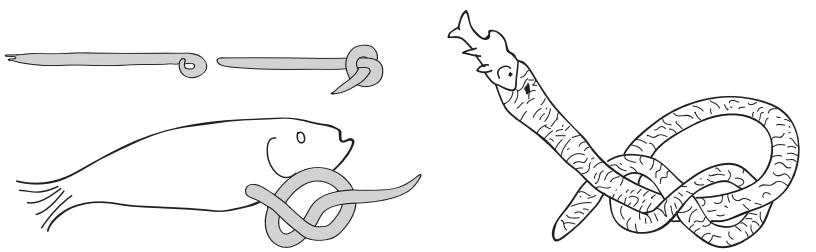

Figure 1.14 Knotting. Left, in the hagfish. The initial stages of knotting (above), about to pull a mouthful from a dead fish (below). Right, the more complex knot tied by the tropical muraenid eel (*Echidna*) to swallow its prey. After Strahan (1963) and Miller (1987).

Lampreys

Lampreys share with gnathostomes a more advanced kidney than hagfish, a photosensitive pineal, a lateral line, radial muscles in the fins, functional eyes, extrinsic eye muscles, neural and hemal vertebral elements formed around the notochord, and similarities in pituitary histology (p. 265). These features have strongly suggested to some that hagfish is the sister group to lampreys and gnathostomes together. The ear, however, has only two semi-circular canals. Like teleosts, in the sea their blood is hypotonic and in freshwater hypertonic (p. 164).

All lampreys are elongate and eel-like (Figure 1.16A); the largest species is almost a meter long, with seven external gill openings, a single opening to the nasohypophyseal sac, and a kind of spiral valve in the ciliated intestine. The Upper Carboniferous fossil *Hardistiella* is similar to living forms, and it remains unclear how (or, indeed, whether) they are related to cephalaspid or anaspid fossil agnathans. Although living forms have an entirely cartilaginous

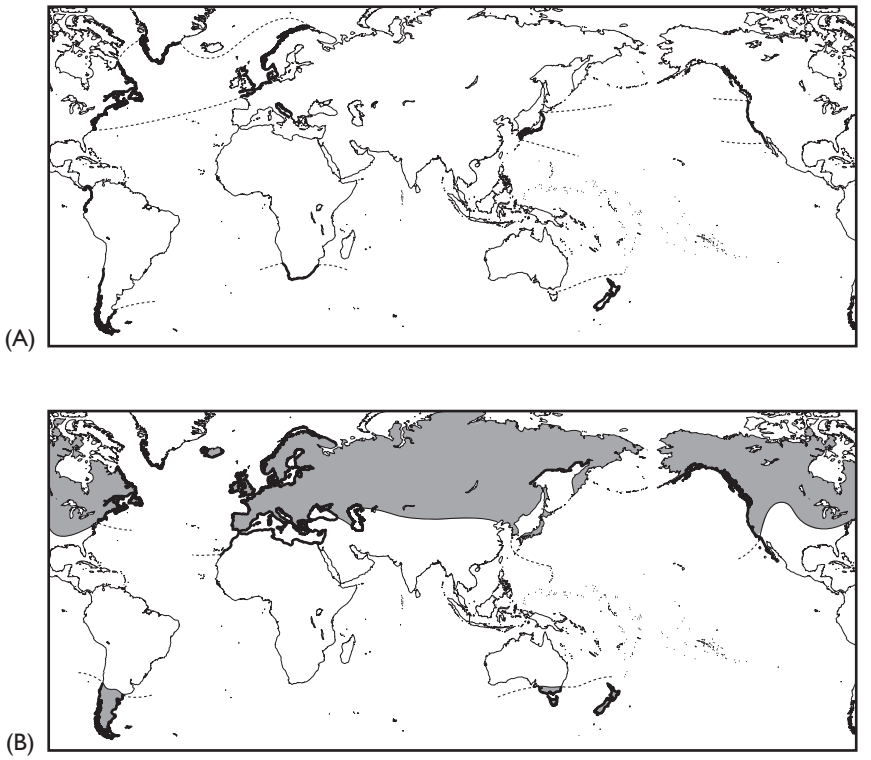

Figure 1.15 (A) Distribution of hagfish species. Usually hagfish occur in deep water. (B) Distribution of lampreys. Note landlocked Great Lakes sea lampreys (*Petromyzon marinus*).

skeleton, with a few cartilaginous "vertebral" elements around the massive unconstricted notochord (Figure 1.16B), under experimental conditions lamprey cartilage retains the ability to become calcified and so the present lack of calcification seems secondary, and is not a bar to a link with the heavily mineralized fossil agnathans.

There are today some 40 or so lamprey species, found in rivers north and south of latitudes 30° north and south (Figure 1.15B), interestingly divided into larger parasitic and smaller non-parasitic species (some not even feeding as adults). All lay small eggs in redds scraped in the gravel of streams and rivers, which hatch into ammocoete larvae that bury in mud. The ammocoetes pump water through the pharynx and filter-feed by using endostylar mucus to trap particles (Figure 1.17). They spend a quiet 5–7 years in the mud before undergoing a radical metamorphosis and swimming out. The adults either spawn in the river soon after metamorphosis, or, like *Geotria australis* and *Petromyzon marinus*, pass down to spend some years in the sea before returning to the river to spawn. In the sea (or in the Great Lakes for the landlocked *P. marinus*) they rasp the skin of other fishes and feed on their blood. *P. marinus* hitches lifts on dolphins and basking sharks, feeding on the urea-rich blood of the shark thanks to special urea-secreting capabilities (p. 166).

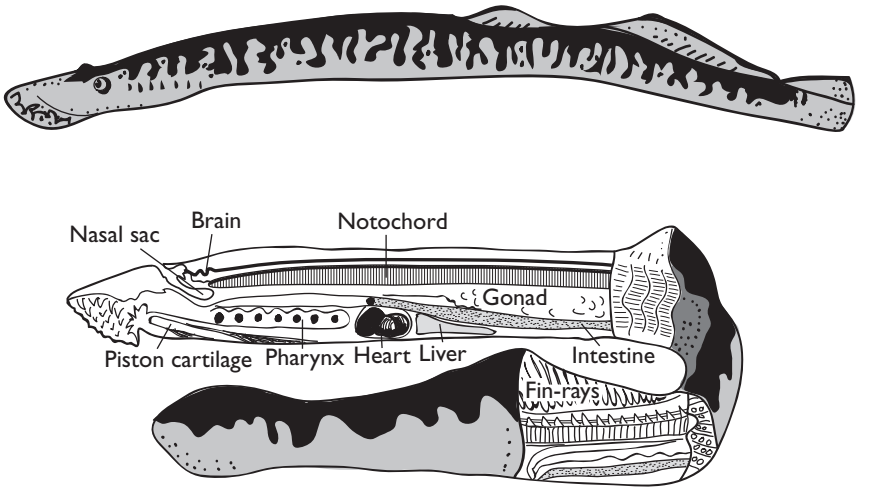

Figure1.16 General organization of lamprey (*Petromyzon marinus*). After Goodrich (1909).

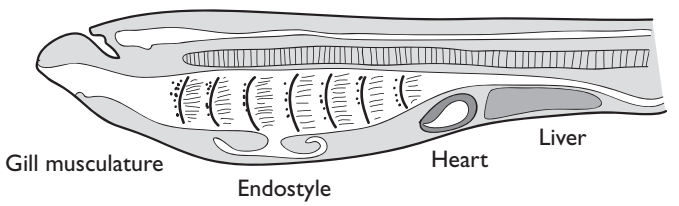

Figure 1.17 Branchial region of lamprey ammocoete larva showing position of endostyle below gill pouches, and in comparison with Figure 1.16, undivided pharynx and absence of teeth and piston cartilage. After de Beer (1928).

Non-parasitic species perhaps evolved from their "twin" parasitic species as a result of isolation in parts of river systems where host fishes were rare; these species do not descend to the sea. There are even examples of parasitic and non-parasitic races of the same species, and evolutionary aspects of lamprey systematics are of continuing interest.

1.6 Elasmobranchiomorpha

The 800 or so sharks and rays and around 30 chimaeroids (Holocephali) are all marine, apart from one shark that ascends far up rivers, and a few freshwater stingrays which are (secondarily) confined to freshwater. All share urea conservation for osmoregulation, rectal salt-secreting glands, and internal fertilization with *Latimeria*, but this does not argue common origins! Internal fertilization in Elasmobranchiomorpha permits a variety of reproductive strategies from laying egg capsules to livebearing (Chapter 8). A huge egg capsule 30 cm × 15 cm was long thought to show that the whale shark (*Rhincodon*) laid eggs, but it now seems it is really a live bearer (p. 234).

It used to be supposed, and some texts still say so, that very small elasmobranchs are not practicable. Urea retention would be too difficult, for as size decreases surface area increases. This argument no longer seems cogent (p. 183), probably never was, but nevertheless there are no very small elasmobranchiomorphs: the little pelagic cookie-cutter squaloids *Isistius* and *Squaliolus* are the smallest at just under 24 cm, while, at hatching or birth, the young of the smaller sharks, such as scyliorhinids, are relatively large.

Elasmobranchiomorphs used to be thought of as primitive fish, with their almost wholly cartilaginous skeletons, and relatively simple viscera (Figure 1.18), features found in the ontogeny of other fish. But their large brains (p. 361) and the diversity of their feeding mechanisms show that they are far from "simple." Their cartilaginous skeletons may make them easy to dissect, but does not mean that they recapitulate the ancestral fish organization. In fact, as well as the elasmobranchiomorph skeleton being strengthened at strategic points by granular or prismatic calcifications (see Figure 1.10), in the dogfish *Scyliorhinus*, lamellar bone overlies the vertebral neural arches and is also found at the bases of the denticles. X–ray diffraction patterns from shark vertebrae are identical to those from bone, and more work is needed to figure out the relations between cartilage and bone in sharks.

Strengthening calcifications are less prominent in chimaeras, which are rather delicate "watery" fishes, with a naked skin, and have denticles only on the curious claspers. However, their rabbit-like tooth plates are supported by special struts within the jaw cartilages. They swim slowly by delicately flapping their large pectoral fins, which ought to have associated proprioceptors as do batoid fins (p. 289), so far we have not managed to demonstrate them. The structure of elasmobranch denticles has been mentioned previously

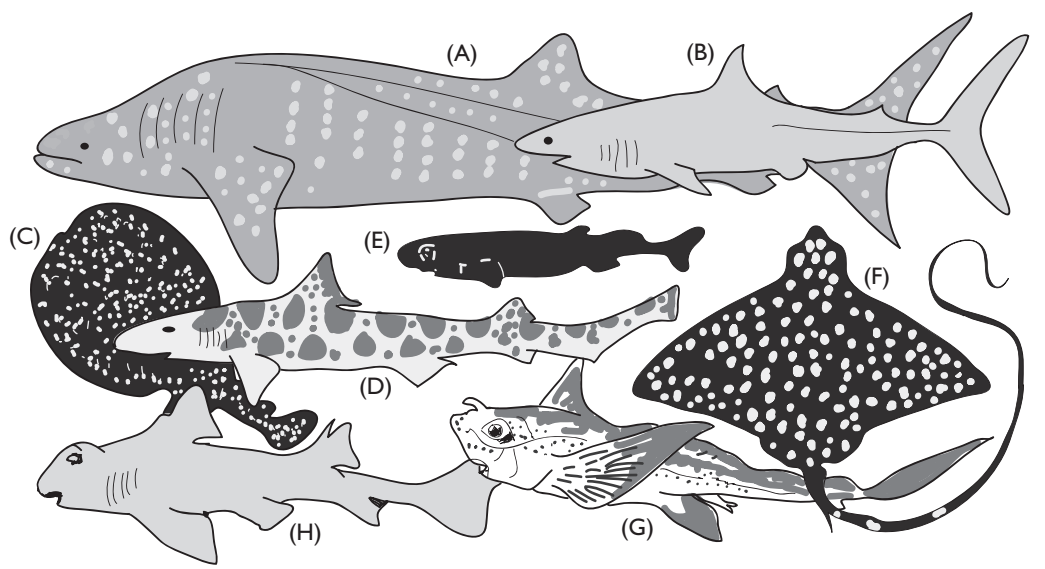

Figure 1.18 Various elasmobranchiomorphs (not to same scale). (A) Whale shark (*Rhincodon*); (B) mako (*Isurus*); (C) electric ray (*Torpedo*); (D) cat shark (*Triakis*); (E) cookie cutter (*Squaliolus*); (F) eagle ray (*Aetiobatis*); (G) rabbitfish (*Chimaera*); (H) Port Jackson shark (*Heterodontus*). After Dean (1906); Daniel (1922); Lineaweaver and Backus (1970); and Marshall (1971).

(p. 16), it may be noted further that true bone tissue may occur in their plate-like bases which support the main dentinal body of the denticle with its enameloid capping. Denticles have been variously modified (Figure 1.19) to form fin spines, flattened scales, and filtering combs (on *Cetorhinus* gills), and lateral teeth on a long flattened saw (*Pristis*). On the jaw, they form teeth. Shark teeth are replaced from behind forwards, with only one or two rows normally being in use at one time. Replacement can be quite frequent, every 9–12 days in sandbar sharks (*Ginglymostoma*), or not so often – two to four times a year in blue shark (*Prionotus*). In cookie-cutter sharks, such as *Squaliolus*, upper and lower sets of teeth are replaced as units, like upper and lower sets of dentures, and swallowed, but this sensible economy is not followed by other sharks, whose teeth simply drop out onto the ocean floor. Since shark teeth are almost the only parts known of fossil sharks, and are very abundant, calculations based on their abundance and on the replacement rates of modern shark teeth have attempted to infer the numbers of sharks in ancient seas.

Holocephalans deal with their varied diet with curious long-lasting toothplates. (Figure 1.19), somewhat reminiscent of rabbit incisors, while rays (usually bottom feeders) have flattened tooth plates, sometimes massive, as in the mollusc-crushing eagle rays (*Myliobatis*). The small butterfly rays (*Gymnura* and *Himantura*) and the huge filter-feeding manta (*Myliobatis*) are pelagic, and mantas have very small teeth.

Just over half of living elasmobranchs are rays, dorsoventrally flattened with ventral gill openings, and pectorals fused to the head. Most, such as *Raja* (the main skate genus), have large pectorals, and swim by undulating or flapping them, and the tail may be much reduced (in rajids bearing a weak electric

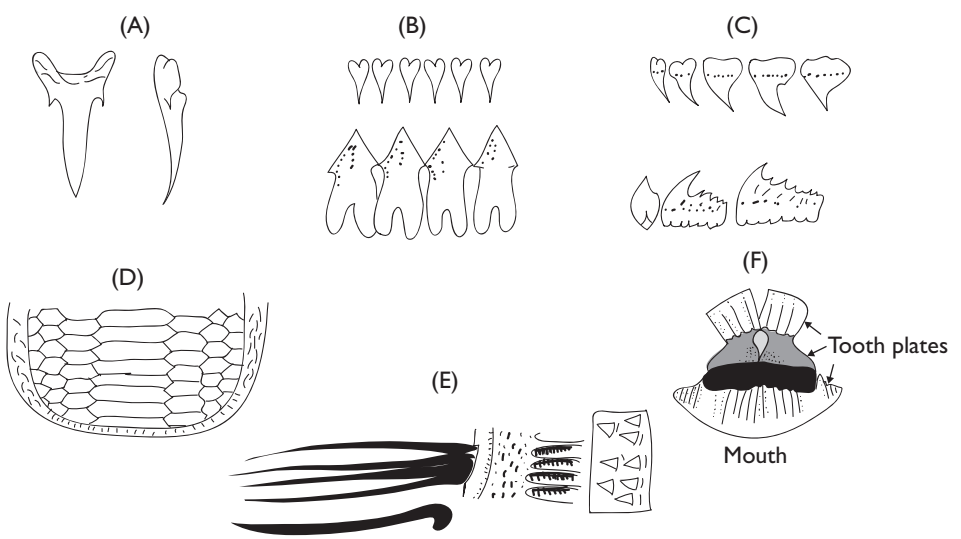

Figure 1.19 Elasmobranch teeth and gill rakers: (A) *Odontaspis*; (B) part of upper and lower set of the cookie-cutter shark (*Isistius*); (C) same for the six-gilled shark (*Hexanchus*); (D) battery of crushing plates from lower jaw of the eagle ray (*Myliobatis*); (E) filtering gill rakers (left), and minute teeth (right) from the basking shark (*Cetorhinus*); (F) toothplates of black ghost shark (*Hydrolagus* sp.). After Bigelow and Schroeder (1948, 1953).

organ, and in dasyatids, venomous spines) and absent in butterfly rays. As well as swimming, some rays walk along the bottom using their pelvic fins as legs (Holst and Bone, 1992). Sawfish and guitarfishes (*Rhinobatus*) have relatively much smaller pectorals, and such fish swim with myotomal muscles oscillating the shark-like tail, rather than by ray-like movements of the pectorals. The flattened angel shark (*Rhina*), looks much like a sawfish without the saw, but its gill openings are lateral rather than ventral. Few visitors to the South of France, even those readers who are habitués of the Côte d'Azur, realize that the Baie des Anges off Nice is named not for its beauty, but after the sometime abundance of *Rhina* there!

Most sharks are gaeleomorphs, a large superorder with smooth dogfish (triakids); there are some 200 carcharinids, such as the tiger (*Galeocerdo*) and gray whaler (*Carcharinus*) sharks, as well as the advanced fast-swimming isurids with warm red muscles (p. 83), culminating in the great white *Carcharodon*. *Carcharodon* has an immense liver to buoy it up, as have the deep-sea squaloids (p. 107), and in the largest specimen recorded, 6.5 m long weighing 3300 kg, the liver weighed no less than 456 kg. The larger basking shark (*Cetorhinus*) also has an enormous liver, for buoyancy, and cruises slowly around filtering copepods with its gill rakers: the teeth are minute (Figure 1.19). The whale shark (*Rhincodon*), even larger, which can exceed 15 m, and is the largest of all fish, feeds on plankton and on shoals of small fish, such as young tunas. The arrival of whale sharks off Ningaloo reef in Western Australia is timed to coincide with the annual gamete release of the corals. Whale sharks are related to the nurse and carpet sharks (*orectolobids*) of tropical seas. *Megachasma*, discovered only as recently as 1976, is a peculiar mid-water filter-feeding shark, which feeds on jellyfish and small crustaceans with a different kind of gill raker to *Cetorhinus*, but just how this works is unclear.

Squaliform sharks include the six- and seven-gilled hexanchids, and pristiophorid saw-sharks (remarkably similar to the batoid pristid sawfishes), but the largest group of squaliforms are the squaloids, such as the spur dogfish *Squalus*. The group has radiated widely in the deep sea, and new species turn up regularly. All are deep brown or black, with retinal reflecting tapeta (p. 317). Hooked and brought up on deck, the attractive feature of their shining greenish-blue eyes is somewhat discounted to Western eyes by their extreme corpulence, resulting from their huge oil-filled livers. This confers neutral buoyancy, and enables them to cruise around above the sea bed.

In older texts it is sometimes stated that sharks need heterocercal tails to produce caudal lift to balance lift from the pectoral fins, such tails with a flexible lower lobe produce lift as well as forward thrust as they are swept across at an angle. The markedly heterocercal tails of the neutrally buoyant deep-sea squaloids demonstrate, however, that they do not inevitably do so (see p. 108). To pile Pelion on Ossa, the angel fish, *Rhina*, is a very dense fish, yet it has a perfectly symmetrical non-heterocercal tail! Evidently, whether lift is or is not produced by the tail depends upon the intrinsic muscles which can tilt the fin. Such observations are relevant to our understanding of early fossil fish, such as the Ordovician *Sacabambaspis*, where although recent study of the best preserved specimen suggests that the tail was basically hypocercal (Pradel *et al.*, 2006), this may not imply that it generated lift.

Sarcopterygii

Lungfish (Dipnoi)

Six species of lungfish in three genera survive today (Figure 1.20) from a group that appeared in the Devonian and was widespread in the Paleozoic. Modern lungfish are in several ways at once specialized and simplified versions of their more completely ossified ancestors. The Australian (Queensland) *Neoceratodus* is the most heavily ossified of living forms, and, although there are differences in the skull and toothplates, it resembles (as its name implies) what is known of the Triassic *Ceratodus*. It is covered with large overlapping scales, the notochord is large, and the girdles and fin skeletons are cartilaginous. The paired fins are lobe-like (sarcopterygian) contrasting with the elongate fins of the other living genera. Young *Neoceratodus* and *Protopterus* "walk" along the bottom of aquaria with their flexible paired fins, much as do urodele amphibians (see p. 355). Unpaired fins are lacking, but it seems that the symmetrical "caudal" may have arisen by the union of dorsal and anal fins, since there is no trace in living lungfish development of the heterocercal tail of fossil lungfish. Lungfish eat small invertebrates and quantities of plant material, which they grind and crush with paired upper and lower tooth plates. The gut is simple and ciliated, there is no stomach nor hepatic cecum, but there is a spiral valve. Both inhalent and exhalent nasal openings to the nasal chamber lie in the roof of the mouth, and, although the exhalent opening was supposed to be the equivalent of the tetrapod internal naris, more recent detailed embryological studies have shown that it is simply the homolog of the actinopterygian posterior nasal opening, secondarily displaced into the mouth. The air bladder is septate and lung-like (p. 143), paired in *Protopterus* and *Lepidosiren*, but single in *Neoceratodus* which normally only breathes air when very active and stressed. Like most fish groups, lungfish have electroreceptors, but studies of *Neoceratodus* snout have shown that interpretations of the cranial tubular systems in fossils as a complex electroreceptor system are probably wrong. The curious vascular loops in *Neoceratodus* skin, such as the fossil tubular systems, are likely related to dermal bone modification as the fish grows. A remarkable feature of *Protopterus* and *Lepidosiren* (but not *Neoceratodus*), is that if the swampy pools they live in dry up, the fish make burrows and estivate (p. 144). This useful habit was also known to fossil lungfish (the burrows of *Griphognathus* are not uncommon) showing that air breathing was early acquired, although water breathing probably was the rule, since there is a large operculum. Estivation is not peculiar to lungfish however (p. 145).

A simple valve in the truncus arteriosus of the heart allows a partial separation of the pulmonary and systemic circulations. In this, and the structure of the brain and reproductive system, lungfish resemble amphibia. They also share with amphibia ciliated skin in the embryos and early hatchlings, and the interesting feature that at these stages the skin propagates action potentials (Bone *et al.*, 1989). *Neoceratodus* simply attaches its globular sticky eggs to water plants and the submerged roots of riverside trees, and then abandons them, but the other living genera make nests.

Coelacanths

The living *Latimeria chalumnae* was first identified off Southern Africa in 1938, and was known from some 200 further specimens all caught off the

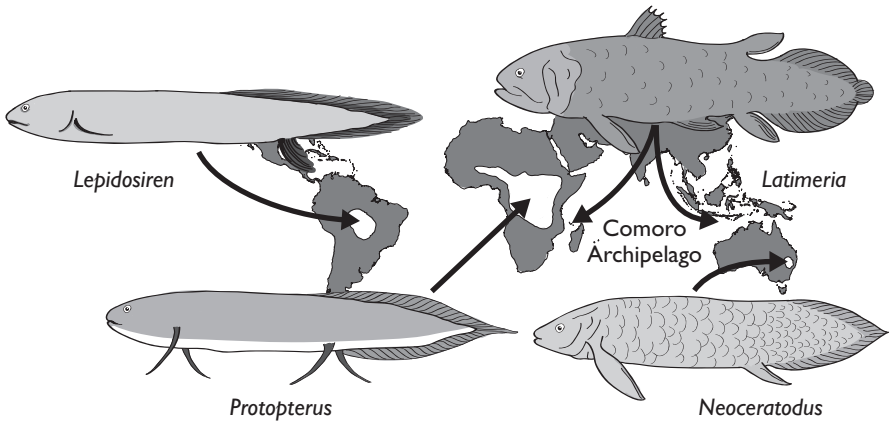

Figure 1.20 The distribution of living lungfish and *Latimeria*. After Norman and Greenwood (1975).

volcanic islands of the Comoro archipelago near Madagascar until a large female was trawled off Mozambique in August 1991. Further search produced more off Sulewesi (Figure 1.20) which externally seemed very much the same as *L. chalumnae*, (Holder *et al.*, 1999) but Inoue and his colleagues (2005) showed that there was a 4.3% difference in nucleotide residues between the Indonesian *L. menadoensis* and *L. chalumnae*, thus establishing the two as separate species. Less certainly, they estimated the split between the two to have taken place 20–30 mya, after the collision of India and Eurasia 50 mya.

L. chalumnae and *L. menadoensis* are (at present) the sole survivors of a specialized crossopterygian group first known from the mid-Devonian. It is much larger (up to 1.6 m) than its forebears, but otherwise very similar to them, covered with large overlapping bony spinous scales, and with the characteristic coelacanth trilobed tail and lobate fins. Like elasmobranchiomorpha, *Latimeria* uses urea as an osmolyte, and has a rectal gland to excrete Cl^-. Recent expeditions to the Comoro islands have successfully used manned submersibles to film *Latimeria* in its benthic habitat around 200 m. Being neutrally buoyant (the swimbladder is lipid filled, see p. 108) *Latimeria* drift around slowly near the bottom, sometimes adopting a head-down attitude, and scull about with their paired and unpaired fins seemingly all having independent actions. Despite the lack of intromittent organs, *Latimeria* bears live young, and the large female caught in a trawl gave birth to 26 young in the trawl. Fossil embryos with yolk sacs are known from the Carboniferous (p. 236).

1.7 Actinopterygii

Chondrostei

The surviving 25 species of chondrostean fishes are divided between the bichirs and reedfishes (18 extant species of the genera *Polypterus* and *Erpetoichthys*) in the Brachiopterygii, and the acipenseriform sturgeons and paddlefishes (*Acipenser, Polyodon*, and *Psephurus*). Both groups share primitive characters

such as spiracles and a spiral valve, but *Polypterus* with its closely-set rhomboid scales covered with shiny ganoin is much closer to its Devonian palaeoniscid ancestors. Indeed, the only speculation that E. S. Goodrich, the most distinguished of all comparative anatomists since Cuvier, ever permitted himself was to entitle a paper "*Polypterus*, a paleoniscid?" (Goodrich, 1928).

Geoffroi St. Hilaire, who traveled with Napoleon to Egypt, said: "If I had discovered only this species in Egypt it would compensate me for the pains usually involved in a long journey." He refused to give his collections to the British after the surrender of Alexandria, finally managing to get them to the museum in Paris where he described the fish (Figure 1.21). Cuvier himself remarked that the discovery of *Polypterus* alone justified Napoleon's Egyptian expedition, (Champollion might have felt that the Rosetta stone took precedence, although this *did* fall into the hands of the British).

The paired lung-like septated swimbladder, connected to the esophagus via a ventral glottis, probably shows the ancestral condition. Bichirs and reedfish are air-breathers and die if denied access to the surface, where they gulp air in, in a curious way. The swimbladder is emptied by intrinsic muscles and refilled by the elastic recoil of the scales. *Polypterus* uses its swimbladder to produce moans and thumping sounds.

Sturgeons have a mainly cartilaginous skeleton, with an enormous unconstricted notochord, but some dermal skull elements are ossified as are a line of lateral body scutes. The swimbladder is not respiratory, but is of some economic importance as it is used in fining wine and beer, and, as aged European readers will recall, in preserving eggs. Of course, the most valuable product from sturgeons is caviar, and a beginning has been made in their aquaculture. Most sturgeons live in oceans or in large inland seas, ascending rivers to breed,

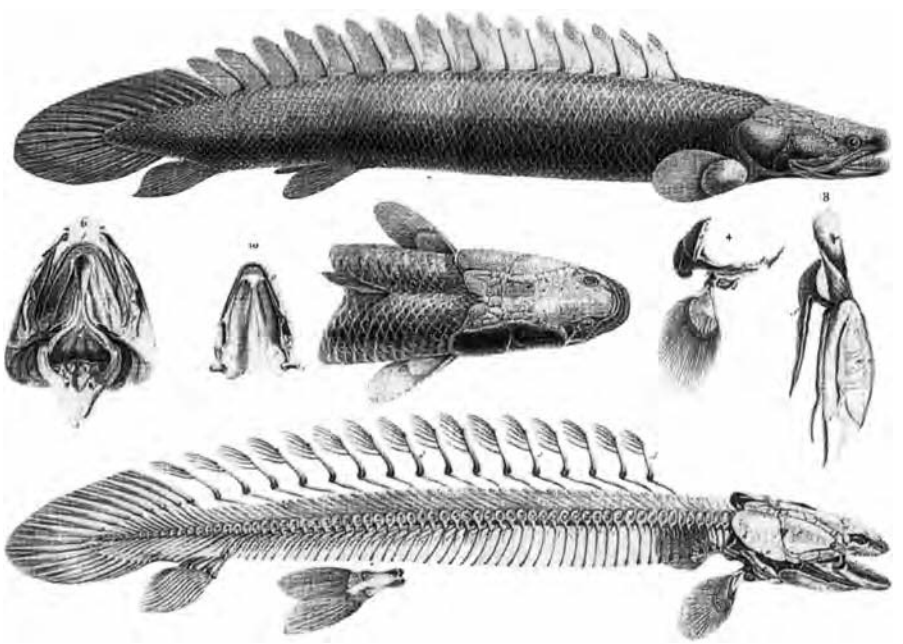

Figure 1.21 The bichir *Polypterus bichir* as figured by St. Hilaire (1802).

where they lay their sticky eggs on the bottom, to be fertilized externally. The spiny larvae that hatch spend a year or so in the river after metamorphosis before descending to the sea. Some sturgeons are large (5 m, 1000 kg) and, while rooting around for their food on the bottom tasting with four large barbels, take fish as well as invertebrates in their diet. In the UK, sturgeons are rarely captured (as by-catch in trawling) and are the property of the reigning monarch. Our research boat at Plymouth (RV *Sarsia*) captured one, so we offered it to the Queen, who served it to de Gaulle at a banquet (but not before we had abstracted two barbels to silver stain receptors).

The Mississipi paddle fish (*Polyodon*) filters plankton from the water with a gill raker sieve, but the Chinese *Psephurus* feeds on small fish and crabs, which it catches with its protrusible jaws. Both, like sturgeons, are equipped with many electroreceptors on their snouts, probably used to detect muscle action potentials from their prey.

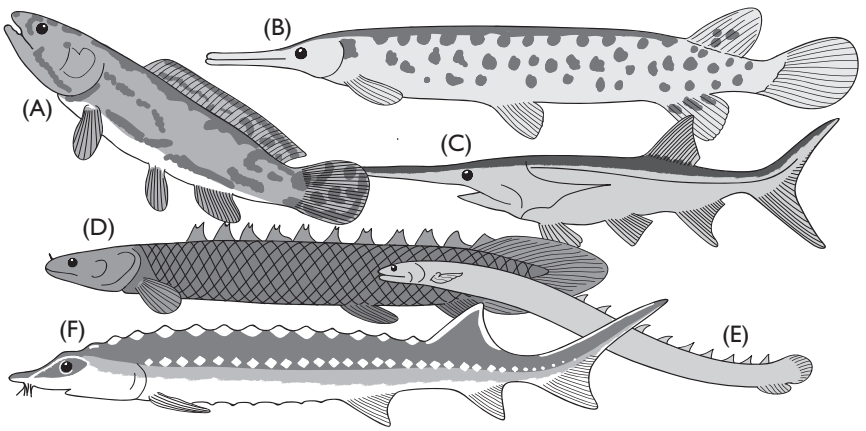

Figure 1.22 Chondrosteans and Holosteans (not to same scale). (A) Bowfin (*Amia*); (B) garpike (*Lepisosteus*); (C) paddle fish (*Polyodon*); (D) bichir (*Polypterus*); (E) reedfish (*Calamoichthys*); (F) sturgeon (*Acipenser*). After Goodrich (1909) and Marshall (1971).

Holostei

Even fewer holosteans than chondrosteans survive today. The bowfin (*Amia*) of rivers in eastern North America, and seven species of garpikes (*Lepisosteus* and *Atractosteus*) from fresh and brackish waters of North and Central America, are all that remain. Garpikes are more primitive than *Amia*, and, as seen in Figure 1.6, form the sister group to *Amia* and teleosts. However, both are much more like teleosts than chondrosteans. The skeleton is strongly ossified, the fins are more flexible, although with fewer fin rays than chondrosteans, and there is no spiracle. The heart has a large conus, and there is a reduced spiral valve in the intestine, but these primitive characters are overshadowed by such teleost features as the development of an eye muscle canal (myodome) in the floor of the skull, the loss of the clavicle from the pelvic girdle, and the freeing of the maxillary from the pre-operculum, strapping together the bones supporting the jaws. *Lepisosteus* retains the thick

rhomboidal ganoin-plated scales, which make the body relatively inflexible and reduce its fast-start performance. It is an ambush predator, hiding among weeds in shallow water, catching its prey with a sideways snap of the jaws. Since the giant tropical alligator gar (*L. tristoechus*) grows to 3.5 m, it is a very formidable predator. To achieve neutral buoyancy to enable garpikes to lurk in position, the airbladder has to be large (12% of body volume) to support the thick dense dermal amour. It is septated and is used also for respiration.

Amia is covered with thin teleost-like bony scales, and is overall much more like a teleost except for the reproductive system, for the ovary is not continuous with the oviduct: like elasmobranchiomorphs and chondrosteans, small eggs are shed into the body cavity before entering the oviduct. Cleavage is total, while in *Lepisosteus* it is meroblastic (partial only), as it is in teleosts.

Teleostei

Of all living fish, 96% are teleosts, an astonishing success since their radiation began in the Cretaceous, and they are very diverse. In size, they range from the largest specimen known of the giant European catfish (*Siluris glanis*) some 5 m long, although swordfish (*Xiphias*) and bluefin tunas (*Thunnus thynnus*) are heavier, to the smallest marine goby (*Trimmatom nanus*) at 8–10 mm, and the dwarf pygmy goby (*Pandaka pygmaea*), about the same size, from Philippine freshwaters. Some 10% of teleosts are 10 cm or smaller, and at least 80% between 10 cm and 1 m, so fitting into many different niches (Chapter 2).

In comparison with holosteans, teleosts are generally more active, faster swimmers and more lightly built, with more flexible fins built from fewer fin rays. Living teleosts are the result of four main radiations, first clearly recognized (on morphological grounds) in the classical paper published by Greenwood and his colleagues in 1966 (Figure 1.3A). One has been far larger than the others. Of these four radiations, that in freshwater containing the electrolocating mormyrids and a few large fish, such as the arapaima (*Osteoglossum*), are regarded as the least advanced. The two other less advanced groups are the Clupeomorpha with the herrings, sprats, and anchovetas, and the Elopomorpha with eels, bonefish, and some deep-sea forms. Clupeomorphs all share good hearing, due to a complex link between the swimbladder and the ear. In some shads this even enables them to hear the ultrasonic hunting cries of dolphins. Elopomorphs, sometimes quite large fish such as the tarpon *Megalops*, all share the ribbon-like flattened leptocephalus larva, in some cases, as in the notacanth *Aldrovandia*, more than a meter long, but in most, as in Anguilla, shorter and willow-leaf shaped.

On the whole, the progressive changes leading to the largest and most derived group, the Euteleosts, from early teleost ancestors have been well summarized by W. B. Stout's famous advice in designing the 1930's Ford tri-motor aeroplane "simplicate and add more lightness." From their paleoniscid ancestors, fishes with thick bony scales, and bones, similar to *Polypterus* or *Lepisosteus*, the modern Euteleosts have thinner scales, and lightened fin rays and skull bones. Although in most teleosts skeletal elements are well calcified, they are lightened by being built from a scaffolding of struts, quite unlike the dense cancellous bone of holosteans. Teleost skulls are like holostean skulls, except that the lower jaw has been simplified to just three components: dentary,

angular and articular. Naturally, there are exceptions to such generalizations, as we might expect, for instance the huge ocean sun fish *Mola* (up to 1500 kg) has hardly calcified its skeleton at all, it feeds on a watery diet of salps and jellyfish, and many deep-sea fish have much reduced skeletal calcification to save weight (see p. 104). In exactly the reverse direction, ostracodont trunk or box fishes live inside a heavy bony cuirass, and many ictalurid catfish are heavily armored.

At the apex of the Euteleosts are the numerous Acanthopterygian species, so diverse in habit and form as to include small gobies; the huge sunfish (*Mola*); mackerel, and barracudas, and over 9000 perch-like fish (Figure 1.23).

In euteleosts, the heart has an elastic bulbus instead of the holostean contractile conus, and there are usually complex gut diverticula (pyloric ceca). Osteoglossids have a spiral valve, but it is lacking in higher teleosts.

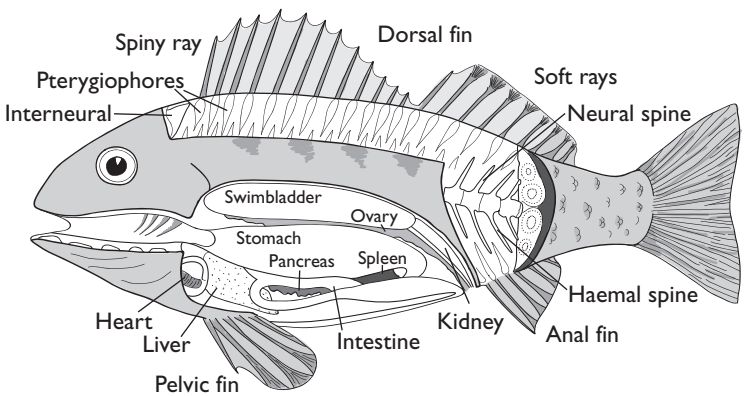

Figure 1.23 General features of a teleost (*Perca*). After Dean (1895).

Envoi

This chapter has covered a large amount of very uneven ground, from what confidence we might place in molecular phylogenies to the briefest glance at teleosts, via some remarks on conodonts, and an aside to the gripping story of the rise of cladistics which, unlike the city of Mahoggony has yet to fall. So this has been, more than most, a chapter to encourage you to think about what you think you know, in different fields.

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For this and all chapters, use search engines on the web to seek for more information on topics that interest you: journals may only give abstracts rather than full texts of the most recent articles, but your university library will have more free access than you have at home.

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2 Fishes and their Habitats

2.1 Introduction

Fishes live in virtually every watery habitat found on earth. The world's deepest living fish (*Abyssobrotula galatheae*, Figure 2.1A) was found in the Puerto Rican Trench at a depth of 8372 meters while the Tibetan stoneloach (*Triplophysa stoliczkai*) lives at altitudes over 5200 meters in the Himalaya (Figure 2.1B). The habitats of fishes vary greatly not only in physical features, such as pH, salinity, temperature, oxygen content, and light level, but also differ immensely in the space available. Some fishes have extraordinarily (and dangerously) restricted distributions, such as the relict populations of 15–20 species of desert pupfishes (*Cyprinodon* spp., Figure 2.1C) endemic to isolated small spring systems in the desert regions of southwest USA and Mexico (Echelle and Echelle, 1993; some species can withstand temperatures up to 44.6°C or salinities greater than 100 ppt), or the equally isolated populations of cave fishes, such as *Lucifuga* (Figure 2.1D) in Cuba and the Bahamas. In the marine environment, living spaces are usually greater, although, even in the marine environment, some fish may be restricted to the reefs around single atolls, or, like the bythitid vent fish (*Thermichthys hollisi*, Figure 2.1E), to thermal vents such as those of the Galapagos rift at 2400 m (Cohen *et al.*, 1990; Nielsen and Cohen, 2005). By contrast, other marine fishes, such as the pelagic blue shark (*Prionace*), range over all the oceans, while the minnow-sized bathypelagic stomiatoids, *Cyclothone microdon* and *C. acclinidens*, are found worldwide from 100 m to below 2000 m, and must comprise many billions of individuals.

2.2 Biogeography

Biogeography (or when restricted to animals, zoogeography) is the study of the distributions of organisms in space and time. Although not the first biogeographer, Charles Darwin was one of the first whose ideas on biogeography were widely disseminated. While some of his conclusions, such as that continental land masses are barriers to the distribution of many species of shallow-water marine fishes, may seem obvious, others, such as that large expanses of open ocean can also be an effective barrier, may have seemed less obvious to readers in his time. Early biogeographers believed that patterns of distribution

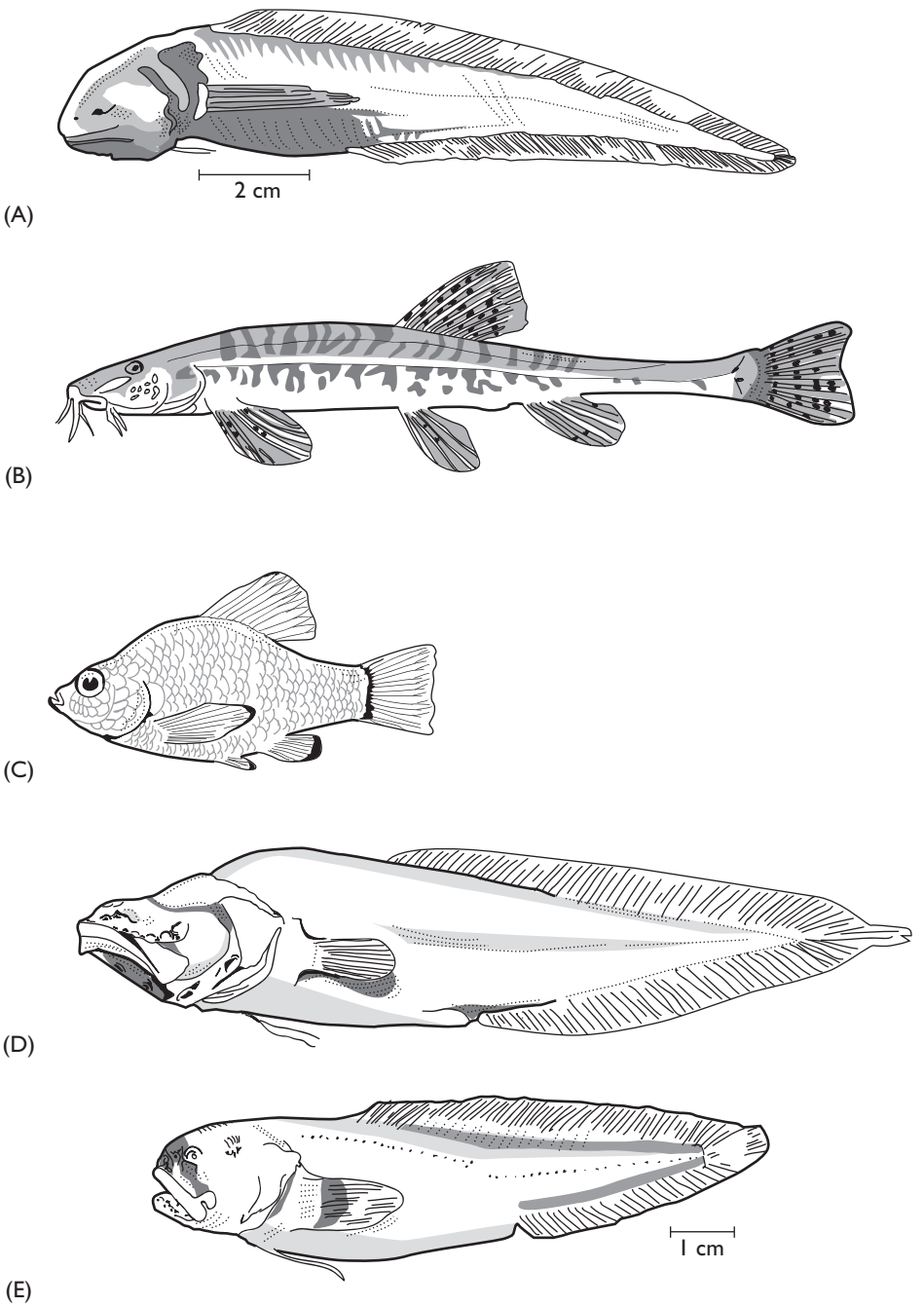

Figure 2.1 Record-setting fishes (A) *Abyssobrotula galatheae* found in the Puerto Rican Trench at a depth of 8372 m; (B) the Tibetan stoneloach (*Triplophysa stoliczkai*) lives in Himalayan streams at altitudes of 5800 m; (C) pupfishes of the genus *Cyprinodon* can survive temperatures and salinities greater than many other fishes, but the distribution may be restricted to only solitary lakes or streams; (D) cave dwelling *Lucifuga*; (E) *Thermichthys hollisi* is common at various vent fields along the Galapagos Rift and East Pacific Rise.

result primarily from the dispersal of organisms from "centers of origin" across barriers. Such dispersals often required the existence of connections (land bridges, which might contain freshwater lakes and streams) or mechanisms (fish eggs adhering to the feet of birds) that are highly improbable or scientifically unsupported. However, in the 1960s, as evidence of sea floor spreading and altered continental arrangements began to accumulate, a new mechanism for explaining biogeographic patterns was proposed. Under the vicariance hypothesis, it's the land masses, not the organisms that have changed and modern patterns are simply the result of the distribution patterns of species, or their ancestral forms. Organisms may disperse within a range, but the subsequent creation of barriers (vicariant events) divides the populations and permits the development of new and different species on either side of the barrier. As with many opposing theories, each school of thought had its adherents and opponents and many refused to accept the possibility that biogeographic patterns could result from the alternative mechanism. There is no good reason to assume the two theories are mutually exclusive, and, as with most such dichotomies, both mechanisms often need to be evoked to explain fully the distributions of modern groups of fishes. Recent molecular studies on the genetics of fish populations have shown that both mechanisms are required to explain adequately the distributions of Atlantic needlefish (garfish) species, or populations of bonito (*Sarda*), and species of the family Sparidae in the eastern Atlantic and Mediterranean. The patterns of distribution of fishes across the 4000–7000 km expanse of deep water separating the eastern Pacific from the central Pacific, which many biogeographers from Darwin to the present have described as "impassable" have also recently been reexamined using mtDNA with the result that we now know that, although infrequent, there is continued genetic interchange between some species across even this vast expanse (Lessios *et al.*, 1998, 2006).

2.3 Marine Habitats

The open ocean

Epipelagic fishes

The open ocean beyond the continental shelf covers nearly two-thirds of the surface of the Earth, and some 2500 species are found there, distributed vertically from the uppermost waters to the greatest depths, about half being benthic, half pelagic. Near the surface is the euphotic zone, where light drives (phytoplankton) photosynthesis year-round in the tropics and sub-tropics, and for the warmer part of the year in cold and temperate waters (Figure 2.2). This zone of primary production (down to 200 m in the clearest waters), supports an epipelagic fish fauna of some 250 species, as well as many larvae of fish from deeper levels (Figure 2.3). Sharks, flying fish, scombroids such as tunas and billfish, halfbeaks, garfish, the large sunfish *Mola*, and stromateoids are typical of this zone, and are usually countershaded, colored dark blue above and lighter below so that they match their background when viewed from any angle. Countershading, which is more fully discussed in Chapter 10 (p. 326), is found in fishes in almost every habitat, but it is most strongly developed in the epipelagic zone where there is little else to hide a fish from

predators. Floating objects attract both smaller epipelagic fishes such as the stromateoid driftfish (*Nomeus*) and medusafish (*Schedophilus*), which hide under medusae for protection, as well as larger scombroids preying on the smaller fishes. Since medusae are found at many levels of the ocean it should come as no surprise that even deep-water medusae have their commensal fish associates (Drazen and Robison, 2004), although, in the case of *Stygiomedusa gigantea*, the fish most likely will be a deep-water species such as the *Merlangius merlangus* or the ophidiform, *Thalassobathia pelagica*. The distinctive Sargassum community consists of a variety of fishes and invertebrates that associates with the pelagic brown alga *Sargassum*. Some, like the Sargassum fish (*Histrio histrio*) are found nowhere else, and have their closest living relatives among the benthic community, but others associate as juveniles with the seaweed, presumably for protection or food (Figure 2.4). The perciform wreckfish (*Polyprion*) is named from its habit of living under floating wreckage, old teacases seemingly being favorite lairs. The epipelagic fauna is richest in warmer regions, but some species, such as the "warm" isurid sharks and blue-fin tuna *Thunnus thynnus* (see p. 82) migrate to colder waters in the productive season.

Mesopelagic fishes

The 900 or so mesopelagic fish species (Figure 2.5) live above the thermocline in a zone where daylight still penetrates; many migrate upwards at night toward the surface, sinking again before dawn, following the migrations of their zooplankton food. Myctophids and several stomiatoids, feeding on copepods and small crustaceans, undertake vertical migrations of 400 m or so up

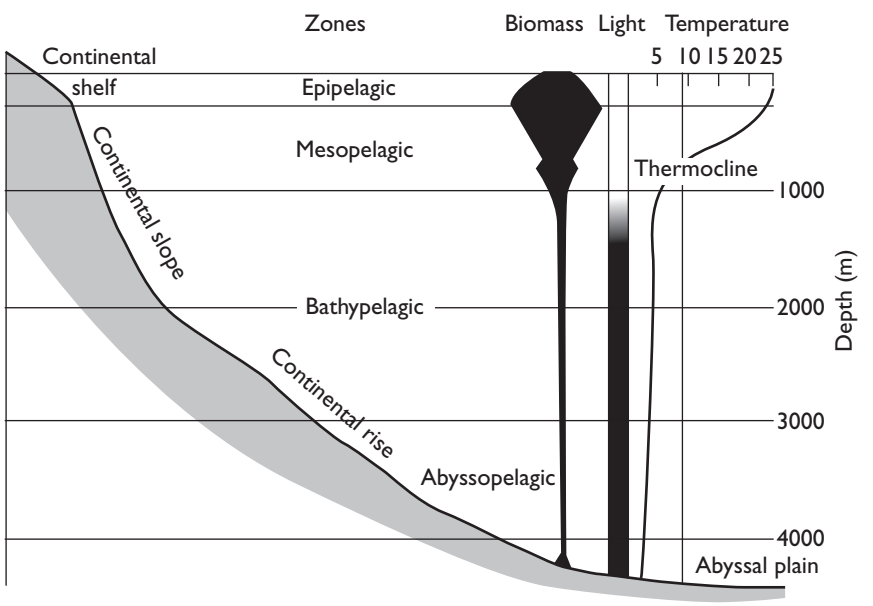

Figure 2.2 Zones of the ocean – shaded area beneath seafloor indicates relative abundance of benthic food. Note that plankton biomass increases close to the ocean floor. Modified from Marshall (1971).

and down each night. Not all vertical migrators travel upward far enough to reach the surface, and differences in the amplitude and timing of such migrations, described as a "ladder of migrations" by Vinogradov (1962; Figure 2.6), effectively partition different feeding levels, so reducing competition. For

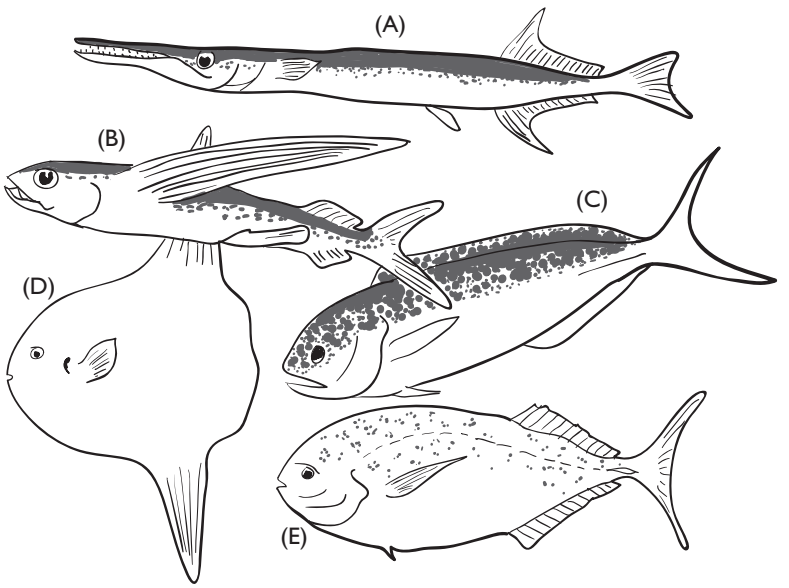

Figure 2.3 Epipelagic fishes (not to same scale). (A) Garfish (*Tylosaurus*); (B) flying fish (*Exocoetus*); (C) dolphin (*Coryphaena*); (D) sunfish (*Mola*); (E) louvar (*Louvarus*). After Fitch and Lavenburg (1971) and Herre (1928, p. 215).

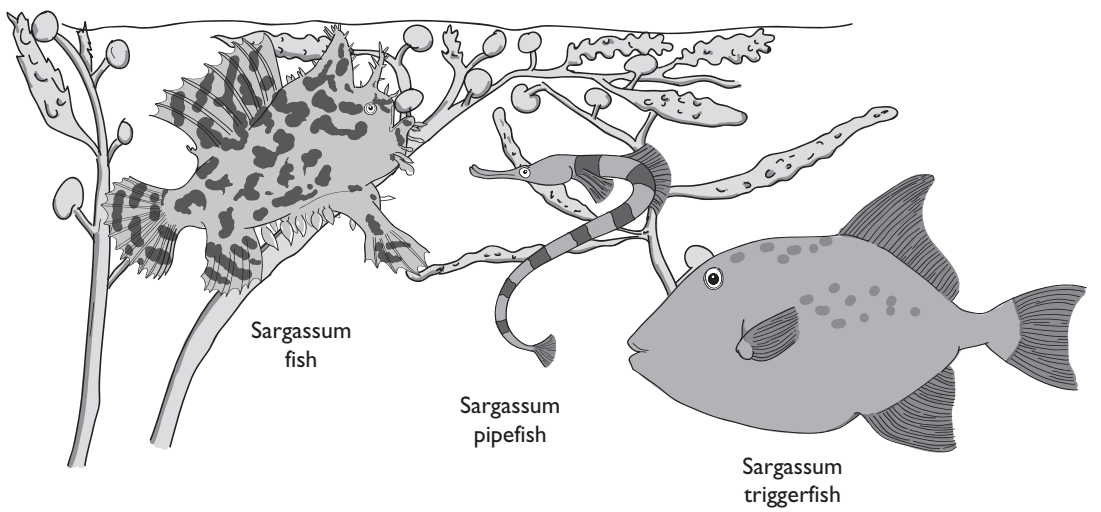

Figure 2.4 Sargassum fauna. Adapted from Thorson (1971).

example, in the Rockall trough in the northeastern Atlantic, west of the UK, as judged by the species composition of the copepods they feed on, the hatchet fish *Argyropelecus olfersi* feeds at lower depth horizons than *A. hemigymnus*, while the third common sternoptychid, *Maurolicus muelleri*, feeds closest to the surface.

The very rare accidental capture of a large mesopelagic fish such as the megamouth shark (*Megachiasma pelagios*) shows us that there are some large fishes in this zone. All biologists who have fished in the open ocean with mid-water trawls are well aware that these devices never catch the large squid which are known to be abundant (from the stomach contents of marine mammals, much more efficient sampling engines), and so it is possible that other large mesopelagic fish remain to be discovered. Those we can catch are almost all smaller than 30 cm, the myctophid lantern fishes (ca. 250 species) mostly being 10 cm or smaller. Larger fishes, such as the alepisauroids (lancet fishes) up to 2 m long, and chiasmodonts (giant swallowers) with larger jaws and distensible gut and body walls, which feed on fishes and other larger prey, are non-migrators. Many mesopelagic fishes have relatively large eyes and are covered with silvery scales and light organs often arranged in patterns for intraspecific communication or in a form of countershading for camouflage (see p. 326), although others, chiefly predators such as the alepocephalids, are dark brown, black, or red (which, because of the absorption of virtually all red wavelengths by this depth, appears black).

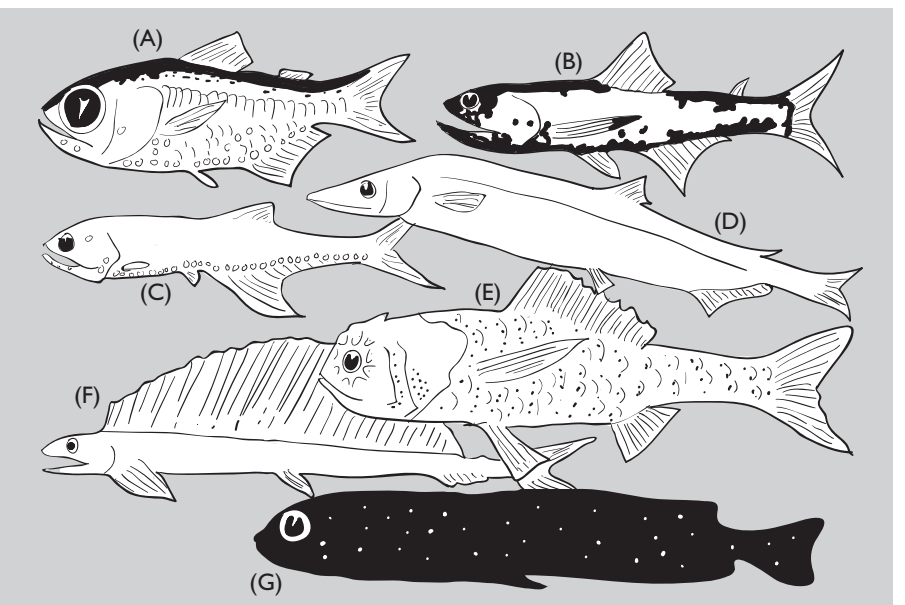

Figure 2.5 Mesopelagic fishes (not to same scale). (A) Myctophid (*Electrona*); (B) myctophid (*Lampanyctus*); (C) stomiatoid (*Bonapartia*); (D) adult *Paralepis* – these are rarely caught, the postlarval and larval stages are elongate and thinner; (E) *Melamphaes*; (F) lancet fish (*Alepisaurus*); (G) alepocephalid (*Xenodermichthys*). After Grey (1964); Rofen (1966); Gibbs and Wilimovsky (1966); Ebeling and Weed (1973, p. 397); and Marshall (1965).

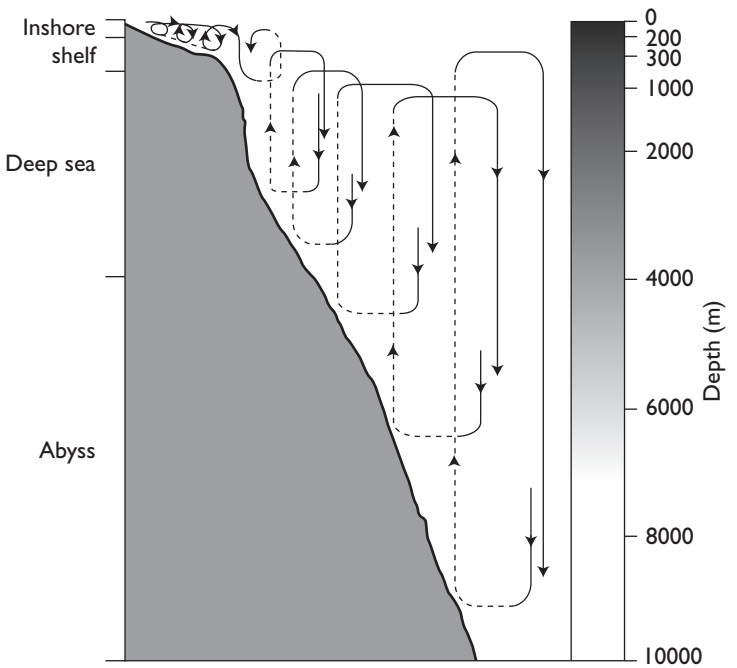

Figure 2.6 Ladder of vertical and horizontal migration as a means of introducing surface production to the deep sea. Changes in relative concentration of particulate matter with depth in the oceans is shown by stippling in the right column. Modified from Conover (1978) after Vinogradov (1962).

Bathypelagic fishes

Compared to the more robust mesopelagic fishes, bathypelagic fishes (Figure 2.7) are "economy" designs, reducing the calcification of their skeletons except for the all-important jaws, and with watery muscles. The water content of such fish is high, for example 95% in the angler fish (*Melanocetus*) and 94% in the gulper eel (*Eurypharynx*). Even without gas-filled swimbladders, they are near neutral buoyancy. Their hearts are very small, they have very little red muscle, and their low hematocrits (packed red blood-cell volumes, a measure of the blood's oxygen-carrying capacity) average 8%, compared with the mackerel with a hematocrit of about 50%. Most of the 150 or so species of bathypelagic fishes are ceratioid angler fishes (about 100 species), but the dominant forms in numbers of individuals are black species of the stomiatoid genus *Cyclothone*. Like angler fishes, *Cyclothone* species have smaller males than females. As several species of this genus have been shown to be protandrous hermaphrodites, the smaller males eventually transform into larger females and so they are not so much reduced, and do not fuse with the females as do ceratioid males. Many bathypelagic fishes must live a rather sedentary life, hanging in the water waiting opportunistically for the occasional meal to come within range of the jaws. *Cyclothone* species live on a diet of copepods and small fish, as do angler fishes, some of which (like *Melanocetus*) can swallow fish two to three times their own length, attracting them within range of the

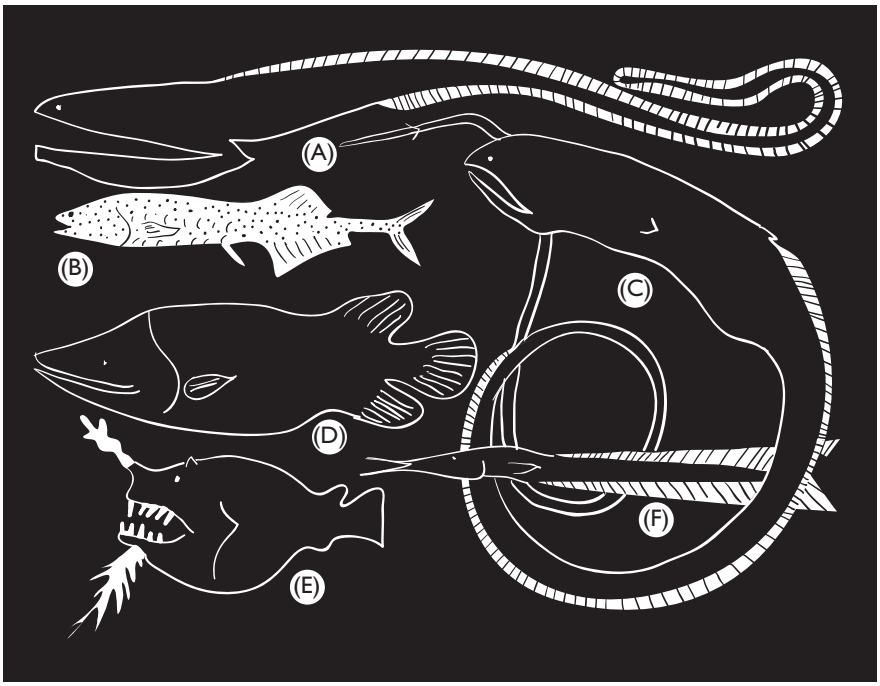

Figure 2.7 Bathypelagic fishes (not to same scale). (A) Gulper (*Eurypharynx*); (B) *Cyclothone braueri*; (C) gulper eel (*Saccopharynx*); (D) whalefish (*Cetominus*); (E) angler (*Linophryne*); (F) snipe eel (*Cyema*). After Böhlke (1966, p. 168); Marshall (1971); and Fitch and Lavenburg (1971).

jaws with luminous lures. Although no daylight penetrates to the deep sea, many bathypelagic fishes have normal-sized eyes often with specializations to increase their sensitivity (see p. 319), and the fitful flashes and glows of bioluminescence must obviously be significant in their lives.

Benthopelagic fishes

Rather surprisingly, the great majority of bottom-living fishes from upper slope levels at 200 m or so to around 8000 m in the deep ocean (Figure 2.8) are neutrally buoyant and live not on the bottom but just off it, like the deep-sea squaloid sharks (floating by means of their large oil-filled livers (p. 103)). Although shallow-water rays are dense fish and rest on the bottom, their deep-sea relatives resemble the squaloids in having very large livers and are also close to neutral buoyancy, presumably also being benthopelagic. Recent analysis of a global data set shows a trend of rapid disappearance of elasmobranch species with depth when compared with bony fishes (Priede *et al.*, 2005). Sharks, apparently well adapted to life at high pressures, are conspicuous on slopes down to 2000 m including scavenging at food falls such as dead whales. It has been suggested that they are excluded from the abyss by their high-energy demands created by the necessity for near-constant swimming and an oil-rich liver for buoyancy, which cannot be sustained in extreme oligotrophic conditions as are found away from the slope environment.

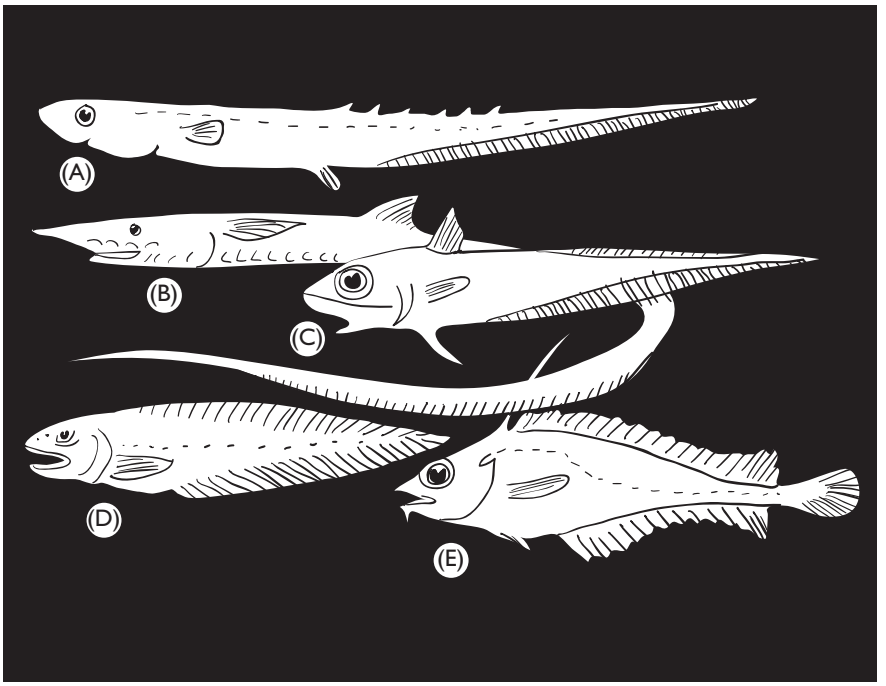

Figure 2.8 Benthopelagic fishes (not to same scale). (A) Notacanth (*Polycanthonotus*); (B) halosaur (*Aldrovandia*); (C) macrourid (*Coelorhyncus*); (D) brotulid (*Bassogigas*); (E) gadoid (*Lepidion*). After McDowell (1973); and Ebeling and Weed (1973, p. 397).

Among the teleosts, cusk-eels (ophidioids) and rat-tails (macrourids) dominate this cosmopolitan fauna, whose biomass may be considerable, and, like the deep-sea cods (morids), deep-sea eels, notacanths, and halosaurs, all have gas-filled swimbladders (Figure 4.7, p. 112). They feed on other fishes, benthopelagic zooplankton, and benthic invertebrates. Apart from those around thermal vents, the benthopelagic and benthic invertebrates ultimately depend on organic matter such as fecal material raining down from surface waters, and it is understandable that the biomass of the plankton decreases rapidly with depth. For example, at 1000 m it is only 1% of that at the surface, and at 5000 m only about 0.01%.

Around thermal vents and methane seeps, however, food for benthopelagic fishes is richer, symbiotic bacteria supplying the energy source for worms, crustaceans, and molluscs.

Benthic fishes

In contrast to the benthopelagic fishes, benthic fishes (Figure 2.9) lack swimbladders and are dense, resting on the bottom, sometimes, like tripod fishes (*Bathypterois* spp.) on stiff elongate fin-rays, sitting aligned into the currents that bring zooplankton to their mouths. Tripod-fish and green-eyes (both chloropthalmids) and lizard-fishes (synodontids) are the dominant forms, and in temperate and polar waters, eel-pouts (zoarcids), and seasnails (liparids) are also important. As with the benthopelagic fishes, benthic fishes of these

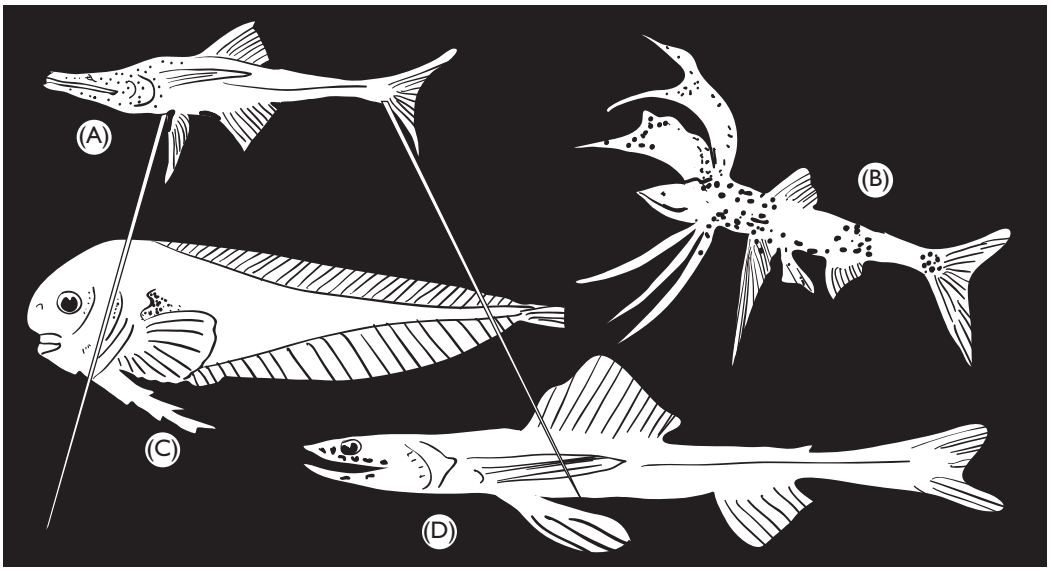

Figure 2.9 Deep benthic fishes (not to same scale). (A) Tripod fish (*Bathypterois grallator*); (B) tripod fish; (*B. bigelowi*) in fishing attitude; (C) seasnail (*Careproctus*); (D) lizard fish (*Bathysaurus*). After Marshall (1971, 1979); Mead (1966).

kinds often show interesting specialization of the eyes (see p. 315), and some, such as *Chloropthalmus*, have luminous organs (see p. 328).

Because of the lack of cues such as light and temperature change, it was once thought that there was no seasonality in the deep sea. This is not so – the fall-out of detritus depends on seasonal production cycles at the surface and, certainly in higher latitudes, there are annual growth cycles in deep-sea fish species.

Shallow seas and coastal regions

Warm-water fishes

By far the greatest number (80%) of the 10 000 or so species of fishes in shallow seas live in warm temperate or tropical waters, most associated with coral reefs and atolls (Figure 2.10), in waters where mean temperatures during the coldest part of the year do not fall below 18°C. Coral reefs are widespread in the Indian and western Pacific oceans between latitudes 30°C north and 30°C south, and there are also large reefs in the Caribbean and around the West Indies. There is a striking difference in the number of species of coral fishes in different regions (Figures 2.11 and 2.12), from the richest central Indo-West Pacific reefs of the Philippines, New Guinea, and the Australian Great Barrier Reef to the less rich reefs around Florida where only 500–750 species live. The decline in species number, seen in Figure 2.12, may reflect the Indo-West Pacific origin of the global coral reef fish fauna, divided into four main regions: Indo-West Pacific, Pacific American (Panamanian), West Indian, and West African (Figure 2.13). The eastern Pacific oceanic barrier (an east–west distance of some 4000–7000 km depending on where measured), between the

Indo-West Pacific and Panamanian faunas, has been crossed by very few species. Some of these are large active swimmers such as the tiger shark (*Galeocerdo*), an important predator on seasnakes, and the spotted eagle ray (*Aetobatis*); others have made the crossing as long-lived pelagic larvae such as the leptocephali of the bonefish (*Albula*), and six species of moray eels, or the larvae of several species of puffers and triggerfishes. On the whole of the Great Barrier Reef 1300 species are known, but at its southern end (the Capricorn–Bunker group, which has been very well sampled, and is the best-known region of the reef) only about two-thirds of these (859 species) occur. This impoverishment, however, seems primarily due to lowered habitat diversity (in the north, there are outer barrier reefs and inshore coastal reefs), and not to within-habitat diversity.

In contrast to the speciose Indo-West Pacific, other tropical regions contain fewer species. The Caribbean/West Indian region represents a secondary center of tropical biodiversity, sharing most of the same families as well as many genera with the Indo-West Pacific. These represent the descendents of a widespread tropical fauna that is believed to have lived in the warm, shallow tropical Tethys Sea stretching from what is now the western Pacific to what is

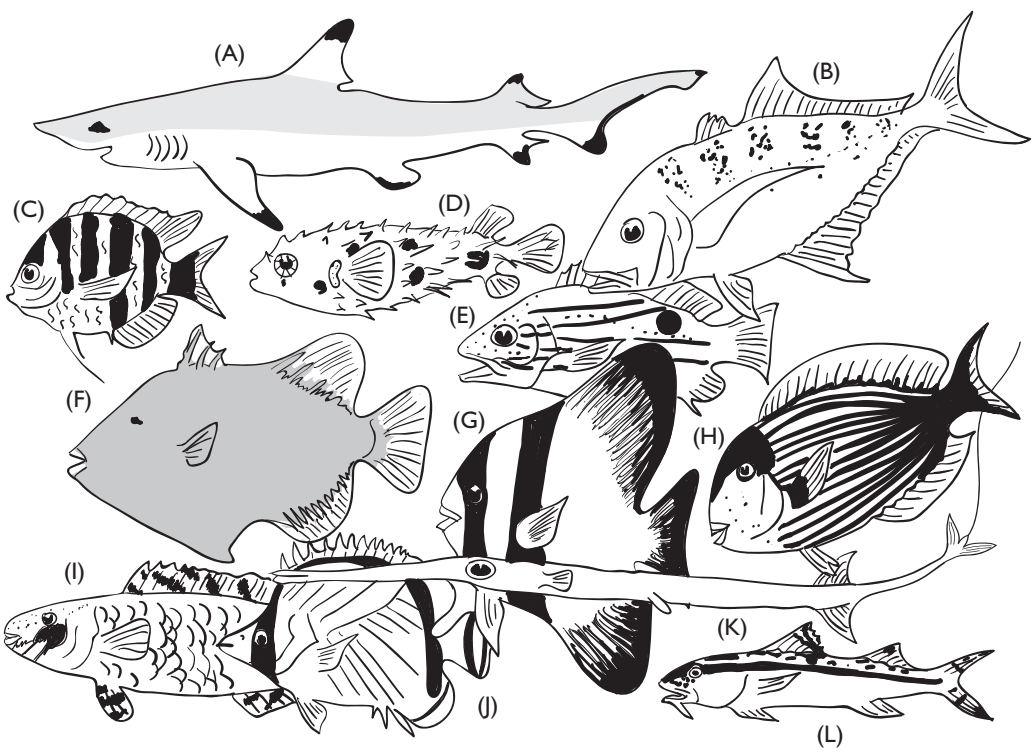

Figure 2.10 Coral reef fishes from the Great Barrier Reef (not to same scale). (A) Blacktip shark (*C. melanopterus*); (B) jack (*Caranx*); (C) damselfish (*Abudefduf*); (D) puffer (*Tragulichthys*); (E) snapper (*Lutjanus*); (F) trigger fish (*Cathidermis*); (G) batfish (*Platax*); (H) sturgeon fish (*Ctenochaetus*); (I) parrotfish (*Leptoscarus*); (J) butterfly fish (*Chaetodon*); (K) cornetfish (*Fistularia*); (L) goatfish (*Upeneus*). After Marshall TC (1965).

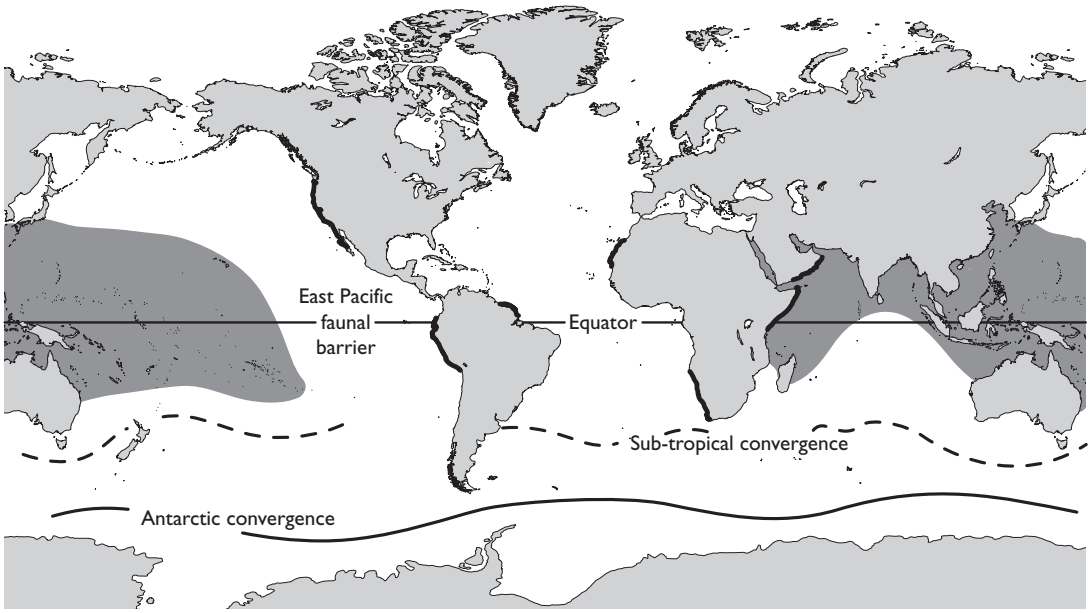

Figure 2.11 The world oceans, showing areas of coral reefs (stippled), coastal upwelling zones of high productivity (black), and the Antarctic and sub-tropical convergences. Modified from Marshall NB (1965).

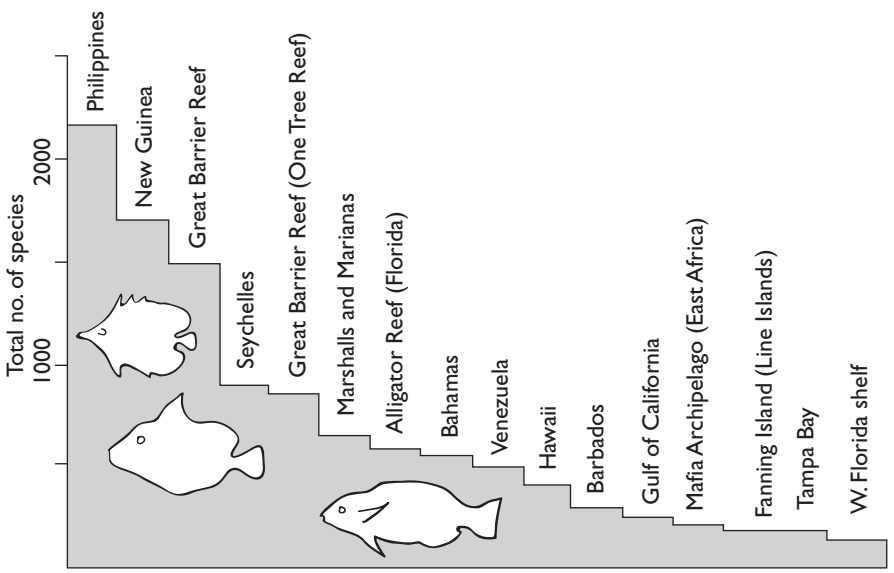

Figure 2.12 Species diversity of coral reef fishes at different reef locations. After Sale (1980).

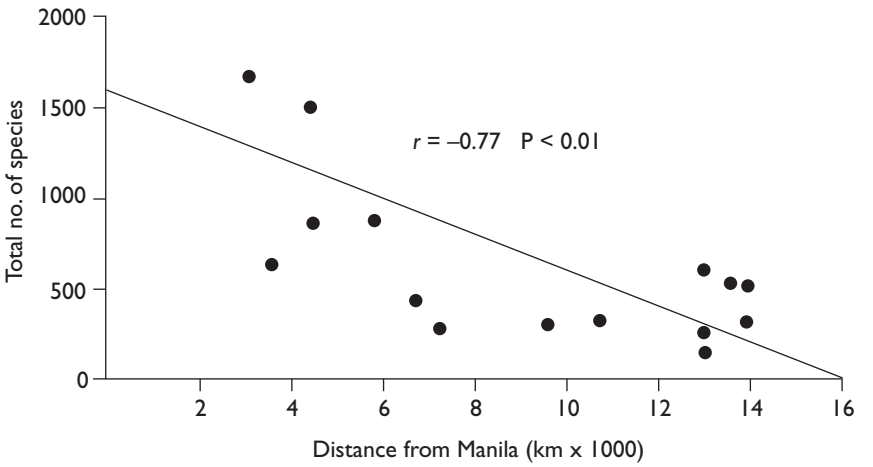

Figure 2.13 Numbers of species of coral reef fishes from each of the 16 sites in Figure 2.12, showing geographical cline in diversity. After Sale (1980).

now the eastern Pacific (Figure 2.14). Closure of the Red Sea land bridge about 18 mya divided this fauna into eastern (Indo-Pacific) and western (Atlantic and Eastern Pacific) components, which were further separated by the Messinian "salinity crisis" (8 mya) in which the Mediterranean basin was completely cut-off from both the Red Sea and Atlantic becoming a hypersaline sea and possibly drying out completely. The opening of the Atlantic Ocean (10–32 mya) separated the geographically restricted and species-poor eastern Atlantic from the larger, richer western Atlantic.

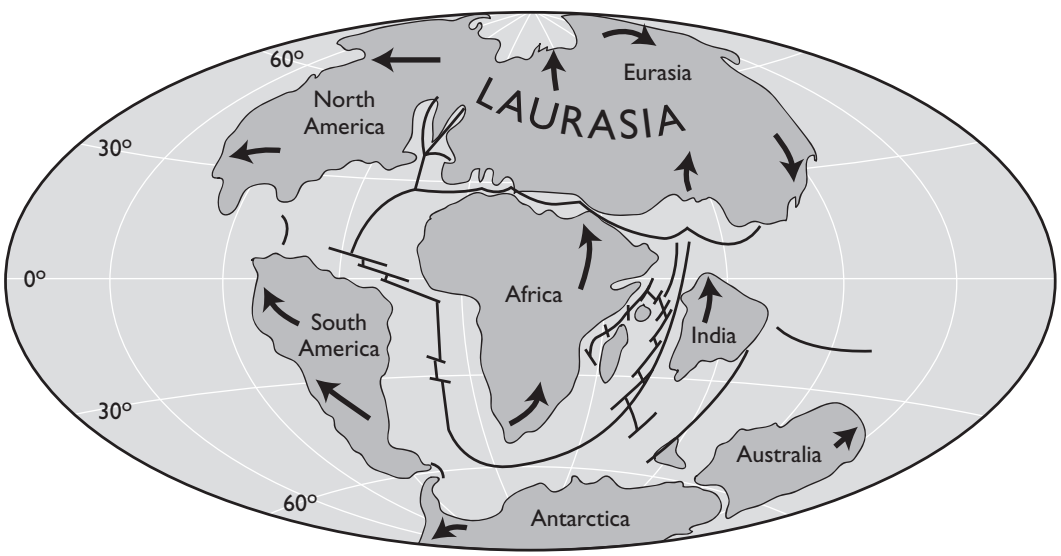

Figure 2.14 Continental and oceanic relationships in the Cretaceous Period showing an open seaway (the Tethys Sea) stretching between the northern and southern hemispheres.

Closure of the Isthmus of Panama about 3.5 mya led to the isolation of eastern Pacific populations and the subsequent evolution of pairs of closely related, and often virtually indistinguishable, geminate species on either side of the isthmus. The emergence of the isthmus was not a singular event, but perhaps as many as four distinct events, resulting in species pairs of varying antiquities.

Nearly all coral fishes are acanthopterygians, and many families such as gobies (Gobiidae), wrasses (Labridae), damsel fishes (Pomacentridae), butterfly fishes (Chaetodontidae), and squirrel fishes (Holocentridae) are represented globally at all coral reefs, although in each faunal area the species are largely different. On the Great Barrier Reef, 43% of all fishes are gobies, while wrasses and damsel fishes each comprise 23%, and butterfly fishes 8%.

Coral fishes make their living in many specialized ways. Some are herbivores, cropping algae, or, like parrot fishes (Scaridae), scraping and biting off coral to obtain the algal symbionts. Others, such as puffer fishes (Tetraodontidae), boxfish (Ostraciontidae), gobies, and some damsel fishes, eat invertebrates, and these include the filefishes (Monacanthidae) and butterfly fishes that eat the coral polyps themselves, picking their food with their forceps-like mouths. Yet others prey on fishes, such as the trumpet fishes (Aulostomatidae) which stalk small fishes by swimming close to other non-predatory fishes. Maneuverability is important in the reef habitat, and, as a result, many coral reef fishes have abandoned normal oscillatory swimming except in emergencies and instead flap their pectoral fins (wrasse, parrot fish, and surgeonfishes (Acanthuridae)) or the dorsal and ventral unpaired fins (triggerfishes (Balistidae)), or undulate unpaired fins (seahorses, pipefishes, and trumpetfishes).

Most coral fishes are dazzlingly brightly colored, and change color during courtship as well as by day and night. During the night, large-eyed nocturnal feeders, such as squirrelfishes and the luminescent pempherids, emerge from their daytime hiding places, while day-feeding parrot fishes retire to sleep in mucous cocoons. As well as the brightly-colored and patterned coral fishes, there are also cryptically camouflaged ambush predators, such as carpet sharks (orectolobids), and frogfish (the Western Australian *Batrachomoeus rubricephalus*, as well as looking rather like a frog, croaks like one).

Fishes of temperate and cold waters

The coastal and shallow-sea fish fauna (Figure 2.15) of temperate regions is much less rich in species than that of warm waters, since there are only around 1000 species in the temperate north Pacific, and many fewer in the temperate North Atlantic. Both regions contain members of the same families, such as the scorpion fishes (Scorpaenidae), kelpfishes (Clinidae), and eel-blennies (Lumpenidae), but in the north Pacific these families are more diverse, and this region also has some endemic families, such as the surf perches (Embiotocidae), and the greenlings (Hexagrammidae).

Although there are fewer species than in warmer waters, temperate and cold waters contain the most important food fishes, such as cod, pollock, and haddock (Gadidae), herrings and their relatives (Clupeidae), and the pleuronectid flatfishes such as plaice, soles, and flounders. Upwelling currents along coasts and mid-ocean convergences bring nutrient-rich deep water to the surface, supporting phytoplankton blooms nourishing the zooplankton on

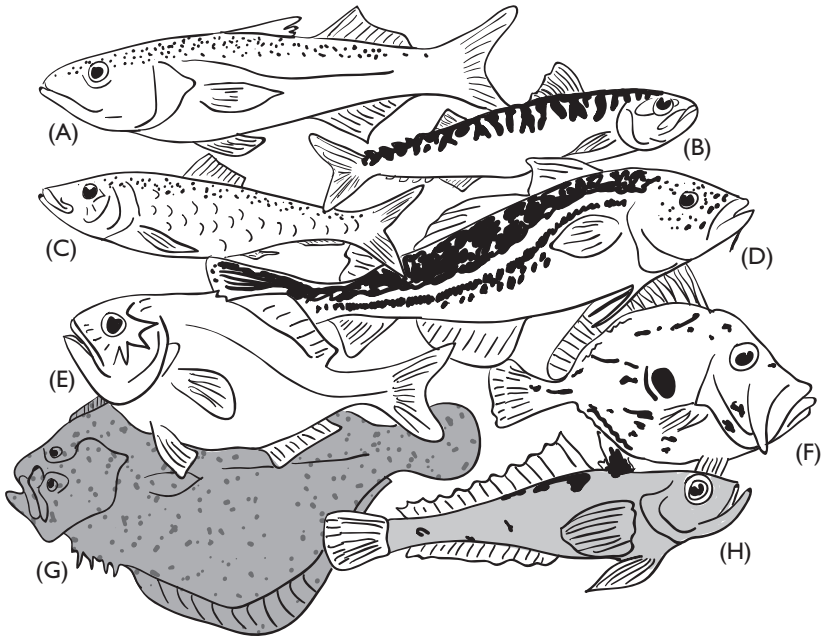

Figure 2.15 Coastal and shallow sea fishes of temperate and cold waters (not to same scale). (A) Bass (*Morone*); (B) mackerel (*Scomber*); (C) herring (*Clupea*); (D) cod (*Gadus*); (E) orange roughy (*Hoplostethus*); (F) John Dory (*Zeus*); (G) turbot (*Scophthalmus*); (H) ice fish (*Notothenia*). After Marshall (1971); Bigelow and Welsh (1925); Hayward and Ryland (1991); Herald (1961); and Whitehead *et al.* (1984–1986).

which fish feed. For example, off the Peruvian coast, upwelling is the basis for the fishery for the Peruvian anchoveta (*Engraulis ringens*), and oceanographic changes (the El Niño Southern Oscillation or ENSO) which reduce upwelling have catastrophic consequences for the fishery and for the seabirds that feed on the anchoveta. On the continental shelf, deeper nutrient-rich waters interact with surface water at fronts, which are regions of high productivity.

Temperate seas vary seasonally in temperature, much more than warm or polar seas which have a nearly constant temperature year-round. In consequence, phytoplankton and zooplankton abundance is seasonal. Thus, for example, Icelandic waters can vary from 0°C in February to 10°C in August, while the more temperate sub-Antarctic waters of the Southern Ocean are in the range 5–15°C. These waters lie between the Antarctic and Subtropical Convergences, and the former, where temperatures rapidly drop by 2–3°C, has long formed a barrier to exchange in sub-Antarctic and Antarctic regions. Once Antarctica had become fully separated from the other parts of Gondwanaland in the late Cenozoic (30–23 mya), the Antarctic circumpolar current decoupled sub-tropical waters from Antarctica, and this isolation permitted the wide adaptive radiation of notothenioid fishes comprising a species flock of over 100 species found only in the south polar ocean (Eastman and McCune, 2000). Molecular evidence indicates that notothenid fishes have been distinct for over 25 and perhaps 55 my (Bargelloni *et al.*, 2000).

Notothenioids are found also on the southern New Zealand shelf and on the Patagonian shelf, perhaps relics of the severe cooling of the Southern Ocean in the Miocene and Pliocene which advanced the Convergence 3–7° north of its present mean position between 8 and 2 mya.

Apart from notothenioids, eel-pouts (Zoarcidae), and seasnails (Liparidae) also occur as coastal fishes in Antarctica, although these are species different from their nearest relatives across the Convergence in the coastal waters of the sub-Antarctic Southern Ocean that are found over the Chilean and Patagonian shelves. The commercially important gadoids, flatfishes (pleuronectids), and clupeids, such a conspicuous part of the fauna of north temperate seas, are poorly represented here, while such fish as the orange roughy (*Hoplostethus*) are presently still commercially important, although overfishing has produced a catastrophic collapse of the New Zealand and Australian stocks of these slow-growing, long-lived fishes to less than 20% of their pre-exploitation levels (see Chapter 14).

Estuarine fishes

Like other estuarine animals, the fishes found in estuaries and river mouths (Figure 2.16) are mainly euryhaline (salinity-tolerant) forms which can live in unstable surroundings, where salinity is variable and the waters are often turbulent and muddy, edged, in the tropics, by mangroves. At any one time, diversity may be low and dominated by just a few species, however, temporal partitioning of the estuarine environment by seasonal migrants, such as herring, sprat, eels, and salmonids, adds greatly to estuarine diversity. In temperate estuaries, typical fishes are grey mullets (Mugilidae), flatfishes such as flounders (Pleuronectectiformes), and shads (*Alosa*), while, in warm waters,

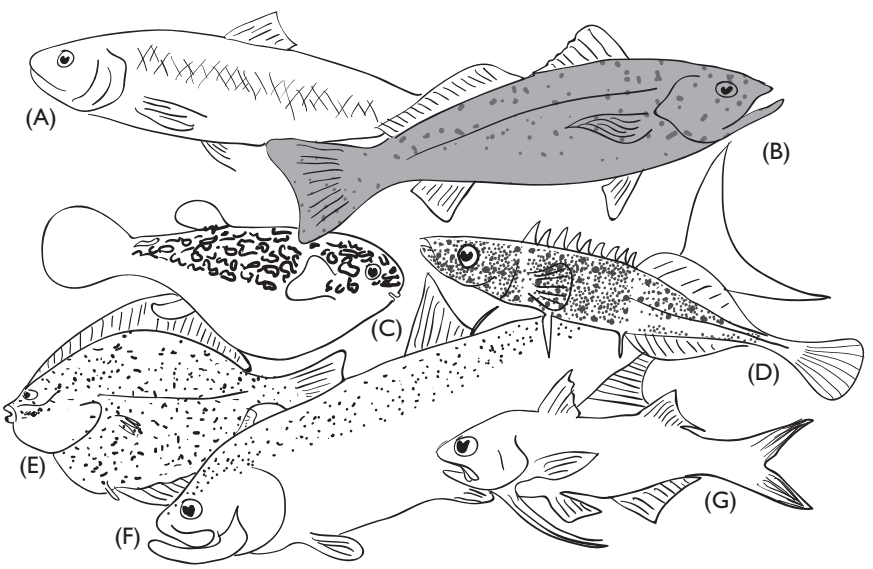

Figure 2.16 Estuarine fishes. (A) Shad (*Alosa*); (B) sciaenid (*Cynoscion*); (C) puffer (*Tetraodon*); (D) stickleback (*Gasterosteus*); (E) flounder (*Platichthys*); (F) tarpon (*Elops*); (G) threadfin (*Polynemus*). After Bigelow and Welsh (1925).

many species of marine origin such as lutjanids, pomadasyids, catfishes, sciaenids, and threadfins (Polynemidae) are found in estuaries, supporting valuable fisheries such as that of the Niger estuary. Fishes of muddy estuaries may look rather like deep-sea fishes, with their long fin-rays and small eyes. The Bombay duck (*Harpadon*), of Indian estuaries, not only looks like a deep-sea fish, but is related to the deep-sea lizard fishes.

In regions of the world were evaporation exceeds freshwater inputs into enclosed coastal bays, hypersaline lagoons represent a special habitat for some estuarine species. The fish community of hypersaline lagoons is usually drawn from the more euryhaline species of nearby estuaries such as mullets, silversides, sciaenids, tilapias, and cyprinodontoids.

Intertidal fishes

The intertidal zone is a demanding environment, where fishes are alternately buffeted by waves and isolated in pools or on mudflats (Figure 2.17). Some intertidal fishes, such as the mudskippers (Periophthalmidae), and leaping blennies such as *Alticus kirki* of the Red Sea, are truly amphibious, emerging from the water to graze on algal films on mud or rock in or above the splash zone. These fishes have remarkable behavioral and physiological adaptations to avoid (or withstand) desiccation and to regulate nitrogen excretion (Chapter 6).

Most intertidal fishes, however, remain in the water, and, to avoid being washed away, generally are dense, small fishes (less than 20 cm), thin or flattened to hide in holes and crevices, or may have the pelvic fins modified into suckers. An interesting and obviously necessary behavioral feature of many intertidal fishes is their pronounced homing ability, particularly striking in

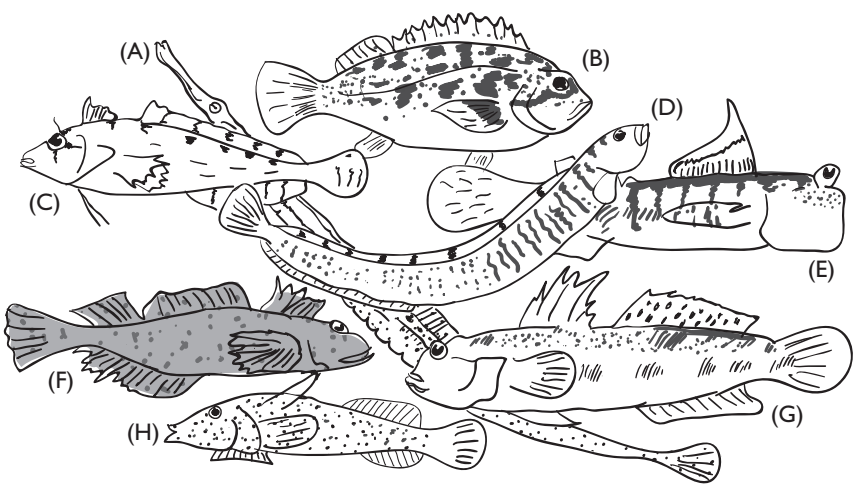

Figure 2.17 Intertidal fishes (not to scale). (A) Pipefish (*Siphostoma*); (B) priest fish (*Sebastodes*); (C) clingfish (*Heteroclinus*); (D) butterfish (*Pholis*); (E) mudskipper (*Periophthalmus*); (F) triple fin (*Tripterygion*); (G) goby (*Gobius*); (H) sucker fish (*Lepadichthys*). After Bigelow and Welsh (1925); Ayling and Cox (1982); Marshall NB (1965).

pool-dwelling blennies that leap into the air to view the surrounding terrain. Intertidal areas are very important as nursery areas for young fishes, for example for young flatfishes in the Wadden Sea, and to avoid stranding, they move up and down as the tide ebbs and flows.

2.4 Freshwater Fishes

Diversity of freshwater fishes

Despite representing only a miniscule (0.0093% according to Horn, 1972) part of the world's aquatic habitat, freshwaters contain a disproportionate number of species (over 40%, again according to Horn, 1972). Unlike the oceans, which constitute broad, uninterrupted expanses of water, freshwater habitats tend to be much smaller in extent, and well separated from other similar habitats by expanses of dry land. Although broad stretches of open ocean may serve as an effective barrier to fish dispersal, dry land is much more effective as numerous zoogeographic studies have demonstrated, making freshwater habitats, to quote Dr. Melanie Stiassny of the American Museum of Natural History, "the ultimate island."

Most of the 8000 or so freshwater fishes (Figure 2.18) known (more turn up each year) live in lakes and rivers out of reach of the sea. Of these, the vast majority (87.5%) are primary freshwater fishes that evolved in freshwater, and exhibit low, if any, tolerance of seawater. Only a very few such fishes have adapted to spawning as well as living in brackish water, such as the cyprinids in the Caspian and Aral Seas, whose eggs can develop in salinities up to 8–10%. By far the majority (over 93%) of primary freshwater fishes are ostariophysan

Figure 2.18 Freshwater fish (not to scale). (A) Arapaima (*Osteoglossum*); (B) elephant snout fish, a mormyrid (*Campylomormyrus*); (C) (*Labeo*); (D) (*Lepidogalaxias salmandroides*); (E) archer fish (*Toxotes*); (F) climbing perch (*Anabas*); (G) paddlefish (*Polyodon*) feeding on plankton; (H) catfish (*Mystus*). After Herald (1961); Merrick and Schmida (1984); and Inger and Kong (1962).

catfish, carps, and characins, the remainder, including the lungfishes, osteoglossids in Australia, South-East Asia, South America, and Africa, the weakly electric mormyrids in Africa, as well as a few widespread larger families such as the pikes (Esocidae), and perches (Percidae). Although most continents possess well-developed primary freshwater fish faunas, isolated regions may lack them. The only two certainly primary freshwater fishes of Australia are the lungfish *Neoceratodus*, and the osteoglossid *Scleropages*, the occurrence of these is believed to reflect the Pangean distribution of these two ancient lineages. The island of Madagascar, despite its proximity to Africa, possesses no primary freshwater fishes nor do the islands of the West Indies or Pacific Oceania.

Secondary freshwater fishes are those whose ancestors entered from the sea in the past and which often still retain close relatives living in the marine environment. Secondary freshwater fishes include the widespread Cichlidae, and numerous cyprinodontiform and atheriniform species, the southerly distributed Galaxiidae, and the Centrarchidae of North America. They mostly now live exclusively in freshwater, and, even though the main groups (cichlids, cyprinodonts, and poeciliids) have species that may have considerable salinity tolerance and can live and even breed in brackish water, most must spawn in freshwater. Several predominately marine families, such as clupeids, puffer fishes (Tetraodontidae), gobies (Gobiidae and related families), and drum fishes (sciaenids), have freshwater representatives, for example the small ctenothrissid clupeids of West African rivers or the Australian clupeid *Nematalosa erebi*. There are even a few entirely freshwater stingrays (Dasyatidae).

Diadromous fishes migrate between fresh and salt waters. Some, like the 19 or so species of freshwater eels (*Anguilla*), leave freshwater to spawn in the sea (catadromous species), making very interesting anticipatory changes in serum ion content, body color, and visual pigments before and during their passage downriver (p. 217). As well as the eels, one or more pleuronectids, scorpaenids, and mullets (Mugilidae) are catadromous, although, aside from the eel, freshwater is not obligatory in most of these species (Dadswell *et al.*, 1987). Many more (anadromous species) make the reverse migration to spawn in freshwater after periods up to 4 or 5 years growing and maturing in the sea. Parasitic lampreys, many salmonids, shads (*Alosa, Ilisha*), and alewives (*Pomolobus*), and smelts (Osmeridae) are all anadromous. A third category of diadromous fishes, amphidromous species, migrates into or out of the sea but do not do so for breeding purposes. For example, the newly hatched larvae of the ayu (*Plecoglossus altivelis*), a fish much prized as a delicacy in Japan, are swept downstream to the sea whence they return as small juveniles to grow and feed for several years before breeding. A similar situation is seen in the southern salmoniform galaxeids. McDowall has examined the systematic distribution of diadromy in teleosts, concluding that it is an ancient life-history style making it difficult to ascertain for modern groups whether they might have had a marine or freshwater origin (McDowall, 2002). Figure 2.19 shows possible steps in the evolution of diadromy in fishes.

As with marine habitats, there are distinctly different types of freshwater habitats: lakes, ponds, pools, rivers, streams, and springs, and within each of these numerous microhabitats may exist a high degree of ecological and genetic diversity among freshwater fishes.

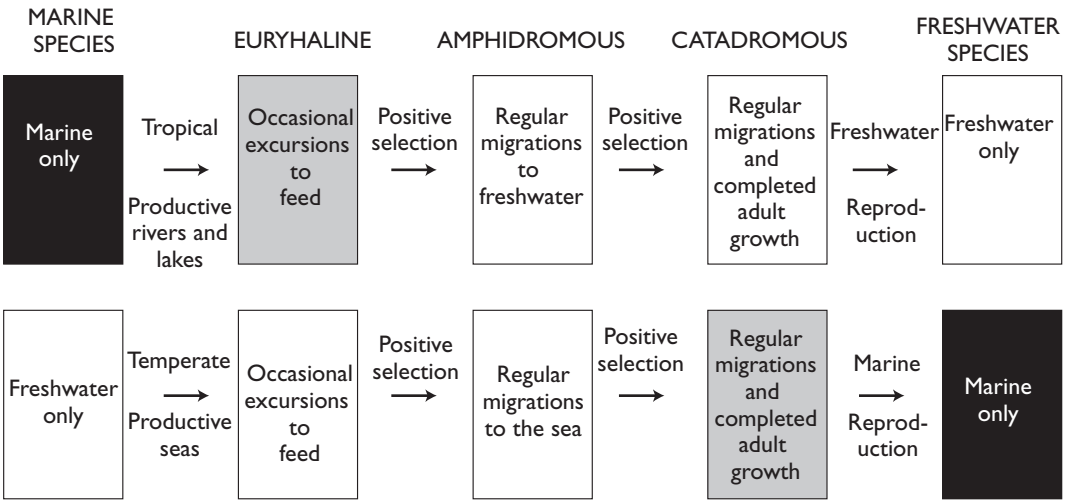

Figure 2.19 Possible steps in the evolution of diadromy in fishes. After Gross (1987).

Lentic systems

Stillwater, or lentic, habitats include lakes, ponds, and impoundments. Although these habitats vary tremendously in their sizes, origins, and ages, all have certain common physical characteristics and are inhabited by similar fish communities.

As in the oceans, deeper lakes may exhibit vertical zonation in their physico-chemical and biotic variables. The shallow zone around the edge of a lake, the littoral, may support a community of rooted aquatic plants, benthic invertebrates (chiefly arthropods and annelids) as well as diverse bottom-oriented fish community (sunfishes, catfishes, and suckers).

In deep lakes the combination of temperature and water density (itself temperature dependent) can create thermal stratification separating a community of planktivorous fishes (atherinids, clupeids, and their relatives) that dwells in the epilimnion, while the deep bottom-water layer, the hypolimnion, provides habitat for predatory as well as bottom-feeding fish species. This stratification can lead to oxygen depletion, even anoxia, in the hypolimnion and to the phenomenon known as turnover when in response to cooling surface temperatures the thermocline gradually rises to the surface permitting the cold oxygen-poor deep waters to mix with surface waters. The resultant drop in dissolved oxygen can produce considerable stress and sometimes massive fish kills. The thermocline zone may contain a fish community distinct from both the epi- and hypolimnion.

In the tropics, stratification can also occur, but the lack of seasonal temperature variations means the thermocline is more stable and turnover rarely, if ever, occurs. This leads to a more-or-less permanently anoxic hypolimnion that cannot support fish or most other aquatic life.

Lotic systems

Flowing water, or lotic systems, such as streams and rivers, exhibit little, if any, stratification, however, longitudinal differences in gradient, temperature,

water velocity, bottom type, width, and depth, and other features create distinct habitats in which different fish communities may be found. In general there is a correlation between stream order and fish species diversity due to increasing complexity and variety of habitats in the downstream reaches.

Since rivers are continuous from their headwaters to their mouths, fish can often pass between adjacent reaches and some broadly adapted species may occur nearly throughout the entire length of the river, however, most rivers can be divided into zones or reaches differentiated by width, bottom type or current, each with a distinctive fish community.

Tropical rivers support a higher diversity of fish species than their temperate counterparts. The world's largest river system, the Amazon, contains over 2000 species with more than 50 new species being described each year (Lundberg and Troll, 2001), while the Congo system in Africa has about 700. In comparison, the Mississippi-Missouri system of North America has fewer than 400 species. As in tropical lakes, speciation has produced a wide variety of trophic and other specializations for living in the slow, turbid, and frequently anoxic waters. Although season flooding plays an important role in the ecology of many temperate coastal rivers, such floods may be more essential in the life cycles of many tropical river fishes where numerous species take advantage of the expanded habitat of the flood plain to spawn and feed. Frugivorous fish species that are almost unique to the tropics also play a role in determining the distribution of floodplain trees similar to the roles of terrestrial bird and mammal frugivores.

2.5 Ostariophysan Success

Ostariophysan fishes dominate the freshwater fish fauna throughout the globe: both Africa and South America having more than 2500 species. In South America, where cyprinoids are absent, characoids have radiated widely, evolving herring-like, mullet-like, goby-like, minnow-like, and salmonid-like forms. There are even characoid flying hatchetfishes (Gastropelecidae) that are reputed to flap their elongated pectorals at high frequency to remain airborne. Catfishes (siluriformes) have also radiated in South America, where there are 14 endemic families and 1100 species, almost as many as characoids (15 endemic families, over 1500 species). In Africa, the different ostariophysan groups occupy different broad habitat types: characoids almost always inhabit sluggish lowland streams, cyprinids higher-level flowing streams and rivers, while catfish occupy most habitats, presumably side-stepping competition from these other diurnal fishes by being active only at night. The 1800 plus acanthopterygian cichlids are mainly found in the extraordinary species flocks of the African rift lakes, such as Lake Malawi, Tanganyika, and Victoria.

The reader may well enquire why it is that the ostariophysi have been so successful in freshwaters, although few (only 140 out of over 2200 catfish species) have become marine. It may seem improbable that the acuteness of their hearing, paralleled in the sea by the clupeids, is the most important basis of their success. However, the distantly related, but ecologically parallel osteoglossid mormyrids testify to the importance of hearing in freshwaters, for, in addition to their sophisticated electrolocation and signaling systems,

they also have gas-filled swimbladder remnants linked to the inner ear (Chapter 10).

The predator-warning pheromone signaling system is a second notable specialization (similar systems are found also in several acanthopterygian families; see p. 338). For schooling fishes, as most characins and cyprinids are, this system must be especially valuable; it is even likely that the evolution of the Weberian ossicle system (see p. 297) was linked to a schooling habit, as excellent hearing seems particularly useful to help fish in a school monitor the movements of their neighbors.

2.6 The Variety and Origin of Some Freshwater Fish Faunas

The variety of freshwater fishes is striking, for they show radiations into different habitats with some of the most extraordinary adaptations of all fishes. The African mormyrids and the South American gymnotids, for example, have independently evolved amazingly specialized electrical signaling systems for use in crowded and turbid waters, and many freshwater fishes have developed different accessory respiratory devices to enable them to live in swamps where the oxygen content of the water is low. Something of this variety is seen in Figure 2.18. The radiations of the cichlid species flocks of the African lakes provide what are probably the best examples of evolution in progress at the species level.

Equally fascinating are the distributions of freshwater fishes, which are closely linked to the geological history of the regions they inhabit, and may, indeed, offer useful clues to the complex history of the changes in the Earth's surface. For example, one scenario for the dominant freshwater group, the ostariophysi, following a relatively simple continental drift scheme, is that ostariophysans arose in Gondwanaland in the early Cretaceous before the South American and African plates separated.

What once seemed to be the most primitive ostariophysans now live in South America, hence the ostariophysi arose in the South American portion of Gondwanaland, and the modern African characoids and siluroids came from ancestors which reached West Africa before the two continents had finally separated. Antarctica, Australia, India, and Madagascar had already separated, hence ostariophysans in these regions came later, such as the two secondary freshwater Australian catfishes. The Laurasian plate also separated early, but the African plate contacted the Laurasian at the end of the Cretaceous, and ostariophysans were able to enter Laurasia. Cyprinoids then evolved from this ostariophysan stock and spread throughout Laurasia and most of Africa, although they did not manage to reach South America (Figure 2.20). Today, South-East Asia has the greatest diversity of cyprinoids.

It will hardly come as a surprise to the reader (who should by now be accustomed to the notoriously revisionist tendencies of fish taxonomists!) that this scenario has been challenged on various grounds. Thus, the "classical" idea that characoids gave rise to cyprinoids has been inverted by Fink and Fink (1981, 1996) to make cyprinoids the basal ostariophysan group, and rejecting the South American portion of Gondwanaland as the site of origin of the group. Additional work on the ostariophysan lineages in the past decade largely supports the Finks' (1996) scheme, even if it does not explain why cyprinoids are absent from South America. The simpler distribution of living

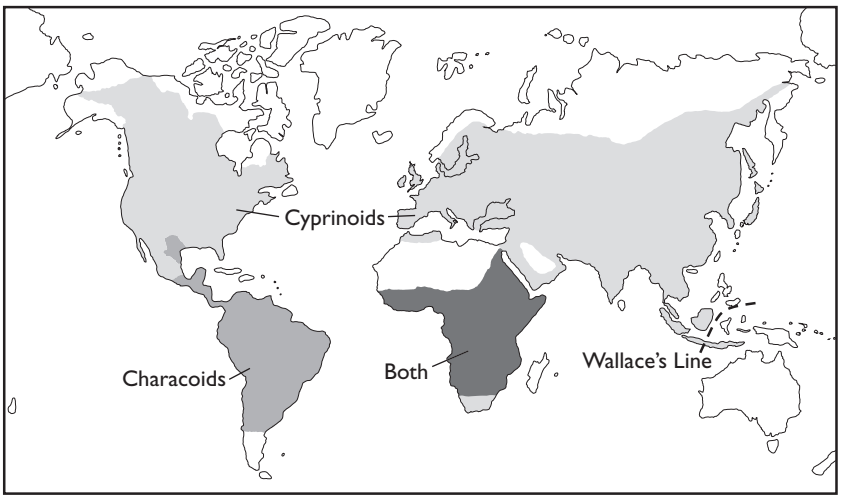

Figure 2.20 The distribution of cyprinids and characoids. After Nelson (1976, 2006).

lungfishes (see Figure 1.15) shows (even if we had no fossil lungfish from Antarctica) that they evolved earlier than the ostariophysi, before the separation of Australia from Gondwanaland.

2.7 Lakes and Species Flocks

Most of the great lakes of the world, the Laurentian Great Lakes of North America, Lake Baikal, the Rift lakes of Africa, and Lake Titicaca in the Andes, as well as many smaller lakes such as Lake Lanao in the Philippines or the Laguna Chichicanarab in Mexico have developed numbers of closely related endemic species (those that occur nowhere else in the world) that are referred to as "species flocks" and are believed to have evolved from only a few ancestral forms that were present in the lake when it was first formed. Large species flocks may occur in larger lakes such as Lake Victoria where it appears that hundreds of species (many becoming extinct before they are even discovered or described) have evolved from a few ancestral forms in less than 0.5 my (some say as little as 15 000 years – Meyer *et al.*, 1990; Salzburger and Meyer, 2004). But after only a few thousand years of isolation, smaller lakes can develop their own distinctive fish faunas, often rich in endemic species. These species flocks typically show great diversification of morphological adaptations, often associated with feeding or reproduction, that permit the occupation of a wide variety of ecological niches very different from the ancestral type. The small Mexican lake, Cuatro Ciénegas provides an example of what may be a best case of a species flock in the process of evolution. The lake's sole native cichlid species (*Herichthys minckleyi*) exhibits at least three distinct morphotypes, each characterized by body form and tooth morphology as well as distinct feeding preferences. All morphotypes can occur in a single nesting and the eventual development of teeth and food habits depends partly on genetics and partly on food availability during the fish's development.

Lake Baikal in Siberia is the world's largest (by volume) and deepest lake. Its fish fauna consists of only about 50 species classified into 12 families. But three of these families (Cottidae, Abyssocottidae, and Cemephoridae) containing 23 species are varieties of sculpin. The Lake Baikal cottoids show distinct depth distributions. Unlike many deep lakes, Baikal's waters are well oxygenated throughout, allowing fishes and invertebrates to occupy habitats down to the maximum depth of the lake, just over 1600 m. Although the shallow-water species are similar in many ways to shallow-water fishes found in other nearby habitats, the deeper dwelling species of Lake Baikal show amazing parallels with many deep-dwelling marine fishes having reduced musculoskeletal and respiratory systems, oil-based buoyancy systems, and eyes adapted for vision in their low-intensity light environments. It seems all that is missing in Lake Baikal fishes are bioluminescent organs to make the comparison complete!

Envoi

Fishes come in a great variety of different species and successfully occupy almost every habitat on earth that contains liquid water. How and when they (or their ancestors) arrived in each habitat, how they meet the many physiological challenges presented by extremes of by temperature, salinity, alkalinity, and other environmental variables, and how similar morphological, physiological, and biochemical adaptations have evolved in discrete phylogenetic lineages afford a variety of challenges and intellectually rewarding experiences for the student of fishes

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3 Swimming

Some fish can fly, others climb trees or, like mudskippers, sit about on mudflats, only occasionally entering the water. But most fish (of all taxonomic persuasions) live and move for all their lives through water. And the great majority of these swim by oscillating flexible bodies that end in a tail fin. This is what the greater part of this chapter is about; it is not an easy system to analyze for several reasons.

3.1 The Problem of Analysis

Essentially, the problem is to understand how movements of the body or fins resulting from muscle contraction affect the surrounding water in such a way as to move the fish through it. Even though this problem was studied for decades, it was not until the appropriate mathematical tools were to hand that real progress was made. Lacking an obvious way of monitoring water movements, the first attempts to tackle the problem were to examine the movements of the fish, and to attempt to deduce from these how thrust was produced. The difficulty here was that analysis was handicapped by the fact that almost all our own machines moving through fluids are rigid bodies with rotating propellors or jets to move them along. It is true that there is a handful of fairly successful mechanical fish that swim by oscillating their bodies (like the roboshark used for photographing shark feeding frenzies), but these all came later.

The hulls of submarines are quite similar to the solid of least resistance drawn from the body of a trout by the great pioneer experimental aerodynamicist Cayley (Figure 3.1). The density and viscosity of the medium dictate the same streamlined shape, but there is an obvious and significant difference between the two. In most fish, the myotomal powerplant itself is involved in the movements that produce thrust, whereas in submarines the engine is held in a non-flexing hull and movements within the engine are divorced from the aft propellor. Consequently, one can make models and test them in wind or water tunnels, and, using well-established formulae, calculate the thrust required (and the power output of the engine) to overcome the drag of the hull and move it forward at any given speed. For fish that oscillate their bodies as they swim, the formulae for rigid bodies are inappropriate. As we shall see later

in this chapter, very fast swimming fish such as the tunas have limited body flexion and have more or less separated body muscle movements from the tail propellor. Again, some fish such as wrasse or trigger fish, and many electric fish (such as gymnotids) produce thrust when swimming slowly by using movements of paired or unpaired fins to generate thrust without moving the body itself. This kind of swimming can be analyzed (Lighthill and Blake, 1990) using the same formulae as for submarines (although the movements of the fins will alter the flow and drag around the body to some extent). However, even these fish that use their paired or unpaired fins as propellors, only do so when swimming slowly, for rapid swimming they change gear and flex their bodies (see for example, Jayne and Lauder (1993) and Korsmeyer *et al.* (2002)). For fish that oscillate their bodies as they swim, the formulae for rigid bodies are inappropriate.

It is a striking tribute to the distinguished small groups of applied hydrodynamicists (mainly at Cambridge and the California Institute of Technology), that undertook the challenging problems of calculating the drag, power output, and efficiency of swimming in different ways, that these problems have now, to a large extent, been solved, although it must be admitted that some difficulties remain (see Lighthill, 1971, 1973). As may well be imagined, movements of the water around the fish as it swims are complex, and could not be analyzed until very recently, when it became possible to view flows around the fish using laser-illuminated small silvered beads monitored by a high-speed video camera. This digital particle image velocimetry (DPIV) provides views of vortex rings shed from the fins or tail, initially in two dimensions only (Figure 3.2), and then in three (see Drucker and Lauder, 1999). From vortex ring direction and momentum so obtained, forces exerted on the water can be calculated. Prior to the advent of this technique, good reviews of the "state of the art" in analysis were given by Blake (1983, and later in 2004); Daniel *et al.* (1992) and Videler (1993).

With most fish we can now make reasonable estimates for what we would like to know about any self-propelled machine: how fast does it go, how much power is needed, and how mechanically and fuel-efficient is it? Unfortunately, the latter considerations do not always enter into the choice of our own transport!

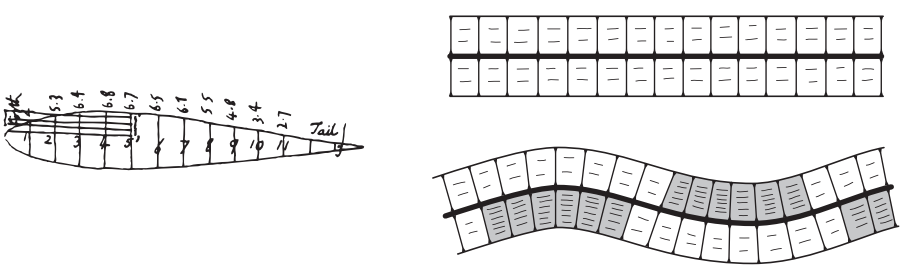

Figure 3.1 Left: drawing of a trout by Sir George Cayley (1773–1857), the "Father of the Aeroplane," who designed and flew in 1852 the first glider to carry a man (his coachman). Right: schematic diagram illustrating bending of a body with a flexible but incompressible backbone (thick black line) by segmented muscle blocks (contracted myotomes stippled). Partly after Gibbs-Smith (1962).

This chapter first examines the muscles fish employ to move their bodies, then how these movements generate propulsive thrust, how fast fish swim and how efficient swimming is, and then how fish may reduce the costs of swimming in fascinating ways.

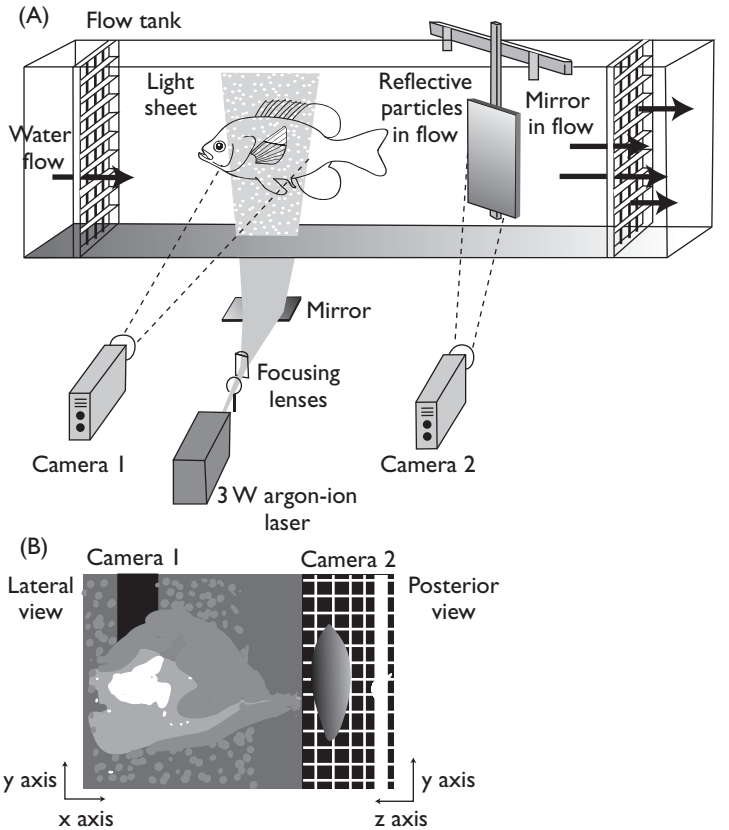

Figure 3.2 Digital particle image velocimetry (DPIV) system. (A) In its depicted configuration, the system allows visualization of water flow in the parasagittal plane behind the left pectoral fin of the bluegill sunfish. The beam of light supplied by an argon-ion laser is focused by means of cylindrical lenses into a thin sheet that is projected into the working area of the flow tank. High-speed video camera 1 records images of reflective particles, illuminated by the laser, moving in the wake of the fin. Camera 2 records a posterior view of the fish via a small mirror mounted at 45° in the flow downstream. For the purposes of illustration, the fish, the reflective particles, and the mirror in the flow are shown at larger-than-actual scale within the flow tank. (B) Signals from cameras 1 and 2 are synchronized and recorded as split-screen images, an example of which is shown. The lateral view of camera 1 shows the silhouette of the ventral margin of the fish in the background, and the bright reflection of the pectoral fin as it breaks the plane of the laser light sheet. Particles suspended in the flow are visible as a cloud of white specks. The posterior view of camera 2 shows, in mirror reflection, the body of the fish and the left pectoral fin as it passes through the laser plane, seen on edge as a vertical white band. The upstream collimator grid is visible in the background. In each case, the plane of view is defined by a pair of perpendicular axes (*x, y, z*). After Drucker and Lauder (1999).

Theoretical models have been tested by a variety of new methods, including high-speed kinematography and particle velocimetry to examine patterns of water flow around and behind fish swimming in tunnel respirometers (Figure 3.2).

3.2 The Myotomal Muscles

Myotomal structure

Oscillation of the body and of the tail fin results from the contraction of the segmented axial musculature, divided into myotomes by the myoseptal connective-tissue partitions on which the muscle fibers insert. Because the supporting central notochord or vertebral column is incompressible, the body bends laterally as the myotomes on one side contract and shorten (Figure 3.2). A simple model of such a system could be made by tying string along either side of a hacksaw blade through the holes at each end, and pulling one or other of the strings (to simulate contraction), when the blade bends but does not shorten. But (unsurprisingly) such a simple model gives no idea of the mechanical subtleties of the myotomal layout, which, after all, has been refined since its origin in the pre-Cambrian. The notochord or vertebral column lies dorsally (with the viscera below) and presumably must always have done so since the origin of the chordate body shape. The consequence of this is that the greater bulk of the myotomes lies below the notochord, and, to avoid simply bending the body downward at either end when they contracted, the ancestral myotomes were V-shaped with the apex of the V at notochordal level and a longer backward pointing arm below.

Although the fascinating *Pikaia* from the lower Cambrian Burgess shales still awaits detailed description, it is clear that its myotomes were of this form, as were those of conodonts and the fish-like chordates from the Chinese fossil Lägerstetten fields. Similar V-shaped myotomes are seen today in amphioxus and in early fish larvae. Once the fish larva begins to ossify the vertebral column, and so restrict its movements to the lateral plane, increased overlap and folding of the myotomes begins, leading to the complex structures of living adult fishes.

Fish myotomes are indeed most complex in shape, and, apart from amphioxus, in all other living adult fishes the outer borders of the myotomes are folded into W-shapes (Figure 3.3), after beginning in ontogeny as simpler V-shapes. The folding and overlap of the myotomes to make a set of nesting cones means that in a transverse section (Figure 3.4) portions of several myotomes are cut across, separated by horizontal septa (extensions of the apices of the myosepta), so the muscle fibers of the myotomes lie in a series of horizontal tubes of connective tissues running along the length of the fish.

It is extremely difficult to make two-dimensional diagrams of such three-dimensional structures so figures such as Figures 3.3 and 3.4 are no substitute for examining them yourself. Probably the best approach is to take a fair-sized fish and lightly boil or steam it to separate the myotomes and then try and model them from modeling clay.

However, even if one succeeds in making simulacra of myotomal shapes, shape is not the only complex feature of the myotomes. The arrangement of

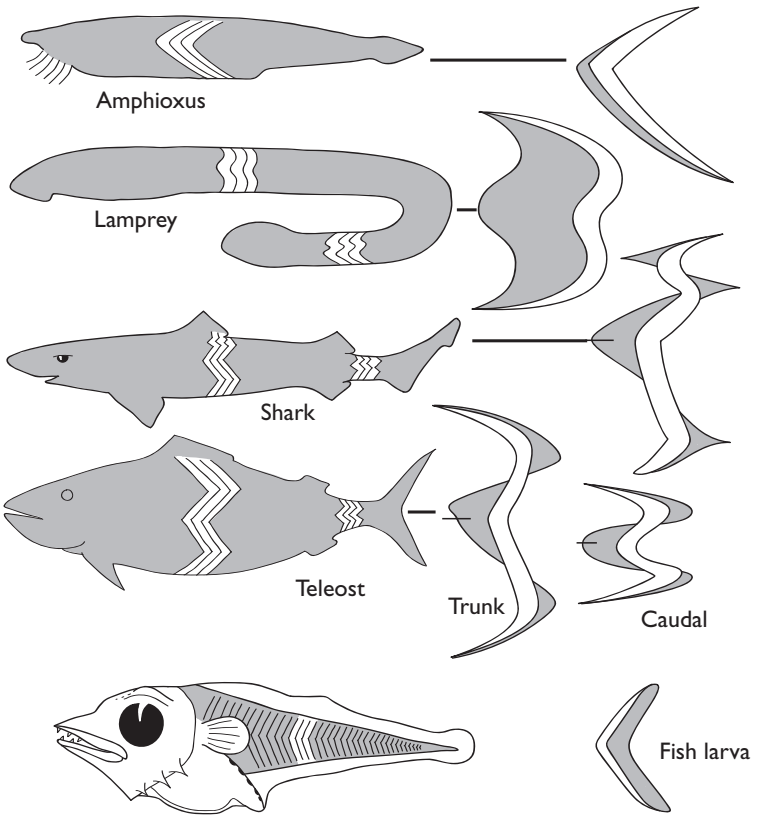

Figure 3.3 Myotomal form in different fishes. Single myotomes at right, their outer surfaces stippled. After Nursall (1956).

the muscle fibers they contain, and that of the tendons within the myoseptal partitions between each myotome is also complicated. We might expect that all the myotomal muscle fibers would lie parallel to the long axis of the fish, which is the arrangement found in amphioxus, where the muscle fibers are very thin flat plates extending from the outer to the inner edges of the myotomes, rather than more or less circular as in gnathostomes. In lampreys and hagfish, the muscle fibers of the myotomes are arranged in a series of sandwiches, most obvious in lampreys where one kind of muscle fiber is flattened (although thicker) as in amphioxus (Figure 3.5). In higher fishes, such as teleosts and elasmobranchs, only the most superficial fibers just under the skin lie along the long axis; deeper fibers spiral in the myotomes at quite large angles (up to 30°) to the body axis (Figure 3.5).

Recently, Gemballa and Vogel (2002) have examined in detail the spatial arrangements of the deeper fibers and the myoseptal tendons in the myotomes of lampreys, hagfish, and gnathostomes. They conclude that in each of these groups the myotomes are arranged differently, and, whereas lampreys and gnathostomes are similar in most respects, hagfish myotomes

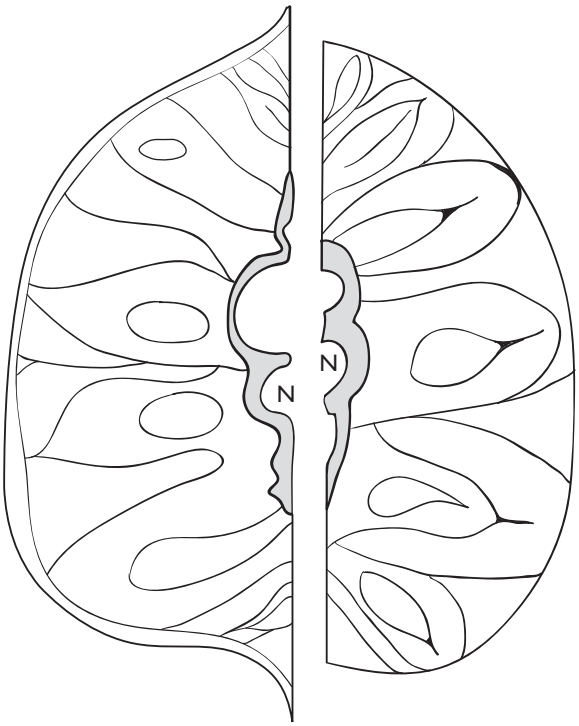

Figure 3.4 Transverse hemi-sections across caudal region of shark (right), and trunk region of elver stage of an eel (left) showing sections of the nesting myotomal cones. N: notochord. After Willemse (1972), and Egginton and Johnston (1982).

are curiously different. For instance, hagfish lack firm myoseptal connections with the skin, an important part of the transfer of force from muscle fibers to the water. Anyone who has ever handled a hagfish will confirm this, for they feel as though enclosed in a floppy sock, (full of blood), quite unlike lampreys. Hagfish blood volume (180 ml kg^{-1}) is high, adding to the sock-like feeling.

In advanced teleosts, the trajectories of the deeper fibers in adjacent myotomes form segments of a helix (Figure 3.6); in sharks they are ordered less regularly. This may seem at first sight a curious arrangement, but it seems to have two important consequences. First, it permits these deeper fibers all to contract to a similar extent for a given amount of bending of the body. Second, while calculations based on the angles of the fibers to the longitudinal axis indicate that the longitudinally running superficial fibers shorten during swimming by about 10% of their resting length, the deeper fibers shorten less (only 2–3% in advanced teleosts) so contraction is near isometric, the least amount of mechanical work is performed, and the fibers operate at the most advantageous point of the force–velocity curve. The analysis of the fiber trajectories in the myotomes may also hint why the myotomes are folded in such a complex way, most likely to allow optimum fiber packing and orientation in a wide myotome.

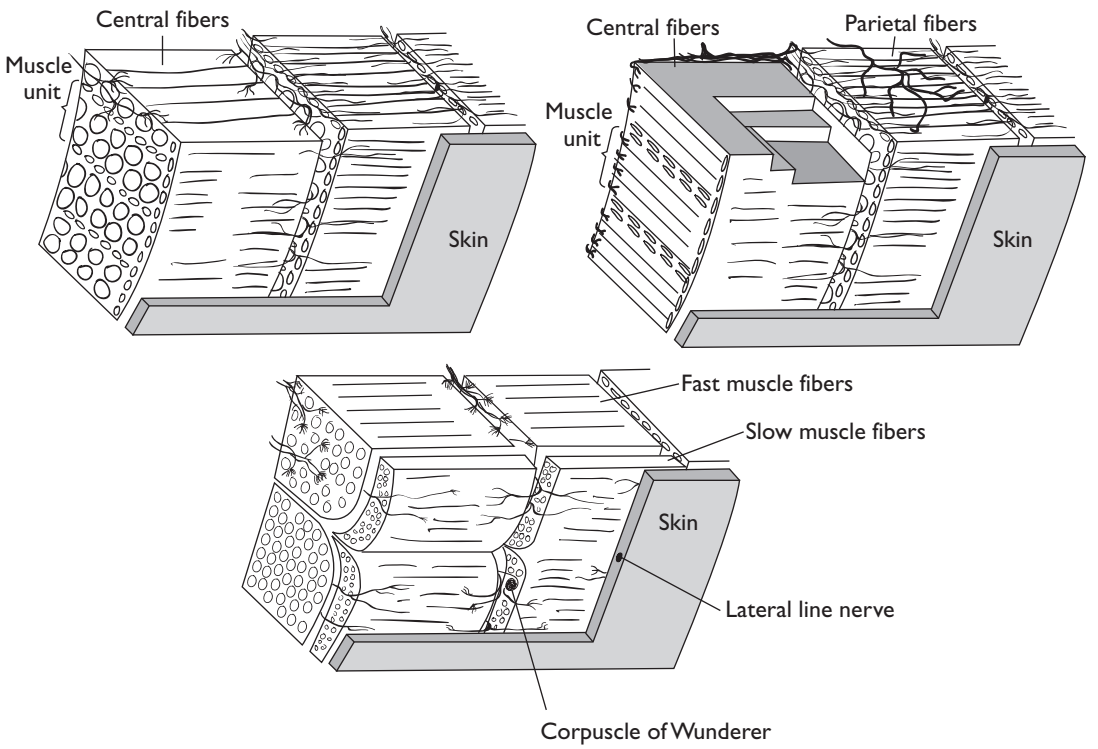

Figure 3.5 Innervation of myotomal muscle. Fiber sandwiches in hagfish (*Myxine*) on left, and lamprey (*Lampetra*) on right, compared with non-sandwich arrangement in dogfish (*Scyliorhinus*) underneath.

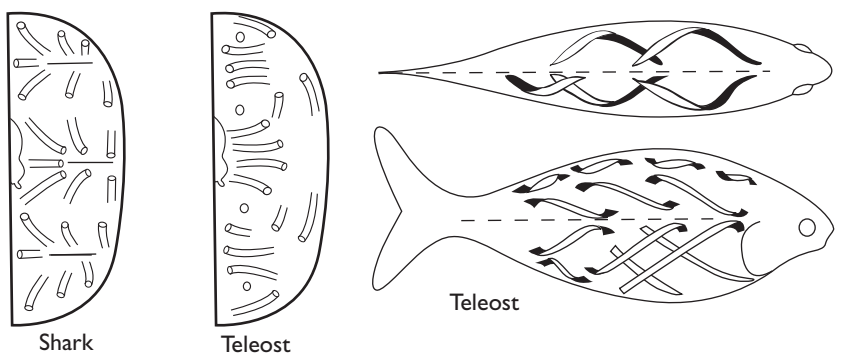

Figure 3.6 Orientation of muscle fibres. Left: thick transverse slices across a shark and a teleost showing orientation of myotomal muscle fibers. Note tendons in shark (horizontal lines). Right: dorsal and lateral views of typical teleost showing course of muscle fibers in successive myotomes along the body. The helices shown were obtained by taking the origin of one muscle fiber from the point at which the muscle fiber in the myotome next anterior inserts onto the common myoseptum, and so along the fish. After Alexander (1969).

Myosepta

The myosepta onto which the muscle fibers attach consist of a meshwork of inextensible overlapping collagen fibers. As a result, the myosepta are like loosely woven pieces of cloth, which can be deformed but not elongated, in many teleosts they are often stiffened (to limit this deformation) by ribs and small intermuscular bones, irritatingly obvious when eating clupeids. Just how these stiffening elements function is not entirely clear nor is it obvious why they should be lacking in other fish (all sharks, for example), but it seems probable that they act to permit only lateral movements when the myotomal fibers contract, and they serve also to reduce the general flexibility of the body. The inner borders of the myosepta attach to the vertebral column, the outer to the connective-tissue layer of the skin, which consists, like the myotomes, of overlapping collagen fibers.

How is the force of myotomal muscle contraction transmitted to the water? When a fiber contracts, the tension produced is transmitted to the collagen fibers of the myoseptal cones, and backward and inward to the vertebral column via the horizontal median septa. This avoids the transmission of force from active muscle to inactive compliant fibers in other myotomes, and (because the myosepta are also attached to the connective-tissue layer of the skin), means that the connective-tissue fibers of the skin become involved in the process of bending the body. The skin connective-tissue fibers are wound around in a helical lattice, making angles of 50–70° to the long axis. Direct measurements in small sharks have shown that intramyotomal pressures in active myotomes (consequent on the increase in diameter of the myotomal muscle fibers as they contract and shorten) rise to as much as 20 times resting values (up to 2.8 MPa) during burst swimming. The pressure imposes a circumferential stress on the skin, shed diagonally (which contributes to the shortening of the skin brought about by the shortening of underlying myotomes), and, at the same time, the contractile force is transmitted to the vertebral column at head and tail.

So the skin plays a role in the bending of the fish, and it is possible (although not yet clearly proven experimentally) that it may also store elastic energy, to aid in accelerating unbending as the myotomes of the opposite side of the fish contract. Long and his colleagues (Long *et al.*, 1996) working with *Lepisosteus*, and subsequently Long (1998) studying segments of eels, obtained interesting and suggestive evidence for elastic energy storage by the skin. Calculations based on biaxial stress tests of shark skin suggest that such energy storage is only likely to be of consequence during burst swimming. In fast-swimming fish, such as oceanic scombroids, the posterior myosepta are very oblique, and produced posteriorly into long tendons, giving rise to a tendinous caudal peduncle (Figure 3.7). This means that the mass and inertia of the caudal peduncle is kept as low as possible to allow increased frequency of tail beat, and its width can also be reduced for hydrodynamic reasons. It is for similar reasons that the legs of fast-running antelopes and deer seem unreasonably slim in comparison to our own rather elephantine legs. Additional benefits of this arrangement are that the inertia of the anterior part of the body is increased, so reducing the tendency of the tail fin to cause yaw of the head as it sweeps across, however, it seems unlikely that there is significant energy storage in these caudal tendons as we might have expected.

Tendons in large scombroids pull across a groove in a joint in the vertebrae of the caudal peduncle, in this way more or less reducing flexure to a single point (Figure 3.7).

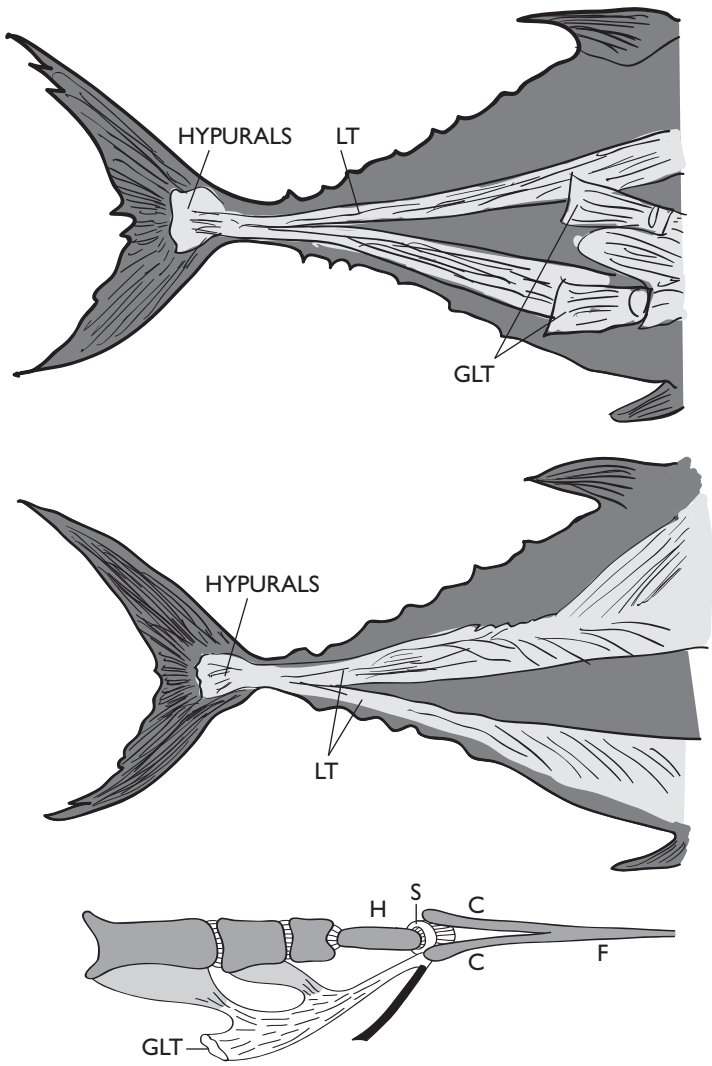

Figure 3.7 Caudal tendons of scombroid fishes. Above: the caudal tendons of yellowfin tuna seen after removal of the skin (upper) where the giant lateral tendon (GLT) links the outer edges of the posterior myosepta to the hypurals. Posterior red muscle (RM) black. After deeper dissection (below) and cutting and reflexing the GLT, the two posterior lateral tendons are seen, linking the myoseptal fibers of the anterior pointing cones of the posterior five or six myotomes with the caudal fin rays. Below: median horizontal section through tail of *Scomber japonicus*, showing two posterior myotomes contributing to the great lateral tendon (GLT) inserting on the middle fin ray (F). The anterior end of the fin ray articulates with the hypural (H) via cartilages (C) enclosing a synovial cavity (S). From Shadwick *et al*. (2002), and Fierstine and Walters (1968).

Muscle fibers

In almost all adult fishes, the muscle fibers of the myotomes are mainly of two quite different kinds, like wines easily distinguished by their color. There are red and white fibers (and in many fish pink or rosé ones too!). This distinction between fibers by color (actually myoglobin content) was discovered by the Italian anatomist Stefano Lorenzini in 1678 and it is easy to observe in fish, for the two main types are not intermingled. Cutting across the post-anal region of any fresh common food fish, such as a clupeid or gadoid, shows a thin layer of red fibers lying just below the skin and covering the main part of the myotome which consists of white fibers. In fixed material or even in smoked fish, such as kippers, the red fibers are brownish, but still easily distinguishable from the underlying paler fibers.

This separation of the fiber types in fish is extremely convenient for biochemists and physiologists, for it enables collection of pure samples of fibers, and makes recording from each type rather simple. In ourselves, however, and in higher vertebrates, biochemical and electrical recording experiments from one muscle fiber type are much harder, for different fiber types *are* intermingled in the muscles, and not easily categorized by color. The reader familiar with higher vertebrates will recall that fibers are instead characterized by their contraction speed and metabolism. For example, as fast oxidative glycolytic (FOG) or slow glycolytic (SG) fibers. These functional categories are more suitable than color since myoglobin content is not necessarily linked with contraction speed. For instance, in bats or pigeons the wing muscles are dark red but these contain several different functional kinds of muscle fiber with different metabolisms. To anticipate a bit, although color and speed are to some extent linked in fish, it is probably best to distinguish the red and white myotomal fibers by contraction speed rather than color, as fast glycolytic (white) and slow glycolytic (red) fibers.

As Table 3.1 and Figure 3.8 show, the fast and slow fibers in many fish are different in most respects, and, in fact, as electromyography shows, are used for different modes of swimming. Even without any direct evidence we might guess that the well-vascularized slow muscle strip was used in slow swimming operating economically by aerobic use of fuel, while the vastly greater mass of fast muscle was only used for short bursts, relying on inefficient anaerobic fuel use. Direct records from the two portions of the myotome in swimming spinal fish have confirmed this guess (Figure 3.9). These records were made by fixing thin insulated copper wires in hypodermic needles with resin, and baring the electrode tips before placing them in fast and slow myotomal muscles of the small shark *Scyliorhinus canicula*. This kind of electromyographic (emg) electrode records differentially between the copper wire tip and the hypodermic needle, and has often been used to detect muscle activity in man as well as in fish. Output from the electrodes fed a Cossor motorized oscilloscope camera which used rolls of paper. Very conveniently, such small sharks will continue to swim for many hours after the brain is destroyed, involving local spinal reflex systems (p. 352), provided water is flowed over the gills. Records from intact small herring swimming freely at different speeds in a tunnel respirometer (Figure 3.10) gave the same result, although recorded this time with a fast pen recorder. Herring are primitive teleosts, and have (like dogfish) focally-

innervated fast fibers. Nowadays, one can easily obtain much more elegant records using multi-channel online computer data acquisition, but these ancient (1965 and 1974) records provided the essential information, even if with rather more effort!

Table 3.1 A comparison between the fast and slow muscle fibers in fish myotomes (Bone, 1978)

Slow	Fast
Smaller diameter (20–50% of fast fibers)	Large diameter (may be more than 300 μm)
Well vascularized	Poorly vascularized
Usually abundant myoglobin, red	No myoglobin, usually white
Abundant large mitochondria	Few smaller mitochondria
Oxidative enzyme systems	Enzymes of anaerobic glycolysis
Lower activity of Ca^{2+}-activated myosin ATPase	High activity of enzyme
Little low molecular wt. Ca^{2+}-binding protein	Rich in low molecular wt. Ca^{2+}-binding protein
Lipid and glycogen stores	Glycogen store, usually little lipid
Sarcotubular system lower volume than in fast fibers	Relatively larger sarcotubular system
Distributed cholinergic innervation	Focal or distributed cholinergic innervation
Propagated muscle action potentials in elasmobranchs but (uniquely) they do not overshoot	Propagated action potentials; may not always occur in multiply-innervated fibers
Long-lasting contractions evoked by depolarizing agents	Brief contractions evoked by depolarizing agents

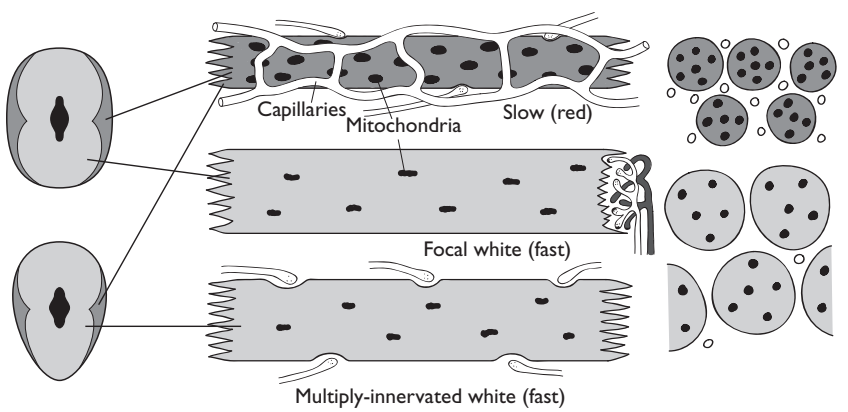

Figure 3.8 Summary of innervation pattern and structure of red and white myotomal muscle. Note dual innervation of white fibers by two separate axons (mid), and, only in higher teleosts, multiply-innervated white fibers (bottom).

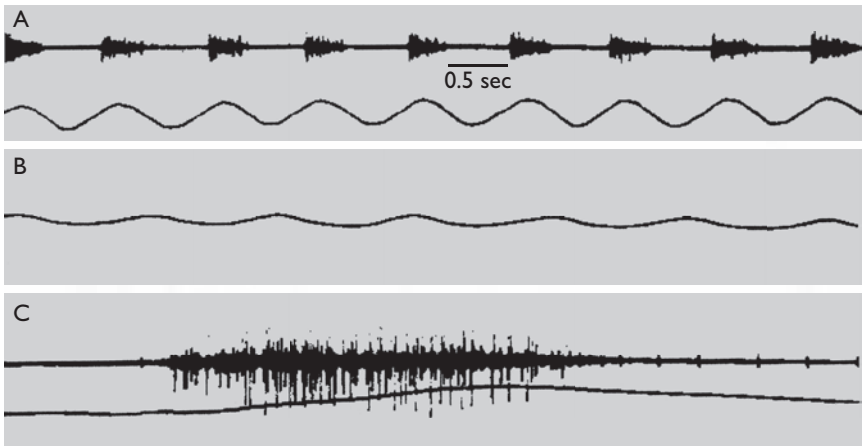

Figure 3.9 Electromyographic (emg) records from swimming spinal dogfish. Upper line in each: electrical activity from muscle fibers; lower line: swimming movements of fish. Top records: slow sustained swimming, electrode tip in red fiber zone of myotome; middle: slow sustained swimming, electrode tip in white fiber zone; bottom: electrode tip in white fiber zone, rapid tail flap evoked by pinching tail. This last recorded at a faster speed than remainder, to show individual white muscle action potentials.

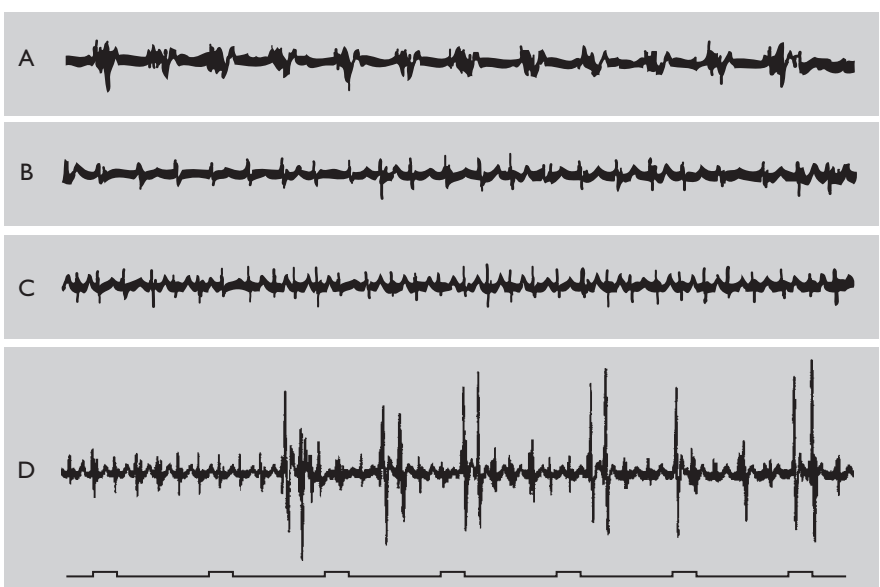

Figure 3.10 Similar emg pattern from electrodes in red muscle of a small Pacific herring (*Clupea pallasi*) swimming in a tunnel respirometer at different speeds. At the higher speed, bursts of fast muscle potentials are picked up from the underlying white muscle. Time marker: seconds. From Bone *et al.* (1978).

Origin of separate motor systems

Unfortunately, little or nothing is known of myotomal muscle fiber operation in adult or larval amphioxus, although short bursts of rapid activity seem all that adult amphioxus is capable of, and (in captivity at least) it does not indulge in slow swimming. The tunicates that swim by oscillating their tails (ascidian tadpole larvae and appendicularians) have only one kind of locomotor muscle fiber, although this is activated by two separate motor axons to give different responses. Perhaps a remnant of this ancestral dual innervation is seen in the dual innervation of fast locomotor fibers (Figures 3.8 and 3.11), found in lampreys, sharks, lungfish, and even amphibia. It seems most probable that the early chordates had a single type of unspecialized muscle fiber, and that the essential division of the myotomal locomotor system seen in fish seems to have been a critical step in the evolution of chordates from the amphioxus-like ancestor, possibly allied to the change from filter-feeding to catching moving prey.

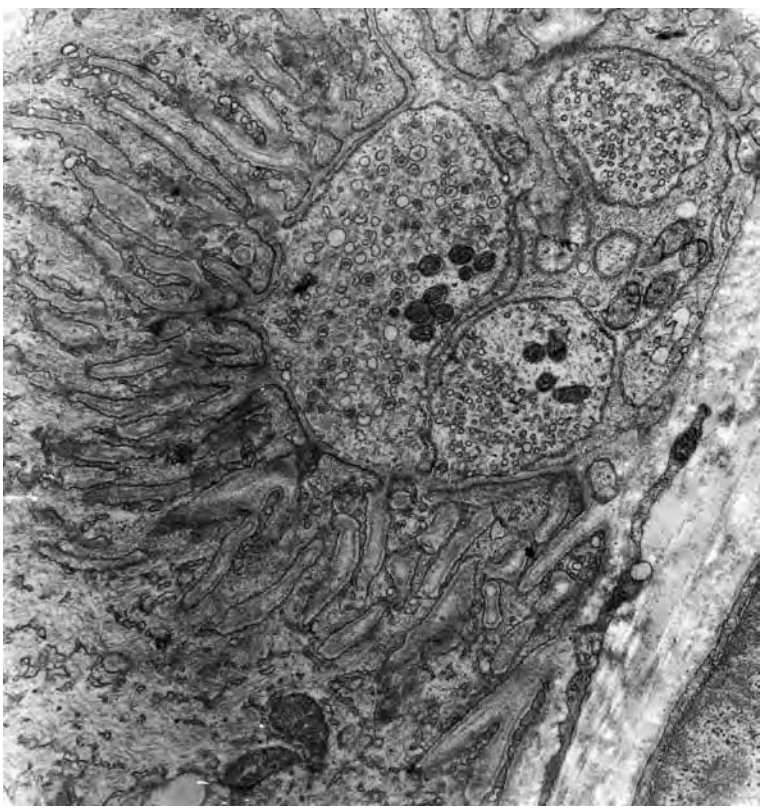

Figure 3.11 Dual innervation of dogfish white (fast) muscle. Electron micrograph of endplates on white fast fibre showing two types of terminals with different synaptic vesicles. Two of the endings seen in the section are from the cholinergic fiber (with smaller vesicles) the other (with dense-cored vesicles) has an as yet unidentified transmitter. From Bone (1972a).

Operation of slow and fast fibers

In the past decades interest has focused on phase relationships between myotomes along the body (e.g. Wardle and Videler, 1993). In the slow muscles of many fish swimming steadily (in flow chambers, tunnel respirometers, or even, as in Figure 3.12, freely in a large aquarium) it is usually found that slow muscle fibers are activated late in their lengthening phase, as the body bends, offset during muscle shortening (see Figure 3.13). Activated in this way, the slow fibers develop maximum force around maximum length, so optimizing power production during steady swimming. It seems, however, that sharks differ from teleosts in the phase of activation of slow fibers along the body, since Donley and Shadwick (2003) found in the leopard shark *Triakis*, forced to swim steadily in the large tunnel respirometer at Scripps that there was no longitudinal variation in the emg/strain relation. This interesting result was later confirmed in the fast-swimming short fin mako (*Isurus oxyrinchus*) using the same respirometer (Donley *et al.*, 2005). The teleosts studied seem to have differences in the timing of activation vis-à-vis the strain cycle along the body (usually activation occurs later in the strain cycle toward the tail), and also differences in activation duration, in contrast to sharks.

More recently, as well as examining fish swimming under steady conditions in respirometers, various studies have examined the rather less tractable problem of fish swimming within more natural turbulent and fluctuating flows. These have provided interesting evidence for the acknowledged ability of salmonids to travel and hold station economically (see Liao *et al.*, 2003).

In sharks and every other fish group, with the single exception of Euteleostei, but including primitive teleosts such as clupeids and eels, the fast white muscle fibers are innervated focally by single motor endplates formed by two separate axons at the ends of the muscle fibers (Figure 3.8), and propagate muscle action potentials along their length. In more advanced Euteleostei the fast fibers are multiply-innervated by numerous nerve terminals, sometimes by many different nerve fibers, sometimes by branches from a single axon, and are also probably normally activated by a single junctional potential at one of the nerve endings giving rise to a propagating action potential. Because all advanced teleosts have adopted distributed multiple innervations for their fast fibers, it seems likely to have a significant selective advantage over the focal pattern seen in all other fish, but it is not yet clear what this may be, particularly because focal innervation of fast fibers is usual in terrestrial vertebrates.

The slow muscle fibers of all kinds of fish are *always* multiply-innervated in the same way, and may also show propagating action potentials. Stanfield (1972) showed with a 2-electrode voltage clamp that at least some dogfish slow fibers should be capable of propagating action potentials, but prior to 1988 when Altringham and Johnston (1988) recorded action potentials from slow myotomal fibers in the sculpin (*Myoxocephalus*) and when one of the authors and his colleagues recorded *undershooting* propagated action potentials from slow myotomal fibers of dogfish, it was supposed that slow fibers did not propagate action potentials. Whether all fish slow fibers normally propagate action potentials is not known, and, indeed, the curious reader familiar with invertebrate muscle physiology might well enquire why they should need to do so.

The basic duality of the slow and fast motor systems seen in the dogfish *Scyliorhinus* (very probably found in all fish with slow and fast muscle fibers in their myotomes and propulsive fins, not just as described in elasmobranchs, Bone, 1999), is to some extent analogous to the jet engines of military aircraft,

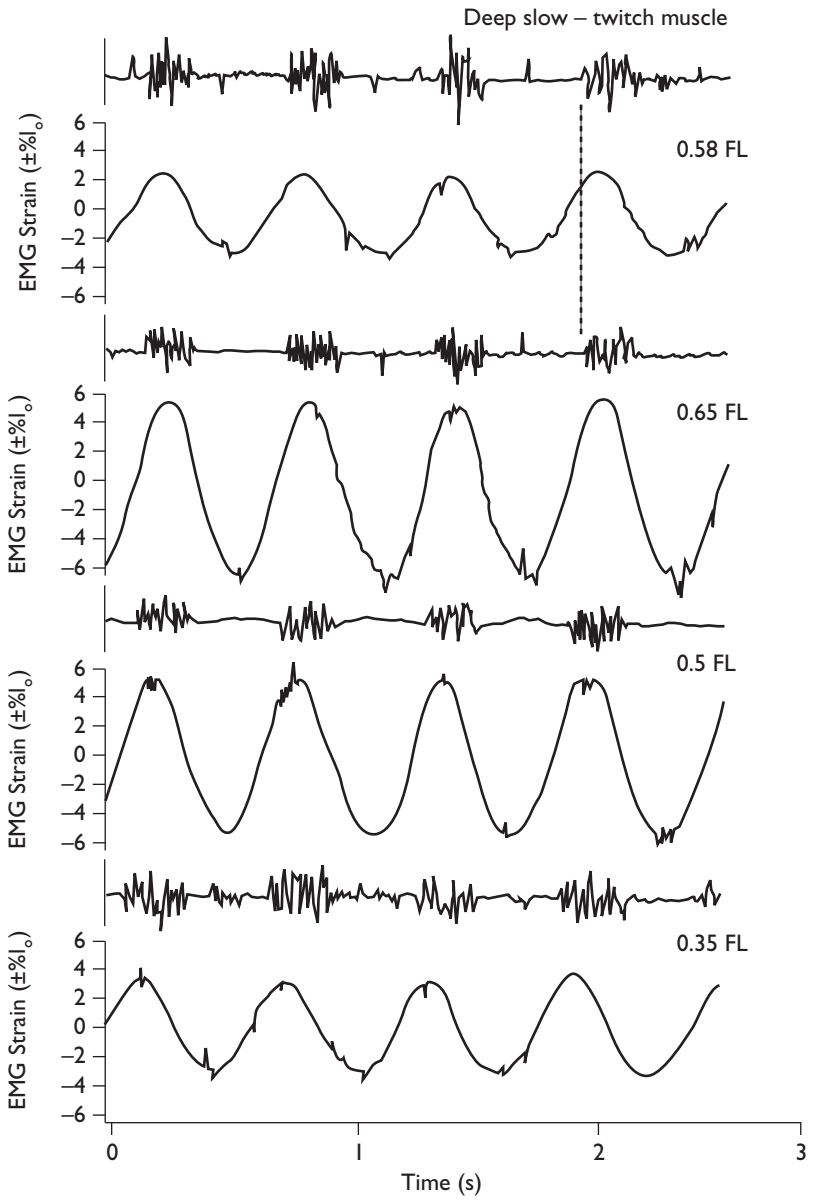

Figure 3.12 Recordings of emg's and strain (by sonomicrometry) in sequence of three steady tail beats from Pacific bonito (*Sarda chiliensis*). The tail is beating at 1.7 Hz. Vertical dashed line emphasizes apparently synchronous onset of emg activity at two points in same myotome but at different places along body axis. FL: fork length, snout to tail fork. After Ellerby *et al.* (2000).

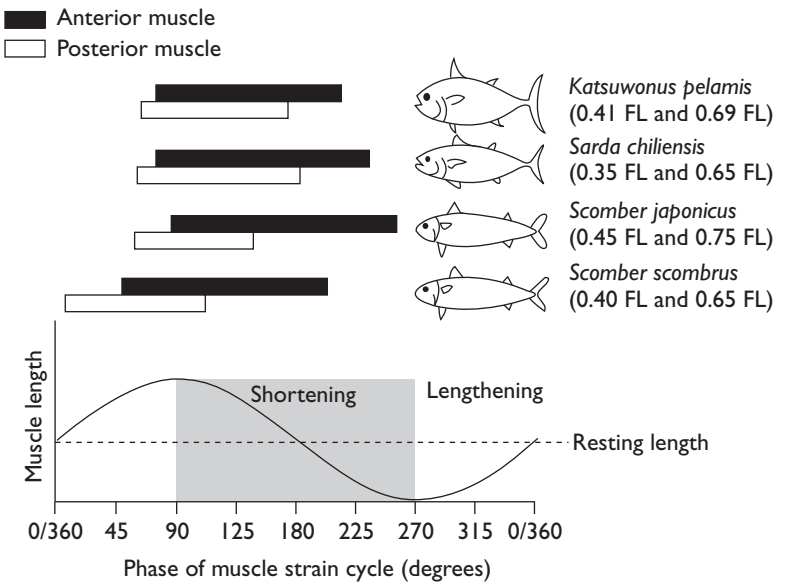

Figure 3.13 Changes in the relationship between electromyographic (emg) activity and strain cycle along the body of four scombrid species. EMG timing data are derived from Knower *et al.* (1999; *Katsuwonus pelamis*), Shadwick *et al.* (1998; *Scomber japonicus*), and Wardle and Videler (1993; *Scomber scombrus*). FL is the body length from snout to tail fork.

where economy is reasonable in the cruise condition, but thrust and speed (and fuel consumption) can be vastly increased for short periods by using reheat. The analogy is not entirely apposite since in aircraft (or in cars where a supercharger can be cut in) the same motor is used in a different way, whereas, in the fish myotome, a different "engine" is used for cruise and burst swimming. Moreover, our engines do not have to repay an oxygen debt! A moment's reflexion will lead the reader to realize that the fish system where a vast mass of fast muscle is set aside only to be active occasionally, during escape or attack maneuvers, would be hopelessly unsuitable for any terrestrial animal. Fish can be designed like this since water buoys them up and there is little weight penalty involved in carrying around a mass of fast fibers for only occasional use. Terrestrial animals not only have relatively less muscle in their bodies (in common domestic animals such as sheep, goats, and cattle, muscle is some 30% of body weight, while in tunas, it is around 60%), but also their muscles are arranged differently, and all fiber types are more similar to each other.

Slow myotomal muscle fibers operating aerobically can contract efficiently for long periods; pelagic fish, such as mackerel (*Scomber*) or spurdogs (*Squalus acanthias*), that are denser than water are obliged to swim forward all their lives to generate dynamic lift (see Chapter 4). In stark contrast, anaerobic fast fibers have a very limited operating duration before glycogen fuel stores are exhausted. In the small shark *Scyliorhinus*, and in herring, this duration is probably only around 1–2 minutes. This is not such a severe handicap as might be supposed at first sight, for it is very rare for any fish to swim at its maximum

speed for more than a few strokes of the tail before gliding to rest or slowing to cruise swimming. Calculations based on the glycogen content of rested *Scyliorhinus* fast fibers show that continuous operation for 2 minutes would allow the fish to travel some 600 m before the system was exhausted, then the fish could only cruise slowly using the slow motor system. Recovery after exhaustion is slow, because it involves oxidative resynthesis of phosphocreatine (PCR), and mitochondria are few in fast muscle fibers. Curtin and her colleagues (1991) have found that 1–2 hours are needed to reverse the breakdown of PCR that takes place in bundles of dogfish muscle fibers stimulated for only 1 minute.

3.3 Swimming Speeds

Cruising speed and slow muscle

Obviously enough, the proportion of slow fibers in the myotomes gives a good indication of the importance of sustained cruising swimming in the life of the fish, so that, for example, spurdogs (*Squalus*) have relatively more slow fibers than the sluggish dogfish (*Scyliorhinus*) and salmonids more than tench (*Tinca*). Next time one chooses to enjoy a proper whole kipper for breakfast (not fillets) it is easy to see that herrings (*Clupea harengus*) must spend much of their lives cruising around. Ambush predators such as the angler *Lophius* or the mudminnow (*Umbra*) have little or no red myotomal muscle. However, sometimes there are surprises: two ambush predators, which in aquaria just sit on the bottom waiting for a meal to swim past, the angel shark *Squatina* and the electric ray *Torpedo*, have a good amount of myotomal red muscle. It transpires that both species undertake migrations, hence require cruising muscle at one season of the year. The opposite and rather mysterious surprise is provided by *Lophius*, for both adults and post-juvenile angler fish have been observed quite often in North Atlantic surface waters.

Sustained swimming

Fish of different sizes and species can cruise steadily at very different speeds. For comparison, therefore, speed measurements are usually given in body lengths per second (BL s^{-1}) as well as in centimeters or meters per second. In most trout- or herring-sized fishes the slow fibers make up around 5–10% of the myotomes, and such fish can cruise at one to two body lengths per second for long periods. Fish that cruise more rapidly have a higher proportion of slow fibers in the myotomes, but there is a limit to what can be done to increase cruising speed by simply increasing the proportion of slow muscle. It needs an abundant oxygen supply that has to be acquired by the gills, and, unless there are special adaptations for oxygen acquisition and transport (as in tunas), around 25% of the total myotomal mass seems to be the limit for red muscle. Maximum sustained cruising speed is limited by the oxygen requirement of the slow muscle, but few fish cruise at this maximum. Instead, as we should expect, pelagic fish such as many sharks and scombroids, cruise at speeds that accord well with the expectations based on minimum energy expenditure for distance travelled, given by $V = O.5L^{0.43}$. For example, a 9 m basking shark tracked while swimming 8 m below the surface swam at 94 cm s^{-1}, very close to the expected 1 m s^{-1}, while 2 m sharks of two carcharinid species swam

close to the expected 68 cm s^{-1}, and (see Weihs, 1973) migrating salmon also swim close to predicted speeds, around 50 cm s^{-1}.

Minimum energy expenditure for distance traveled is, however, not the only criterion by which cruising speeds are determined. Recent direct measurements of swimming speeds in blue marlin (*Makaira nigricans*), a large scombroid, show that these fish (1.5–2.0 m long) cruise at relatively low speeds (15–25 cm s^{-1}) when within 10 m of the surface, while at depths greater than 50 m, they cruise at higher speeds, up to 120 cm s^{-1}. Short bursts up to 2.25 m s^{-1} were recorded, usually when changing depth. In schools, yellowfin tuna 48–79 cm long, swam between 1.6 and 5.5 m s^{-1}, well above the predicted speed, but these fish were cruising to catch food and hence swam faster than for minimum energy expenditure.

Maximum speeds of fishes

Maximum burst speeds are hard to measure, for burst speeds are naturally only sustained for brief periods of a minute or less. A variety of approaches has been employed to estimate burst speeds, including measuring the height to which fish of known weight jump, or their ability to catch bait trolled at known speeds. For example, using a line coated with iron filings run out from a reel with a magnetic counter, Walters and Fierstine (1964) found that a wahoo (*Acanthocybium*) 113 cm long achieved a burst speed of no less than 19 L s^{-1} (76.8 km h^{-1})! As they pointed out, this figure was likely biased low for the fish had to overcome the additional drag of the line. The record speed measured (by line run-out) for a fish was 68 mph or 110 km h^{-1}, for the larger sailfish *Istiophorus platypterus*, some 2.5 m long, equivalent to 12–15 Ls^{-1}. A quite different approach, by Wardle and his colleagues (1989), was to measure the twitch contraction time of the fast muscle fibers, since it is this that will limit the tail beat frequency (which increases linearly with speed). Larger fish beat their tails more slowly than small ones, but if account is taken of this by including fish size (Figure 3.12), we see that the fish moves forwards for the same fraction of body length for each tail beat. In most fishes, this distance (the "stride") is around 0.7 × BL. To swim faster, then, a fish increases its tail beat frequency, so increasing the number of strides in a given time. Since maximum tail beat frequency must be limited by the duration of the white muscle twitch contraction, by measuring contraction times of isolated white muscle bundles, the maximum possible burst swimming speed can be calculated from:

$$M = \frac{AL}{2T} \text{ (where M = speed, A = stride length, and T = muscle contraction time)}$$

Excellent agreement was given between the maximum speeds given by this formula, and those obtained from videos of fish swimming at burst speeds. Scombroids differ from other fish (for a reason not fully understood) in having stride lengths close to unity rather than 0.7, and using this for A in the formula above, with minimum muscle contraction time (0.026 s) gave 19 BL s^{-1} for mackerel (*Scomber scomber*) 30.5 cm long. The highest speed recorded for the same fish swimming at maximum speed in a large tank was 18 BL s^{-1}.

For the much larger bluefin tuna (*Thunnus thynnus*) 2.26 m long, T was 0.05 s, and from this Wardle and his colleagues concluded that the upper limit of tail beat frequency was 10 Hz. Setting A at unity, as in mackerel, suggests

that this fish could achieve 22.6 ms^{-1} or 81.4 km h^{-1} as the maximum burst speed, equivalent to 10 BL s^{-1}.

Maximum and sustained speeds are not everything

Just as with our own vehicles, before choosing a new car we might enquire whether it could cruise economically, or whether it could achieve the maximum speed of a Bugatti 35B, but we should probably also like to know if it could go around corners without leaving the road, and what kind of steering lock it had (although if it was any kind of Bugatti we need not ask). Locomotor performance in fish involves such things as fast C-start avoidance of predators, maneuverability in confined spaces, that is, unsteady maneuvers involving acceleration and turning. Gerstner (1999) examined four coral reef fish of different body shapes, concluding that differences in turning ability were linked to pectoral fin morphology, damsel fish being the most agile of the fish examined. As one might guess, turning ability is related to fish length, and Domenici (2001) gives an interesting discussion of the relations between predator and prey length.

Not so simple: overlap of the two fiber systems

Up to now, we have considered the fast and slow motor fibers of the fish myotome as two quite separate systems. It would be extremely surprising to any biologist to find that there was such a clear-cut division between the slow and fast motor systems, and (reassuringly) in fact there is not. The simple dichotomy of function of the fast and slow muscle fibers is blurred in two ways. First, in the myotomes of most fish, including small sharks such as *Scyliorhinus*, there is a gradation across the mass of the slow fiber type in enzymatic activity, mitochondrial content and vascular supply such that the outermost fibers are richer in each than the innermost region (see Figure 3.14). It is still unclear whether this implies that this varied range is used for different roles. For example, we might guess that over the range of possible sustained cruising speeds, the outer layers of slow fibers alone are used at low speeds and that, to increase cruising speed, the inner slow fibers are recruited. But no-one has done the (not very difficult) experiments to test this idea.

Second, especially in higher teleosts, there is an intermediate class of fibers between the outer layer of slow fibers and the inner fast fibers, and electromyography of fish swimming in water tunnels has shown that these are recruited as swimming speed rises (Figure 3.15). Such intermediate fibers have a less rich oxidative enzyme profile than those of the slow fiber zone, but much richer than the deep fibers of the fast fiber zone. Although measurements on intact swimming fish have only been carried out on deep fast fibers and superficial slow fibers, these showed (hardly surprisingly) that the velocities of muscle fiber shortening during fast and slow swimming are those at which they operate at peak mechanical power and efficiency. It seems reasonable to assume that the presence of gradations of enzyme content across the slow muscle, and of intermediate fibers in many teleosts is a reflection of tuning fibers to operate at different contraction velocities to provide optimum efficiency.

Tunas swim rather differently to other fish, and in two ways their slow and fast muscles interact more closely than in other teleosts. First, the tunas have

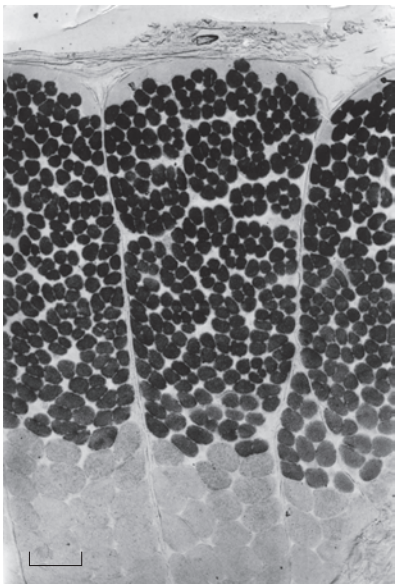

Figure 3.14 Myotomal fiber types in *Scyliorhinus*. In this 25 μm cryostat section stained for succinic dehydrogenase (SDH) activity (= mitochondrial content), the outermost fibers (probably postural fibers, and not found in most fishes) (top) are unstained; the red smaller fibers are much darker; and the inner larger white fibres only weakly. Note that the inner red fibers are larger and less stained than those in the major part of the red muscle, while the outer white fibres are smaller and more stained than those deeper. SDH activity. Scale bar: 300 μm.

developed structures that permit them to run their muscles above ambient temperature (as have lamnid sharks), and, second, the fast muscle is well vascularized and also contains much myoglobin, so that recovery from bursts of speed fuelled by anaerobic glycolysis is rapid, and, also, there is a considerable capacity for aerobic activity, supporting the slow muscle during high speed cruising.

The development of the slow and fast system in teleosts

In the fish larvae studied, the slow muscle eventually concentrates along the mid-lateral line of the flank but in plaice (*Pleuronectes*) the slow muscle monolayer is still present after metamorphosis. Unlike elasmobranchs, however, teleost myotomal muscle fibers not only increase in size after the end of the yolk sac stage, but also increase in number throughout life.

In addition, there are changes in the contractile proteins of the muscle fibers during development. Herring, seabass (*Morone*), barbel (*Barbus*), and plaice, and, no doubt, all fish larvae, have muscle fiber types with embryonic or larval isoforms of myosin and other contractile proteins. These fibers arise from pre-myoblast cells in the embryo somites well before hatching, but their number and diameter depend on hatching temperature. As the larvae age and metamorphose, the isoforms of the myofibrillar proteins change with age. Presumably contraction speed (apparently linked to the nature of the myosin

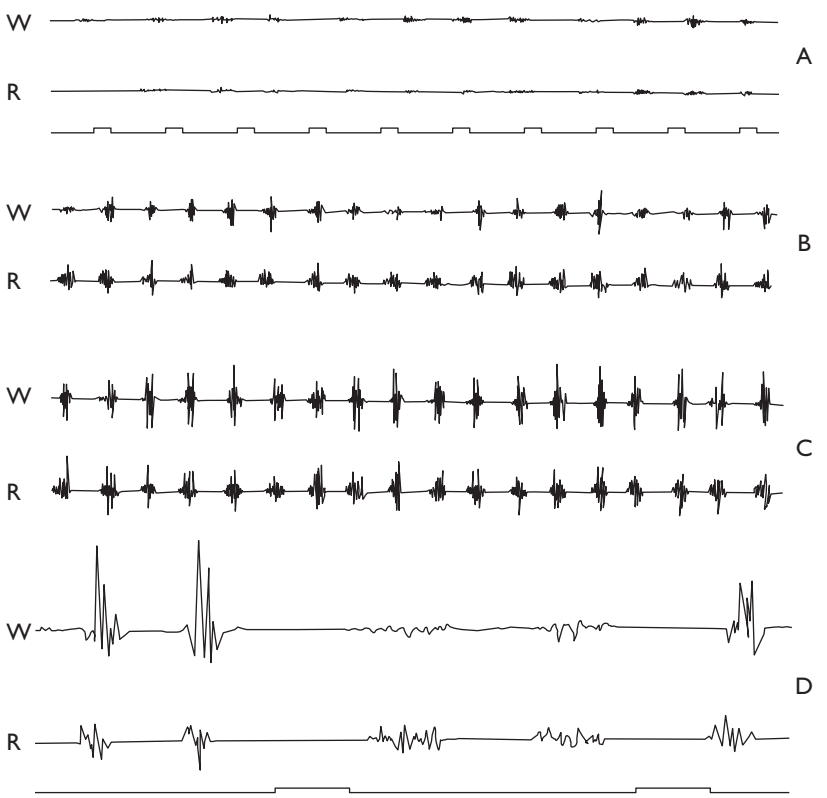

Figure 3.15 Muscle electrical activity from carp swimming at different speeds in tunnel respirometer. Note activity from white (fast) muscle at intermediate speeds. From Bone *et al.* (1978).

heavy-chain isoforms) changes as the fish increases in size and its muscle fibers elongate and increase in diameter.

As development of the larva proceeds, other changes occur in relation to locomotion. The fin fold (see p. 13) is gradually replaced by median dorsal, anal, and caudal fins, although the lateral fins, especially the pectorals are likely to be present at an early stage to give stability. A major event in development is the flexion of the caudal fin when the tip of the notochord turns up and hypural finrays form the base of the caudal fin. At this time (certainly in the longer, slimmer larvae), locomotion changes from an eel-like mode, where locomotor waves pass along the whole body, to oscillations of the caudal region alone, and swimming becomes more efficient.

3.4 Warm Red Muscle

All kinds of fish (with two independent parallel exceptions) operate with body and muscle temperatures the same as the ambient seawater. There is no problem in generating heat, indeed it is an inevitable by-product of muscular contraction, which is why we and large moths shiver to try and warm up. The

problem for a fish in conserving heat is that water has a high heat capacity (four times that of air), and the fish is in contact with it, not only all over the body surface, but also most intimately at the gills, where the blood has to be very close to the water whence it obtains oxygen. The gills act rather like car radiators, but, surprisingly perhaps, only about 30% of the heat generated in the body is lost at the gills, much more is lost from the body surface.

Remarkably, the more advanced scombroids, such as the skipjack, bluefin, and yellowfin tunas, and lamnid and isurid sharks, such as the great white (*Carcharodon*), are able to run their slow muscles above ambient temperature. To do so, the two groups have evolved similar convergent special modifications of the muscular vascular supply (see Block *et al.*, 1993). Blood warmed by slow muscle activity leaves the muscle via a special network of venous capillaries that run closely parallel to a similar network of arterial capillaries supplying the muscle with cool oxygenated blood (Figure 3.16). In these special retia mirabilia, heat is exchanged to the incoming blood, and so the muscle is not cooled by arterial blood as it is in other fish. Measurements on albacore (*Thunnus alalunga*) have indicated heat transfer efficiency to be around 98%! The bluefin tuna (*Thunnus thynnus*) can maintain internal muscle temperature up to 21°C above the ambient water, while in the skipjack *Katsuwonus* and other tuna species, muscle temperatures during continuous swimming are half this or less.

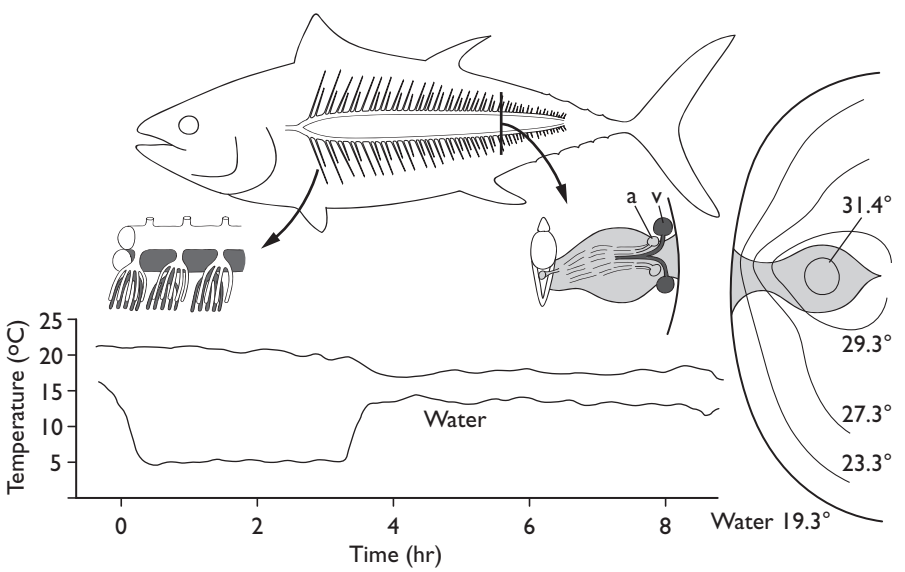

Figure 3.16 Organization and function of the retial thermoregulation system in the bluefin tuna (*Thunnus thynnus*). Upper: bluefin showing lateral vessels (details of branches to left, position of lateral vessels and retial system in red muscle to right; a, artery; v, vein). Right: thermal profile obtained with thermistor probes, red muscle stippled. Bottom: records of water temperature (lower line) and stomach temperature (upper) obtained by telemetry from free-swimming bluefin, showing independence of body temperature from changes in water temperature. After Carey *et al.* (1971), and Gibbs and Collette (1967).

Similar retia to retain heat are found in the vascular supply to the brain and eyes in swordfish (*Xiphias*) and marlin (*Makaira*), where the heat is produced by thermogenic tissue specially modified from eye muscles (p. 363), and there are suprahepatic retia warming the viscera in lamnid sharks. Of course, the best known retia are those of the swimbladder gas gland (p. 115).

Curiously enough, warm muscles were found in bonito as long ago as 1835 by Humphry Davy's brother John on a voyage to India. He obviously inherited the same enquiring spirit as the better-known Humphry, who was a distinguished chemist (he discovered sodium and potassium and invented the miners' safety lamp against firedamp). Shark warm slow muscle was discovered in the 1960s (Carey and Teal, 1969), but it is still not entirely clear just *why* all such fish have evolved such complex arrangements, which must involve considerable added vascular resistance. Warm skipjack tunas (*Euthynnus pelamis*), of the same size as cold bonito, (*Sarda chiliensis*) cruise at about the same speed, but the skipjack's burst speed is nearly twice that of the bonito. It seems possible that the "embedded" slow muscle mass within the fast muscle not only enables it to produce more power, but also warms the fast fibers to increase their power and hence burst speed.

The salmon shark (*Lamna ditropis*) lives in the cold waters of the North Pacific but some migrate south to warmer waters around Hawaii, as shown by archival tags (Weng *et al.*, 2005). It seems clear that, in this fish (the warmest shark known), the central red axial muscle core, which is at 26°C with the ambient water at 6°C (Figure 3.17), can *only* produce any power when it is warmed well above ambient (Bernal *et al.*, 2005). The white (fast) muscle is not so dependent on warming, and can operate around ambient temperatures, (although it is warmed somewhat by the underlying red muscle) to enable

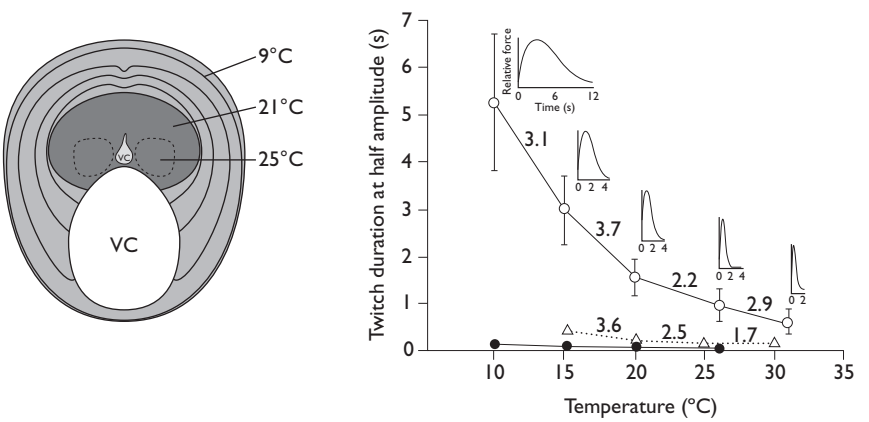

Figure 3.17 The warm salmon shark (*Lamna ditropis*). (A) Transverse section of body showing temperature gradients from warm red muscle. (B) Muscle twitch duration (scale: 5) of red fibers (above) and white fibers at different temperatures. Number above each interval are Q_{10}. h. From Bernal *et al.* (2005).

short bursts of very rapid swimming, (said to be over 90 kph). The salmon shark thus cruises on obligatorily warm red muscle, if this were cooled to the ambient 6°C, the fish would glide to a halt. In fact, it is permanently warm-blooded, at least in part (visceral retia keep the stomach warm as do cranial the brain) although the heart capably continues at low temperatures (why should the heart have had to evolve this ability?).

For other sharks (such as the great white), and for tunas, however, it seems most probable that warm red muscle was evolved in order to enable them to cruise into colder waters. Katz (2002) considers these possibilities.

The more advanced bluefin tuna (*Thunnus thynnus*) not only can warm the slow muscle but can control the temperature, an obvious advantage when migrating into cold and warm waters where muscle enzyme systems can always be used at their thermal optima. Block and her colleagues (Block *et al.*, 2005) have tracked bluefins in the Atlantic (again with archival tags) from the American east coast to the Mediterranean spawning grounds, on one occasion following the same fish for four and a half years (Figure 10.19, p. 310). Once having evolved a counter current heat exchanger to retain metabolic heat, there is the obvious danger of overheating, but this is avoided by having vascular shunts permitting the heat exchanger to be bypassed when necessary.

3.5 The Generation of Thrust

To generate forward thrust, fish must transfer the forces resulting from the contractions of the body or fin musculature to the water. Traditionally, there are two ways of analyzing this process: (1) by a lift-based or vorticity approach; and (2) by a bulk momentum or added mass method. The overwhelming majority of fish produce thrust by the passage of transverse body waves along the body toward the tail (as does a trout or an eel), and this way of generating thrust has been analyzed by the second method. However, it is easiest first to consider the most advanced and most rapid swimmers, such as the tunas, where a caudal oscillating propellor is attached to a more or less rigid body since these are closer to our own rigid vehicles.

Caudal fin oscillations

A fish such as a bluefin tuna (*Thunnus thynnus*) is essentially a more or less rigid mass of myotomal muscle (with some viscera) attached to a stiff lunate (crescentic) foil by a narrow neck. With striking success, it has succeeded in separating the myotomal motor from the thrust-generating caudal propellor. Interestingly, swifts (Apodidae) which have rigid bodies containing the wing muscles, also have evolved lunate wing propellors for the same reasons as tunas have lunate tails. The myosepta are prolonged into caudal tendons passing across a flexible joint in the narrow caudal peduncle (see Figure 3.7), so that as the muscles contract, the foil oscillates from side to side while the greater part of the body remains more or less rigid (Figure 3.18). Because the foil oscillates in the vertical plane rather than rotating as do our propellors, it is of symmetrical section, each side alternately leading and trailing, and as the tail fin comes to a halt, as it were, at the end of each stroke, the thrust produced is oscillatory, although smoothed by the high frequency of tail beat.

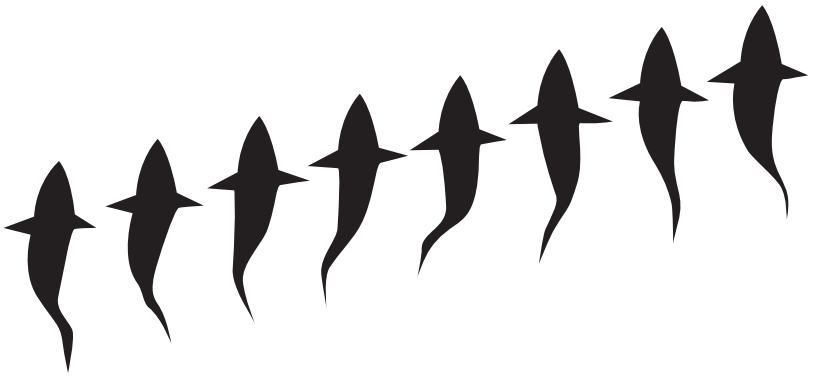

Figure 3.18 Successive dorsal views of tuna (*Euthynnus affinis*) at intervals of 10 ms, swimming at 8.2 body lengths s^{-1} (approximately 4 m s^{-1}) in an experimental tank. After Fierstine and Walters (1968).

The way in which this oscillating foil generates thrust is well understood, for it is the same process whereby airplane wings generate lift and rotating propellors generate thrust. In fact, the successful hydrodynamic analysis of fish swimming like tunas, with lunate tails, used oscillating aerofoil theory.

Circulation, lift, and thrust

The forces acting on a foil (or any object) as it passes through a fluid arise from the displacement of the fluid by the foil; they are of two kinds. Frictional forces (arising from the viscosity of the fluid) are only indirectly concerned and may be neglected for the present. Lift or thrust forces can only result from a net difference in the pressure of the fluid on either side of the foil. As an airplane wing passes through the air, it is both sucked upward from above, and pushed up from below, for lower pressures are found above the wing, and higher pressures below it (Figure 3.19). How do these pressure differences arise?

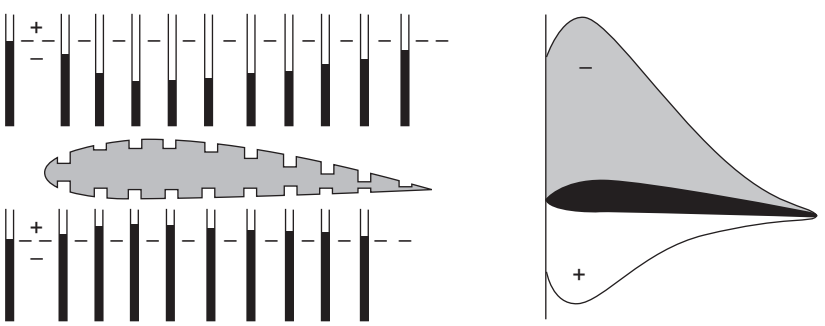

Figure 3.19 Pressure distribution around a foil at a positive angle of attack in a wind or water tunnel. Left: manometers connected to openings in upper and lower surfaces show pressures above and below ambient (dotted line). Right: typical pressure distribution around such a lifting foil.

The relationship between pressure and velocity in the flow of an incompressible fluid is given by:

$$P + \tfrac{1}{2}\rho V^2 = \text{constant}$$

where P = static pressure, ρ = density, and V = velocity. The quantity $\tfrac{1}{2}\rho V^2$ (often abbreviated to q), is the dynamic pressure, the force experienced if one puts one's hand out of the window of a moving car, and it is this that is the fundamental source of aerodynamic or hydrodynamic forces. As we shall see, q will appear in formulae for calculating thrust, lift, and drag. At the speeds fishes swim, fluid density remains the same, so the differences in pressure above and below a lifting foil in a fluid (such as an airplane wing, or a shark pectoral fin) must mean that there are differences in the velocity of flow on the two surfaces; the velocity of flow must be higher above a lifting foil than below it. Lanchester's circulation theory (see below) showed how these velocity differences arise. When a lifting foil is moved through water, it is possible to visualize the displacement of the water caused by the passage of the foil using a high-speed camera or video system to track small particles such as polystyrene beads distributed in the water. Of course, the more convenient (and equivalent) experimental arrangement is to fix the foil and flow water past it using a water tunnel. If we monitor the displacement of two particles (A and B, Figure 3.20) from their initial positions before the water reaches the leading edge of the foil, until their final positions after they have passed it, we find that they have followed curved paths of opposite sense.

This results in a net clockwise rotation of circulation. Because it is in this sense, the relative velocity of flow of the fluid above the foil will be greater than that below it, hence lift will be generated. Evidently, the stronger the circulation, the greater the lift.

Although we have been considering circulation and the origin of lift for a lifting foil, the generation of thrust by propellors (whether rotating or oscillating) of course has the same basis. This circulation theory of lift was first described at the end of the last century by one of the very greatest English engineers, F. W. Lanchester, also known for his radical early motor car designs, and for his invention of contra-rotating balance shafts (still used in recent Alfa engines). His seminal paper was rejected by *Physical Reviews*, and he was obliged to publish it himself in book form! He disliked the way in which his business partners kept changing their minds about the company operation, remarking "If I had minds like theirs, I would change them as soon as possible."

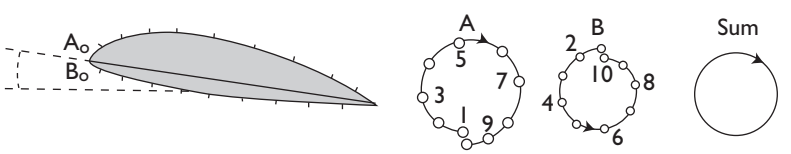

Figure 3.20 Circulation and lift. Two particles A and B are caused to change position as the foil passes through the fluid. Right: the successive positions of the two particles (numbering as on left). The sum of their movement is a net clockwise circulation producing lift.

Body waves, and bulk momentum thrust generation

Eels swim in a way quite different to tunas: instead of separating the propulsive muscles from the thrust-generating high aspect ratio (AR) caudal foil as tuna do, they have no caudal foil at all, and the movements of the body themselves produce thrust. As Gray (1933) showed, when eels swim, they pass transverse waves of increasing amplitude down the body (Figure 3.21). These pass backward faster than the fish swims forward. Eel swimming ("anguilliform locomotion") can be seen in terms of the transverse motions of short segments of the body (e.g. those shown on Figure 3.21) as the propulsive waves pass backward, but, more recently, elongate body theory (a version of a reactive slender-body theory) has proven a more fruitful and appropriate approach, which has been used not only to calculate drag and thrust requirements in steady swimming but also during acceleration and maneuvering. In essence, this approach (mainly due to Lighthill, 1971, 1973) deals with the forces met by the water masses next to the body surface. These result from the inertia of the water next to the fish and are proportional to the rate of change of relative velocity of the fish surface with respect to the water next to it. As the locomotor wave passes backward along the fish, it increases the momentum of water passing backward (as it is "pushed" by the inclined planes seen in Figure 3.21); the rate of shedding of this momentum into the wake (as vortices) is proportional to thrust. While elongate body theory correctly predicts the power shed into the wake, it underestimates the lateral jets of fluid in the wake that become two vortices shed each time the tail beats in the opposite direction.

Although eels travel huge distances to spawn, it was supposed that they are relatively inefficient swimmers, because, although long, the body is rounded in section, and so a relatively small mass of water is affected by the oscillating body of the eel through it. Recently, however, several studies by Tytell and colleagues (2004a, 2004b) suggest that as eels swim faster the cost of producing the wake behind the fish rises less than might be expected.

An even more striking result has been obtained by van Ginneken *et al.* (2005), who swam eels in a respirometer for the equivalent of 5500 km (the distance for the fasting European eels to reach the spawning grounds in the Sargasso Sea). The experiment lasted for 173 days of continuous swimming (at 21.5 cm s^{-1} = 0.5 BL s^{-1}), during which oxygen consumption was monitored, and, at the end of the experiment, food reserves were measured in a bomb

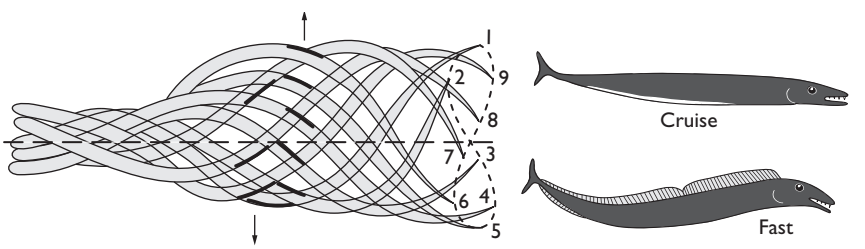

Figure 3.21 Left: drawings from frames (1–9) of film of young *Anguilla*, superimposed to show short sections of the body (thick lines) as planes inclined to axis of progression when the eel moves forwards. The oblique dashed line is the axis of progression, the tail tip describes a figure-eight pattern. After Gray (1933). Right: the two swimming modes of the black scabbard fish (*Aphanopus carbo*).

calorimeter. This provided two independent estimates for the cost of transport, from oxygen uptake and from the mass loss, body composition, and energy conversion factors from the calorimetry. Both estimates were similar, and gave a cost of transport of 0.42–0.62 kJkg $^{-1}$km^{-1}. Surprisingly, the cost of transport for trout swimming continuously was much greater, 2.73 kJkg^{-1}km^{-1}: eels swim 4–6 times more efficiently than trout or other similar fish. At present, as van Ginneken *et al.* (2005) remark, the source of the eel's remarkably high efficiency remains unknown. Certainly, it is neither predicted by theoretical analyses, nor by studies of the flows around swimming eels!

Deepening the body to give a deep flat cross-section like many carangids or the elopomorph leptocephali larvae, much increases the virtual mass of water given momentum by the swimming movements, producing greater thrust. Many fishes that swim by passing waves along their bodies (even if less than a whole wavelength is formed along the body) deepen the body section and have median fins of various kinds to extend body depth. These not only stabilize the fish in roll but are important in thrust generation. It is not necessary to have a continuous fin, for provided the spacing of the fins along the body is suitable, as it is, for example, in many sharks or in gadoids, momentum shed from the anterior fin is "passed" to the next posterior. Interrupting the series of fins is, in fact, advantageous, for it incurs less drag (see Section 3.6).

If we consider what fishes need to do in their lives (e.g. Castro-Santos, 2005), we see that almost all have to compromise between a body shape suitable for low- or high-speed cruising, and a shape suitable for rapid acceleration and maneuver. Some actually adopt different shapes for the two requirements. The deep-sea scabbard fish (*Aphanopus carbo*) has a long body ending in a very small caudal fin of fairly high aspect ration. It swims slowly by oscillating this foil at the end of the rigid body. To accelerate rapidly, it unfurls the long median dorsal fin to deepen the body section, and passes locomotor waves backward along the body (Figure 3.21). Amputation experiments on salmonids and gadoids have shown that caudal fin removal has little effect on steady swimming speed. Most of the thrust generated must be provided by the locomotor waves passing back along the body. Amputation of the fins, however, especially the caudal fin, has a marked effect on acceleration, so it seems that the wide deep caudal fins of many fish such as barracuda (*Sphyraena*) are adaptations for rapid acceleration as are the deep bodies of carp (*Cyprinus*).

Although, as we saw earlier, bulk momentum theory has usually been used for body wave swimmers, such fish produce alternating vortices which are shed into the wake from the caudal fin, and these might be analyzed by a lift-based method.

3.6 Drag

There are two kinds of drag forces which retard the forward motion of fishes or indeed any other object in a flow. The different kinds of pressure drag result from the pressure at the nose of the fish being higher than at the tail, owing to vorticity in the wake. Skin friction drag results from the viscosity of the water making it stick as a boundary layer to the fish surface.

Total drag, therefore, is the sum of pressure drag (form drag depending on shape plus drag associated with circulation and lift) and skin friction drag.

How do these different kinds of drag arise, and how can they be minimized? Any drag-reduction achievable will be greatly advantageous since it will mean that, for a given power output, a fish can travel faster, or for a given speed need less power.

Pressure drag

Unstreamlined objects (such as bricks) passing through water leave a large turbulent wake behind them because what little boundary layer can form near the leading edge soon separates to give rise to the wake. A large wake means a large pressure difference between the nose and tail, and so a large form drag is incurred. This can be very greatly reduced by streamlining to avoid abrupt changes of contour and awkward excrescences. We have seen that trout approximate to the solid of least resistance, and scombroids such as mackerel have transparent eye fairings. Drag tests on dead mackerel show that the drag incurred is almost exactly that of a flat plate of similar wetted area; this is because form drag is virtually absent, so excellent is the streamlining. When the mackerel swims, however, some part of the vortices trailing in its wake arise from the generation of thrust and lift, so that this second vortex drag component is an inevitable consequence of these processes.

Readers familiar with tropical waters may well wonder why some fish do indeed rather resemble bricks than trout. Curiously, model tests on various ostraciid (boxfish) species have shown them to have low drag coefficients, and excellent self-trimming abilities as a result of leading edge vortices on the keels along their bodies (Bartol *et al.*, 2005).

Vortex, induced, or lift (thrust) associated drag and circulation

The brief account of circulation theory in Section 3.5 dealt with the lift generated by circulation around the main part of a foil. So what happens where the foil ends at the tip? This is an important question, for while circulation around the body of the foil generates lift (or thrust), at the tip it gives rise to drag. The circulation of the fluid over the main part of the fin continues downstream at the tip in a series of tip vortices, giving rise to upwash just behind the tip, and downwash across the span, decreasing the root of the foil (Figure 3.22). Vortex production requires energy; the energy expended to maintain the tip vortices is one component of the pressure drag incurred as the foil passes through the fluid, called, variously: induced, lift-associated, or vortex drag (D_v; proportional to $1/V^2$).

Since the tip vortices depend upon the circulation, the stronger the circulation, the greater D_v incurred. Like aeroplanes, fishes take advantage of two possibilities to reduce this component. First, since the same lift or thrust can be produced by a weak circulation over a long span as by a strong circulation over a short span, doubling the span halves the D_v. This is why the caudal fins of fast-swimming fishes such as tuna or blue sharks (*Prionace*) are long and thin, as are the wings of efficient sailplanes and the lifting pectorals of dense fast-swimming fishes. It would be interesting to know why the albacore (*Thunnus alalunga*) has such elegant elongate pectorals (Figure 3.23) compared to other tunas. Not all fish that swim with outspread pectoral fins use them to generate lift however; sturgeons angle their bodies to generate lift and the pectoral fins are used only when changing direction in the vertical plane.

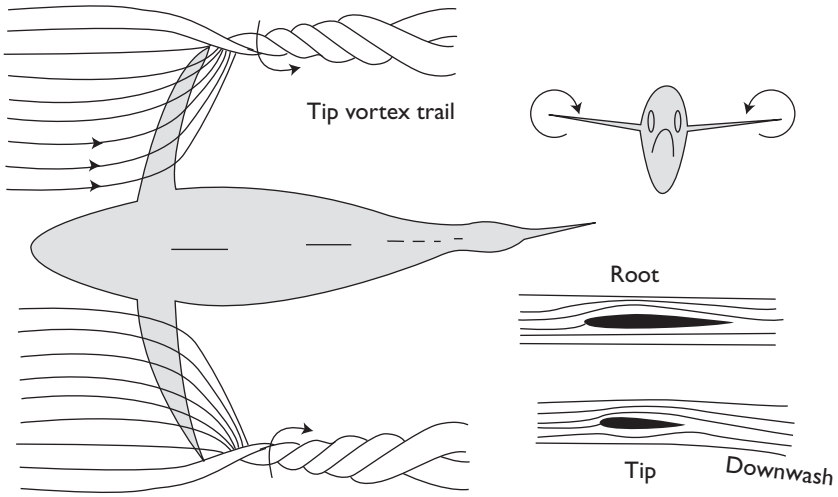

Figure 3.22 Flow patterns around a gliding skipjack (*Katsuwonus*). Tip vortices cause vortex of lift-associated drag. Lower right: flow over root and near tip of pectoral fins showing downwash near tips.

The same is probably true for hammerheads, which gain dynamic lift from the head and use the pectorals for maneuvering.

The aspect ratio, AR (span^2/area), of tuna caudal fins is up to 6–7, much lower than that of sailplane wings; marlin (*Makaira*) caudal fins are just over 10: probably as high as can be arranged without sacrificing the structural strength needed to cope with the forces involved in transmitting thrust.

Second, it can be shown that for a given AR, D_v, is least if the spanwise distribution is elliptical; this can be conveniently achieved by using an elliptical planform, as R. G. Mitchell did for his hard-to-manufacture but efficient Spitfire wing. All fishes which normally cruise at high speed, like scombroids or fast sharks, have lunate elliptical caudal thrust-generating foils and lifting pectoral foils (Figure 3.23). So far as is known, no fish fins are bent upwards at their ends (as are the wings of newer jumbo jets), a device to reduce tip vortices, but the authors have long been on the lookout for such a modification!

High AR elliptical caudal and pectoral fins are designed to minimize D_v in fast-cruising fishes. But such fins are neither suitable for rapid acceleration nor for agile maneuvering, and fishes such as salmonids which need these capabilities have much broader lower AR fins.

Why are high AR fins unsuitable? Symmetrical section foils (almost all fish fins are like this) have to be operated at an angle of attack to their axis of movement, or circulation will be equal above and below the foil, and no lift or thrust will result. At small angles of attack, the fluid will flow around the foil in an ordered way, but, if the angle of attack is increased above a certain limit, the flow above the foil will separate from it: it will stall with loss of lift and greatly increased drag. A long thin foil of high AR is much more prone to stall as the angle of attack is increased than is a short wide low AR foil, and it is when accelerating or maneuvering that the angle of attack of the fins is greatest. So

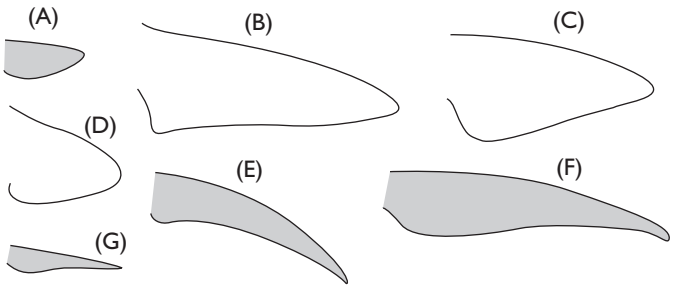

Figure 3.23 The lifting pectoral foils of different fishes (teleosts stippled). (A) Mackerel (*Scomber*); (B) *Carcharhinus longimanus*; (C) basking shark (*Cetorhinus*); (D) dogfish (*Scyliorhinus*); (E) carangid (*Trachurus*); (F) longfin tuna (*Thunnus alalunga*); (F) swordfish (*Xiphias*).

the high AR design of tuna fins or albatross wings is not suitable for trout or pheasants which need rapid acceleration from rest and both have low AR, short, broad designs. The convergent designs of the lifting foils of birds, and the lifting and thrust-generating foils of tunas and similar fast-cruising fish, are not the only convergences between fish, birds, and aeroplanes, as we shall see.

Skin friction drag, boundary layers, and Reynolds number

The most important drag component incurred by fish results from the viscosity of the water. Water tends to stick to the surface of the fish as it moves forward, forming a thin boundary layer in which there is a steep velocity gradient between the still water carried along by the fish, to the water going past in the free stream outside the boundary layer (Figure 3.24). This velocity gradient means that there is a large shear stress in the boundary layer, resulting in skin friction drag, D_{sf}.

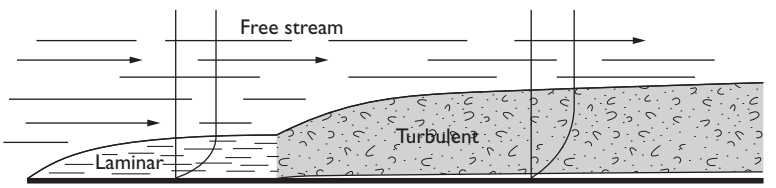

Figure 3.24 Development of boundary layer on a flat plate in a fluid flowing from left to right. The anterior thinner laminar layer has a less steep velocity gradient than the posterior turbulent layer.

The properties of the boundary layer, including its thickness and the D_{sf} incurred, depend on the ratio between the viscous and inertial forces acting on the fish, or submarine, or airplane, or indeed any object moving through a fluid. These are given by the useful Reynolds number, Re (introduced by the distinguished fluid dynamicist Osborne Reynolds who experimented on flow in tubes). For general flow conditions this number is defined as:

$$\text{Re} = \frac{\text{Length (L)} \times \text{Velocity (V)} \times \text{Density of medium } (\rho)}{\text{Dynamic viscosity of medium } (\nu)}$$

The *kinematic* viscosity of the medium, ν, is dynamic viscosity/density (for water; constant at any given temperature, approximately 0.001). Re is usually given as LV/ν, which in dimensional symbols is:

$$\text{Re} = \frac{L^2T^{-1}}{\dfrac{ML^{-1}T^{-1}}{ML^{-3}}}$$

where L = length, T = time and M = mass.

Re is thus dimensionless and so, whatever their size, if two similarly shaped objects of different sizes pass through a fluid at different speeds so that Re is the same, the flow regime around them will be the same, and flows can be tested with models. If we know Re, we have at once a good idea of what the flow pattern around an object (such as a fish) will be. In practice, at Re below 5×10^5 viscous forces predominate and the boundary layer on a flat plate will be laminar. At higher Re, over 5×10^6, inertial forces are more important, and flow within the boundary layer will be turbulent. The two kinds of boundary layer flow are seen in Figure 3.24, while Figure 3.25 shows the relation between Re and fish swimming speeds. Evidently, fish larvae will operate at low Reynolds numbers, and, as they grow in length and in the speed at which they swim, Re will increase.

On a flat plate of sufficient length placed in a water tunnel, the boundary will be laminar near its upstream (front) edge but it will thicken downstream

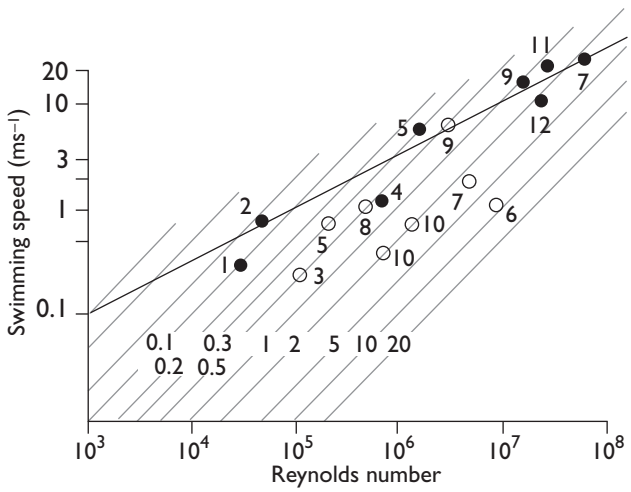

Figure 3.25 Relation between Reynolds number and swimming speeds (open circles: cruising speeds, filled circles: burst speeds). Oblique lines: fish lengths (m). (1) *Pholis*; (2) *Clupea*; (3) *Scyliorhinus*; (4) *Anguilla*; (5) *Scomber*; (6) *Cetorhinus*; (7) *Thunnus thynnus*; (8) *Salmo*; (9) *Thunnus albacares*; (10) *Makaira*; (11) *Acanthocybium*; (12) *Isurus*. Note that the bluefin tuna (*T. thynnus*) when sprinting operates at a Reynolds number of 6.5×10^7. Partly after Webb (1975).

(aft), and change to a turbulent boundary layer. The point of transition depends much on the smoothness of the surface, for the laminar layer is rather sensitive to any adverse pressure gradients produced by surface irregularities, because energy to maintain it can only diffuse across the layers within it.

Skin friction drag on a flat plate is given by:

$$D_f = \tfrac{1}{2} \rho V^2 A C_f$$

where ρ = density, V = velocity of flow, A = wetted area and C_f is a drag coefficient that depends on the type of boundary layer.

Attention to streamlining, and to the planform and AR of lifting and thrust generating foils will minimize pressure drag. What can be done to minimize skin friction drag; almost always much more important than pressure drag?

3.7 Mechanisms for Reducing Skin Friction Drag

Reduction of wetted area

Anything that reduces wetted area (A) is worth doing, for this reason scombroids have fins that retract into slots when not required for maneuvering, and, instead of continuous fins, body depth in fish making lateral body movements is increased by a discontinuous series of fins rather than a single elongate fin. Similarly, caudal fins are often scooped out at the back to reduce the wetted area.

Reduction of lateral movements

Fish that make large lateral movements as they swim incur greatly increased D_{sf} compared to that of a flat plate of equivalent wetted area, or indeed for the same fish gliding with rigid body. This is because the transverse movements of the body thin the boundary layer on the leading side, so increasing the velocity gradient and thus the stress within it. Oxygen consumption measurements and thrust calculations suggest that this increases D_f over the flat plate value by around 5 times. So it is small wonder that all the fastest swimming fishes use a caudal propellor and minimize body movements.

Boundary layer control mechanisms

Most fish are small enough that at burst speeds they operate in a flow regime where Re does not exceed 5×10^5 and boundary layer flow will be laminar. But larger and faster fish operate at Re above this, where inertial forces are more important and where the boundary layer might be expected to be largely turbulent. The drag coefficient (C_f) for a laminar boundary layer is much smaller than that for a turbulent boundary layer, so that anything the fish can do to maintain a laminary boundary layer and delay transition to a turbulent layer is worthwhile. Sharks have denticles with low sharp-edged ridges parallel to the direction of motion (Figure 3.26). Similar ridges have been made on flat plates (reducing drag by 10–15%) and ridging of this kind has been used on yacht hulls. The ridges are believed to delay turbulence by reducing microturbulence in a laminar sub-layer, delaying transition. Another possibility is to delay transition by moving maximum body thickness rearward so that adverse pressure gradients are avoided as long as possible. Tunas do this, just as the efficient

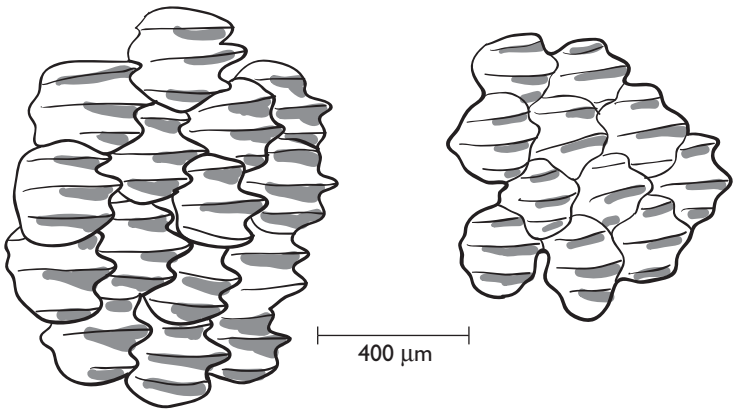

Figure 3.26 The ridged scales of rapid sharks. Anterior to left.

P-51 Mustang long-range fighter did with the first laminar flow wing. Laminar flow profiles are not always desirable however. Some fish seem to have adopted boundary layer control devices designed to *induce* transition to the higher drag turbulent boundary layer. This is because the turbulent boundary layer is less sensitive to disturbance than the laminar layer, and so is less prone to separate from the body. Separation is to be avoided at all costs, as it produces a large pressure drag penalty in the shape of a vortex-laden wake. For some fish it is advantageous to induce transition to the higher drag of the turbulent boundary layer rather than risk separation.

Mucus injection to the boundary layer

Low concentrations of mucus scraped from different fish reduced drag by up to 70% when added to water flowing through thin pipes. The most effective drag-reducing mucus was found on large barracuda (*Sphyraena*) and other fish with rapid acceleration to burst speeds, larger fish having more effective mucus than smaller. Here, what seems to be happening is that injection of long chain mucus polymers into the boundary layer stabilizes a turbulent boundary layer, delaying separation. This might seem to be an excellent strategy for reducing drag on torpedoes, hence increasing their speed or range, but the snag was that the polymer used left no room for the warhead.

Vortex generators and fluid injection

The large mesopelagic castor oil fish (*Ruvettus*), so-called because its tissues, especially the bones, contain a low-density purgative oil, has a most complex and fascinating integument seemingly with two separate mechanisms for maintaining a turbulent boundary layer. The very sharp pointed spines of the ctenoid (comb-like) scales all over the body project about 1.0 mm above the body surface and act as vortex generators, entraining fluid from the free stream as seen in Figure 3.27.

This input to the boundary layer helps to stabilize it and prevent separation during the accelerations and bursts of speed of this big predator. A less complex vortex generator array is often seen on the upper surface of the wings of

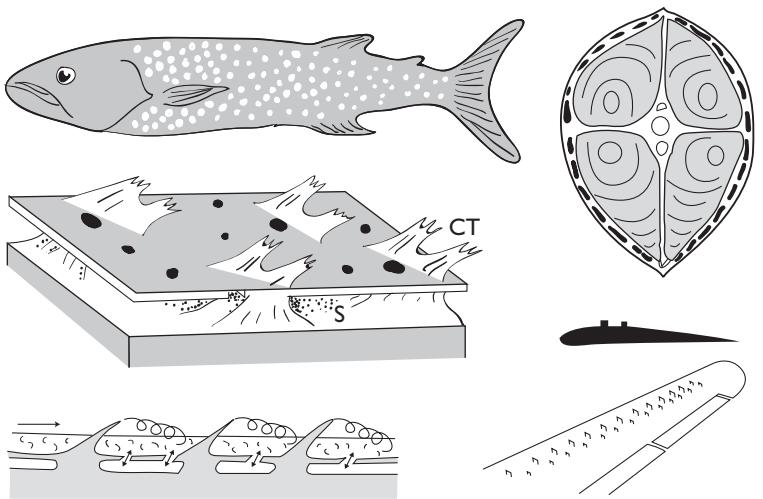

Figure 3.27 The specialized integument of the castor oil fish *Ruvettus*. Upper left: ctenoid scales (white dots) form a regular pattern over the surface. Upper right: transverse section of body showing sub-dermal spaces (black). Middle: stereogram of integument showing ctenoid scales (CT) and sub-dermal spaces (S). Anterior to left. Lower left: assumed operation of integument injecting momentum into boundary layer (see text). Lower right: vortex generators on aircraft wing. After Bone (1972b).

airliners, at the point on the chord where separation (stall) needs to be avoided during large angles of attack at take off and landing.

Vortex generators seem such a simple idea, and it may be that all fish with ctenoid scales use them as vortex generators, although this remains to be proved. They would not be suitable for fishes for which cruise efficiency is important, since they increase drag at low speeds.

Ruvettus also injects fluid into the boundary layer. The bases of the ctenoid scales form pillars supporting the outer skin away from the connective-tissue layer over the muscles; the resultant sub-dermal spaces communicate with the boundary layer by backward-facing openings (Figure 3.27). As the fish flexes its body, seawater will be alternately sucked into and squeezed out of these openings, so energizing the boundary layer and keeping it attached. This is certainly the most complex integument of any fish yet examined, but analogous simpler injection systems are seen in some stromateoids, such as the salp-eating *Tetragonurus*. Fluid injection into the boundary layer became common naval aircraft practice with "blown" flaps and control surfaces, once these could easily be fed from jet engines

Drag-reducing behaviors?

Fish denser than water could save energy, as many small birds do, by alternating propulsive body movements and periods of gliding with the body rigid when D_{sf} is less. An advantage of 20% or more could result, but we do not know whether dense fish (like many scombroids or the pelagic blue shark (*Prionotus*)) actually swim such an undulating glide-power cycle. Small whales and dolphins reduce the energy costs of swimming by "porpoising" in and out

of the water, and it seems that the pop-eye mullet (*Rhinomugil nasutus*) of Northern Australia which "porpoises" as it enters estuaries on the incoming tide, does so to reduce D_{sf}. For simplicity, as Liao (2004) recently pointed out, studies of fish swimming have so far been concerned with fish swimming at a constant speed in low turbulence conditions, but in nature fish will meet turbulence in the surrounding water flows, and, as we would expect, in flowing waters tend to swim where they need to expend least energy to hold station. Trout are adept at this, and can capture energy from the vortices around rocks to reduce their tail beat frequency and even perhaps any energy devoted to swimming whilst still holding station.

3.8 Efficiency

Efficiency is essentially measured by the ratio of input to output; there are various measures of efficiency according to the input:output property considered. We could compare the efficiency of the swimming fish to that of our own machines, such as motor cars, which generate power (as does the swimming musculature) by converting chemical to mechanical energy, and then transmit this power to the road to drive themselves along. For such machines, engineers define two principal kinds of efficiency.

1. *Brake horsepower* × *100*. Mechanical efficiency (η_m) = Indicated horsepower.
   This is the ratio of the power developed at the pistons vs. the power available at the output shaft after frictional losses in bearings, gear trains, etc. In practice, the mechanical efficiency of a motor car is usually around 80–85%, highest where roller bearings are liberally used in the engine, as in the Lory-designed 1927 Delage racing car where there were no fewer than 30!
2. A second measure of efficiency is the ratio of one kind of energy, heat, supplied during the combustion of fuel in the cylinders to the useful energy obtained in the form of work done by the engine driving the wheels.

$$\text{Thermal efficiency } (\eta_t) = \frac{\text{Work done} \times 100}{\text{heat supplied}}$$

Diesel engines have efficiencies around 40%, petrol engines rather less. Overall efficiency, *N*, is the product of the two, so *N* of a well-designed car will be something like 30%. How do fishes compare with this? Compared with land animals, fishes have efficient locomotion, as measured by the energy used per unit of body mass required to travel a distance, for as Schmidt-Nielsen (1971) pointed out, in fishes this "cost of transport" is about 10 times less than that land animals require. In practice, the cost of transport is determined by measuring oxygen consumption for fish swimming under controlled conditions in water tunnels or flumes, and is expressed as calories g^{-1} km^{-1}. In the white crappie (*Pomoxis annularis*) for example, swimming at its most efficient speed of 20–25 cm s^{-1} (1–1.5 BL s^{-1}) the total cost of transport was found to be 0.72 cal g^{-1} km^{-1}, whereas in the much larger hammerhead shark (*Sphyrna tiburo*), which cruises continuously, the cost of transport for small specimens (body length 34–95 cm) cruising at 29–67 cm s^{-1} was significantly lower

(0.4–0.67 cal g^{-1} km^{-1}). We have already seen (p. 87) that eels have an unusually low cost of transport. *N* may be considered as the product of the efficiency of transfer of momentum *m* from the fish body and fins to the water (usually called η_p), and that of the conversion of chemical to mechanical energy in the muscles (called η_m)). Values for *N* obtained from oxygen consumption measurements during sustained aerobic cruising by salmonids work out between 5% at *low* speeds to around 20–22% at the maximum sustainable speeds. Knowing *N*, we could obtain ε_m or ε_p, if we knew one of these. Unfortunately, few measurements exist of ε_m for fish muscle. Thus T1 has to be estimated using values for ε_m known for other animals such as frogs. Fish muscle is not very different in structure to frog muscle, so this is not an unreasonable assumption to make, and if we do so, estimates of ε_n range up to 75% in trout swimming at 2 BL s^{-1}. It seems highly probable that tunas and other lunate-tail swimmers will have a higher ε_p than this (perhaps 85%), and that *N* will be higher than for trout, possibly greater than 30%. So fish compare quite favorably with motor cars!

Envoi

Fish swimming has long intrigued scientists and advances in our understanding have come from advances in technique, both mathematical and experimental. In many respects, because they are both designs for operation in fluids, fish and airplanes have much in common. Most of the advances in boundary layer control and drag reduction used by both have, up to now, been understood from our own machines, but we await with much interest future work showing novel features first understood in fish, and later applied to submarines and aeroplanes.

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4 Buoyancy

4.1 Dynamic Lift

We saw in the previous chapter that some fishes are denser than the water in which they swim, and have to generate dynamic lift like airplanes either by using their outspread pectorals as lifting foils, or by inclining their bodies at an angle of attack to the horizontal. These processes inevitably generate drag, which makes a significant contribution to the total drag and energy requirement of such fishes. For example, a mackerel (*Scomber scomber*) weighing around 25 g in seawater has to generate some 1.2×10^4 N of dynamic lift during level swimming – such mackerel are effectively climbing a 1 in 15 hill all their lives!

But increased energy expenditure is not the only drawback to keeping aloft in the water by generating dynamic lift. Dense fishes must maintain a minimum forward cruising speed to generate enough lift to prevent them sinking, so they cannot hover or swim backward. The swimming rhythms of spinal sharks (35–40 tail beats min^{-1} in spinal *Scyliorhinus*, see p. 356) are apparently just below this minimum swimming speed. For benthic fishes that rest on the bottom and only swim occasionally, dynamic lift generation is appropriate, but, for other fishes, a better solution would seem at first sight to be the storage of light materials to provide static lift (as do airships and submarines) and thus avoid the drawbacks of continually generating dynamic lift. But little is as it seems at first sight, and since there are many superbly adapted pelagic fishes such as tunas that rely solely on dynamic lift, static lift is not the best solution for all, and even those that adopt it require to "invent" remarkable tricks to surmount the problems involved with static lift.

4.2 Static Lift

Most of the materials making up a fish are denser than water. For example much of the body consists of locomotor muscle (density 1050–1060 g l^{-1} in common marine fishes). Skeletal tissues loaded with heavy mineral salts are correspondingly denser, around 2040 g l^{-1} for typical teleost bones. Nevertheless, water is so much denser than air that even without special stores of low-density materials, fish are not so much denser than the water in which

they swim. They have the option (denied to birds) of storing sufficient low-density material that they can make themselves the same density as the water; they can thus achieve neutral buoyancy and need expend no muscular energy to keep station in the water.

Fishes use two quite different materials to provide static lift. Gas is efficient in giving lift, since its density is very low; small wonder that most teleosts possess gas-filled swimbladders. Usually the swimbladder is about 5% of the body volume of marine fishes, and about 7% in freshwater fishes, and provides enough lift for neutral buoyancy. The heavily armored *Lepisosteus*, however, needs a swimbladder around 12% of its volume for neutral buoyancy (p. 28). Fish swimbladders cannot significantly resist changing in volume as the fish swims up and down in the water and the ambient pressure changes; indeed, the swimbladders of almost all teleosts obey Boyle's law nearly perfectly (Figure 4.1). So if a fish with a gas-filled swimbladder is to remain neutrally buoyant at different depths, it must secrete or absorb gas to keep the swimbladder at constant volume as the ambient pressure changes. To regulate the mass of gas within the swimbladder in this way requires complex mechanisms of great physiological interest. For daily vertical migrators, such as many lantern fish (myctophids), maintaining neutral buoyancy as they make depth changes of several hundred m or more probably poses an insurmountable problem as will be seen in Section 4.4. As one reviewer remarked of swimbladder reviews, "they are as common as butterflies on a buddleia bush," and the reader may find it instructive (and even amusing) to examine an early and a more recent review to see how ideas have changed. For instance, Denton (1961); Alexander; (1966); Fänge (1966); and more recently Pelster (2004); Berenbrink *et al.* (2005); Phleger (1990); Phleger and Grigor (1990).

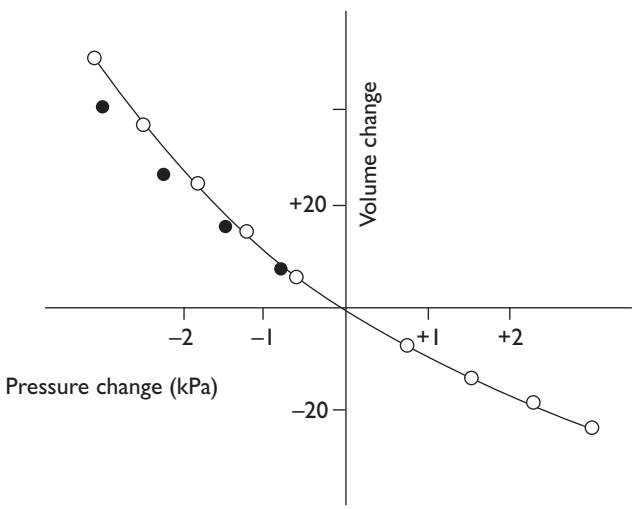

Figure 4.1A Volume changes of bubble (open circles) and fish swimbladder (filled circles). After Alexander (1966).

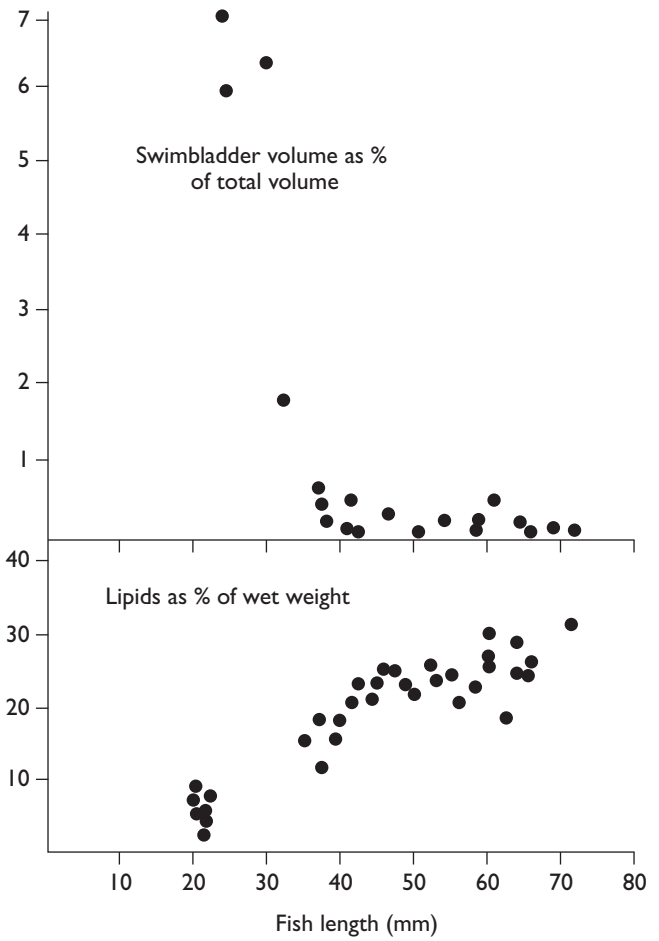

Figure 4.1B Changes in swimbladder gas and lipid content during growth of the myctophid *Diaphus theta*. After Butler and Pearcy (1972).

4.3 Lipid as a Source of Static Lift

Fats and oils are also used by fishes as sources of static lift. These are much less efficient and much bulkier for a given amount of lift. However, they have the great advantage that the lift provided varies little with depth, because changes in ambient pressure have relatively little effect on the volume of the fat or oil; if a fish using lipid as a source of static lift is neutrally buoyant at the surface of the sea, it will also be close to neutral buoyancy at the sea bed, even at considerable depths.

In the short term, then, lipid provides fewer problems of buoyancy regulation than does gas, but in the longer term difficulties arise because the lipids stored to provide lift may have other functions as well. For example, sharks which store oil in the liver and muscles may have to draw on this store as a fuel for continuous swimming, or as food reserve for either developing embryos or the adult. Where lipid is the sole source of static lift, there will certainly be

complexities in the regulation of lipid metabolism, and the fish will find it difficult to adjust its density rapidly to cope with the short-term density changes resulting from feeding and parturition.

Fish using gas as a source of static lift can afford to support the heavy components of their bodies without difficulty, but, where lipids are used, the safety margin is not so great, and many fish using lipid for static lift can only achieve neutral buoyancy by reducing the dense components of their bodies and so reducing the lift they require (Figure 4.2). Their skeletons are reduced and poorly calcified, and they even reduce the amount of protein in the muscles, which are weak and watery. Mesopelagic and bathypelagic fishes without swimbladders have an extremely high water content, e.g. *Melanocetus* (angler fish) 95%, *Eurypharynx* (gulper eel) 94%, *Photostomias* 92%, *Gonostoma elongatum* 90% and 88%. Those with swimbladders like the lantern fishes and hatchet fish (sternoptychids) are 70–85% water. The first group has very small hearts, and very little red (cruising) muscle: they are little more than floating traps or ambush predators, welcoming their prey with luminous lures, and quite unable to pursue potential food. Lantern and hatchet fish have larger hearts and more red muscle, so are able to chase their copepod prey. Larval fishes before ossification of the skeleton are watery and have their own characteristic buoyancy balance sheet (Figure 4.2). The elongate leptocephali larvae of eels, the bone fish (*Albula*), and the tarpon (*Elops*) are somewhat watery, but are also buoyed up by special gelatinous tissue in the gut.

But some fish without swimbladders can achieve neutral or near-neutral buoyancy without going in for high water content and reduced skeletons: they store enough lipid that they need not reduce their denser components.

In five unrelated fish groups (and perhaps in others) enough lift is provided by stored lipids to achieve neutral buoyancy (Phleger, 1990). Interestingly enough, the lipids stored are not biochemically similar (Figure 4.3) but with one curious exception, they are all of particularly low density and so most

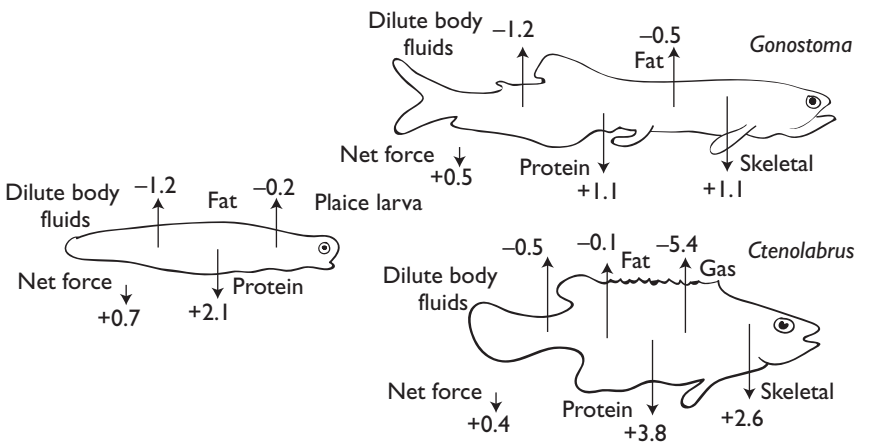

Figure 4.2 Buoyancy budgets of a mesopelagic fish (*Gonostoma*), a shallow-water acanthopterygian (*Ctenolabrus*) and a fish larva (plaice, *Pleuronectes platessa*). All upward (–) and downward (+) forces are in arbitrary units. After Denton and Marshall (1958), and Blaxter and Ehrlich (1974).

efficient in providing lift. Fish lipids vary in density from around 930 g l^{-1} (cod liver oil, and the oil of dogfish livers) to the wax esters of myctophids, gempylids, and the coelacanth *Latimeria* (densities around 860 g l^{-1}), and the hydrocarbons of some sharks (860 g l^{-1}). The difference in specific gravity between cod liver oil and the wax esters or shark hydrocarbons may not seem very striking, but 1 g of the less dense oil will provide 0.1675 g of lift in seawater (density 1027.5 g l^{-1}), whereas a gram of the denser oil will only provide about half as much lift (0.0975 g). It is thus well worthwhile for fishes to store these lighter lipids rather than the more common triglyceride metabolic reserves, as almost all the fishes that achieve neutral buoyancy using lipids actually do.

The exceptions are the two pelagic Antarctic notothenioids (other members of the family are benthic): the toothfish (*Dissostichus*), and the silver fish (*Pleurogramma*; Figure 4.4 A and B). Both fish have large amounts of triacylglycerols with similar density to cod liver oil (930 g l^{-1}), that is the lipid is not especially designed to give maximum possible lift, for just below the ice in the waters of McMurdo Sound (1028 g l^{-1}) 1 g of this lipid provides only 0.098 g of lift. The juveniles are benthic and dense (Figure 4.4C) but, as the adults increase in size and swim in the water column, they contain progressively more and more long chain unsaturated fatty acids, and become neutrally buoyant. It seems probable that this lipid is derived from their copepod food. Both fish have reduced dense components as far as possible, ash content of the skeleton being only some 0.2% compared with 2–3% in a normal fish.

Squalene

The first fishes found to use lipid to achieve neutral buoyancy were the deep-sea squaloid sharks (Figure 4.5). Unlike the spurdog (*Squalus acanthias*) of the same family, these fish live near the bottom in deep water, and the habitat is

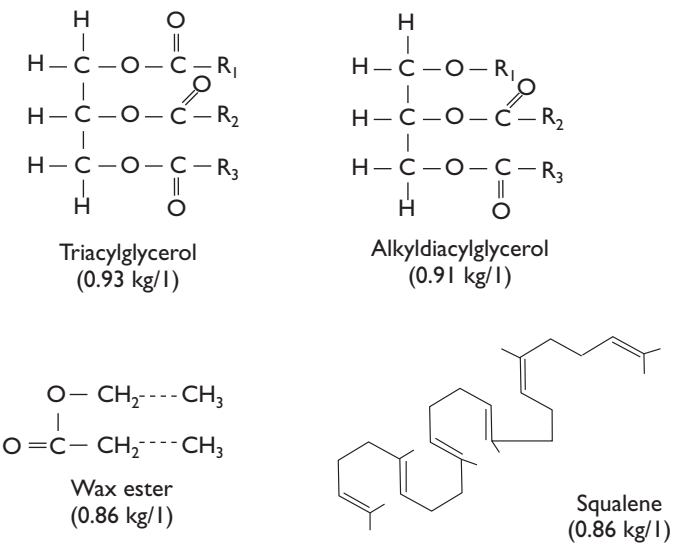

Figure 4.3 Chemical structure and density of lipids used by fish for static lift. From Pelster (1998).

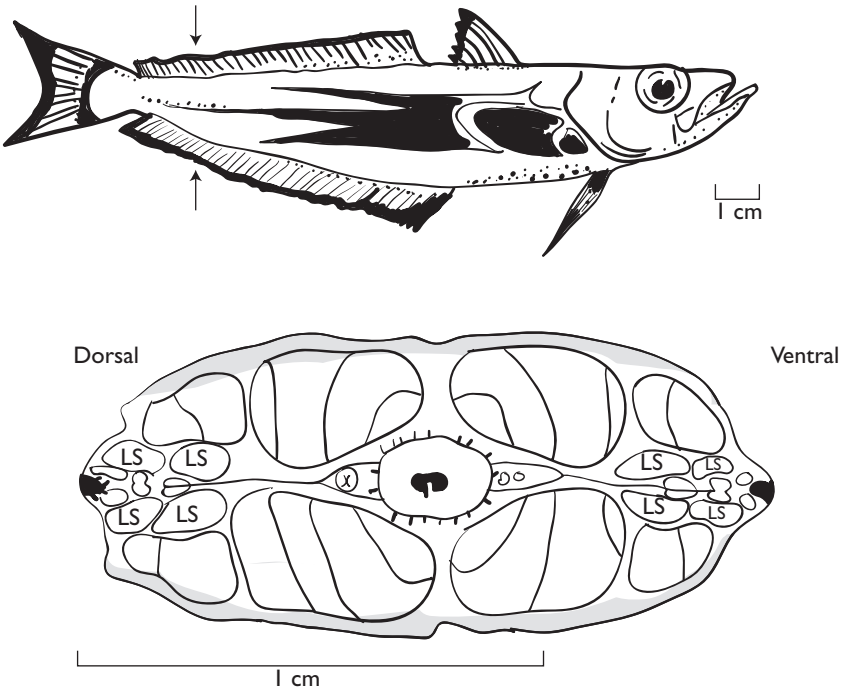

Figure 4.4A The pelagic Antarctic silverfish *Pleurogramma antarcticum* which stores buoyancy lipid in subcutaneous sacs (above, black dots) and in intermuscular sacs seen in cross-section below (LS). De Vries and Eastman (1978).

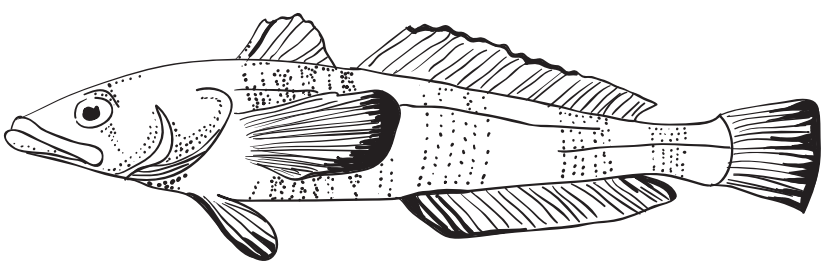

Figure 4.4B *Dissostichus mawsoni*. Eastman and de Vries (1981).

one where the family has successfully diversified, for there are many genera known. All share three striking characteristics: very large livers (making them grossly corpulent), very small pectoral fins, and blue or green eye-shine (see p. 317). In most animals, including ourselves, the liver is around 4–6% of the total weight, but, in these fish, it may be more than one-quarter of the total weight, because it contains an enormous amount of pale yellow oil, valuable at one time for lubricating wooden mill machinery. On this oil the fish literally float – when their livers are removed, they sink. In all species examined, the

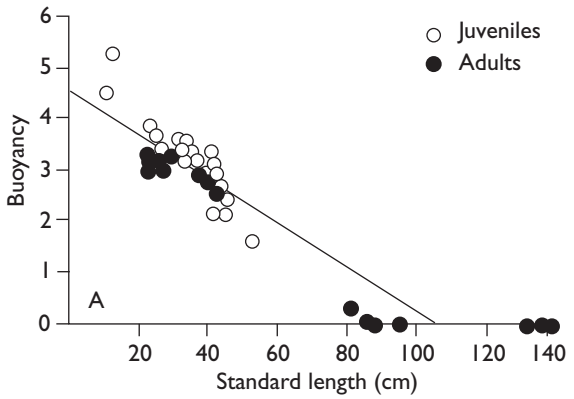

Figure 4.4C Increase in density as *Dissostichus mawsoni* grows and changes from a pelagic to a benthic life. Ordinate: buoyancy expressed as (weight of fish in water/weight in air) × 100. Abscissa: standard length (cm). Near *et al.* (2003).

liver oil is of low density (870–880 g l^{-1}) because it is mainly composed of the hydrocarbon squalene. Squalene, which was first isolated from such sharks, is formed by the condensation of isoprene units on the pathway leading to cholesterol. It has the low density of 860 g l^{-1}, and so is admirably suited to provide static lift. Curiously, it is a main constituent of human sebaceous gland secretions.

We do not yet know how sharks regulate their liver lipids so as to balance their weight in water, but it appears from experiments on *Squalus* that the fine adjustments required for neutral buoyancy may depend not on changing amounts of squalene, but rather on varying the other less-abundant lipid constituents of the liver oil. By attaching weights to *Squalus* in an aquarium, it was found that the fish responded by increasing the amount of low-density alkoxydiglycerides (also known as diacylglyceryl ethers or DAGE) in the liver oil, compared with the control fishes which had larger amounts of the denser triglycerides. Further experiments of this kind deserve to be carried out to examine the cues which induce preferential production of DAGE, which is lighter than the triacylglycerols in the liver oil.

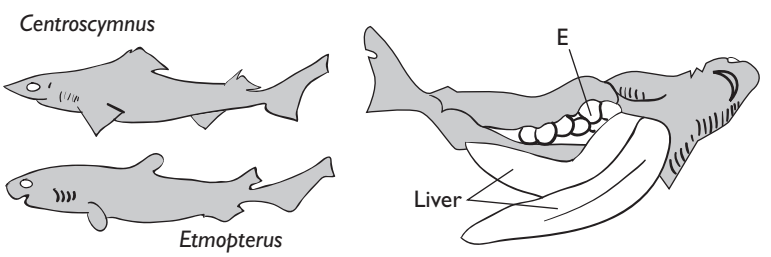

Figure 4.5 Neutrally buoyant deep-sea squaloid sharks. Right: female *Etmopterus* opened to show huge liver and large eggs (E). From sharks caught on a deep-sea long line by G. R. Forster in the Bay of Biscay from RV *Sarsia*.

Deep-sea squaloids bear live young and have very large eggs (about the size of a billiard ball in a shark a meter long); sensibly enough, the eggs contain squalene and are neutrally buoyant themselves, so that pregnancy does not increase the density of the mother.

It is obvious why these fishes need only have relatively small pectoral fins; they are used only during maneuvering, not for lift generation. Although the tails in most genera are markedly heterocercal, they evidently do not generate lift (p. 24). Why do deep-sea squaloids need to be neutrally buoyant if they live on the sea bed? The answer is that they hover just *off* the bottom (as we know from photographs and videos taken by deep cameras), unlike the dense bottom-dwelling elasmobranchs of shallow water, which rest on the bottom and are invariably very dense, like most rays or the angel fish (*Squatina*).

Deepwater Holocephali evidently live in a similar way to the deep-sea squaloids, and like them, are close to neutral buoyancy, although they only manage this by virtue of reduction of dense components, and have poorly calcified skeletons. Their liver oil consists largely of squalene and this is also the main source of static lift in some teleosts. Eulachon (*Thaleichthys*), for instance, contain 15–20% of lipid by weight. Dried eulachon can be ignited and used as candles, and their grease was an important trading commodity for Fraser River Indians. They do not have a gas-filled swimbladder, and during their spawning migration, squalene forms a higher proportion of the total lipid than at other times; it seems that the fish metabolizes reserve triglycerides during these migrations and become denser, so that, in this case, lipid metabolism is not sufficiently well regulated to cope with buoyancy and metabolic demands upon the total lipid pool, while maintaining neutral buoyancy.

Wax esters

A little squalene is also found in the living coelacanth *Latimeria*, but most of the massive amounts of lipid stored are wax esters. These make up 30% of the wet weight of the ventral musculature and over 60% of the wet weight of the swimbladder (which contains only lipid, no gas), and in the pericardial and pericranial tissues, the percentage is even higher. We know a little about the habits of *Latimeria* (from excellent films taken from a submersible), its fins certainly seem unsuited to provide dynamic lift, and the curious variety of attitudes in life strongly suggest neutral buoyancy (see p. 27).

Wax esters are probably stored to provide lift in many families of mesopelagic teleosts, but the only ones examined so far are the beryciform orange roughy (*Hoplostethus atlanticus*), gempylids and a few of the numerous kinds of myctophids. The orange roughy has extracellular wax esters that are solid at 6°C where the fish normally live. They are stored in the bones, swimbladder, and neurocranial cavity, and confer neutral buoyancy (Phleger and Grigor, 1990).

The gempylid *Ruvettus* (which has the remarkable integument described on p. 94) is loaded with low-density oil (density 870g l^{-1}) which has purgative properties, hence the common name of castor oil fish. The cranial bones have been modified as oil tanks, and are the least dense tissues of the body. This large (1 m) predatory fish ranges from depths of 15 m to over 500 m, and is very close to neutral buoyancy, feeding on smaller fishes which undertake diurnal vertical migrations.

The most remarkable of these are the myctophids, some undertaking daily a double journey of 500 m up and down in the water column. Although all myctophids have gas-filled swimbladders as larvae, in many species the swimbladder shrinks and becomes invested with more and more lipid as the fish gets older, until no gas remains (Figure 4.1B). As in *Latimeria*, lift from gas is replaced by lift from lipid, which may eventually make up 15% of the wet weight of the fish. As we should expect, the species with a high lipid content are neutrally buoyant, and store low-density wax esters. Why should many myctophids have abandoned gas as a source of static lift? Although the evidence is only circumstantial, it seems a reasonable guess that it is because there are difficulties in regulating buoyancy over a wide depth range when gas is the source of the lift. Those species which as adults have much low-density lipid and are neutrally buoyant, have a greater depth range than the juveniles, which still have gas in the swimbladder and similarly, species which have gas-filled swimbladders as adults undergo less extensive vertical migrations than those which rely on lipid only.

On the whole, it seems that lipid storage for static lift is a secondary phenomenon in myctophids, and that the most "advanced" species in the family are those which have abandoned gas altogether as adults; it is interesting that within the family there are fishes showing all stages in this changeover adapted to different lifestyles (Figure 4.6).

Insufficient static lift for neutral buoyancy

So far, we have considered fishes of different groups which store low-density lipids to attain neutral buoyancy. What of fish that use lipid for static lift, but are not neutrally buoyant? Many teleosts are certainly in this category. Mackerel (*Scomber*) vary in density at different times of year because they have mainly lipid stored in the muscles; such a system is evidently a primitive one, for lipid is not stored to maintain a constant density. Rather, the lipid store is

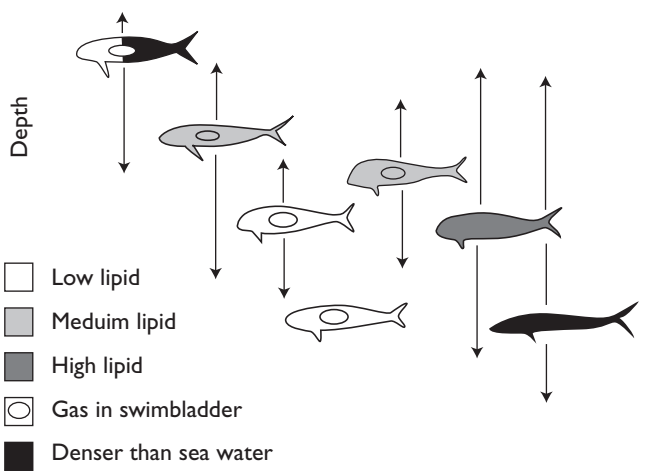

Figure 4.6 Functional types of myctophids. Vertical arrows indicate known or assumed extent of vertical migration. After Bone (1973).

at the mercy of metabolic demand, and reduction in density, valuable as it must be, is simply a side effect of the storage of lipid for metabolic purposes.

We know that a wide range of elasmobranchs, including large pelagic sharks, are each of a characteristic density, some being fairly close to neutral buoyancy, others being very dense. Liver lipid is an important factor in determining the density of some species, but in most it is the density of other tissues (amount of fat in the white muscle, mineralization of the skeleton) that determines the density of the species. Not only do different elasmobranchs store different amounts of lipid in their livers, but the stored oil is least dense in the least dense species. It seems that elasmobranchs have managed to set apart the lipid used for density regulation, whether in the muscles or liver, from metabolic stores, so that the fish can regulate its density to a characteristic figure, whatever metabolic demands are made. How this is done remains to be discovered.

At the beginning of this section, the advantages of neutral buoyancy were extolled, and it seems odd to find fishes using static lift to maintain a fixed density below that of neutral buoyancy. The regulatory mechanisms are there, and, at first sight, it seems a harder problem to maintain a constant density (say around $1.030\,g\,l^{-1}$) than to regulate to neutral buoyancy, and what is more, dynamic lift must still be generated, with the penalties that this incurs. However, it is not hard to see why many pelagic sharks use lipid to reduce their density, but not so far as to become neutrally buoyant.

Shark fins vary a good deal in flexibility and in their stiffening by skeletal elements, but the basic design, unlike that of teleosts, makes them impossible to retract; they are not of varying geometry. If we bear this in mind, we can see how considerations of dynamic lift generation lead to different densities in different species, depending on their mode of life. Reduction in density by lipid storage means that less lift needs be generated in level swimming, so that the shark can either reduce the size of its pectoral fins, or it can cruise more slowly without stalling. Since sharks use their pectoral fins for maneuvering (to turn, change in pitch, and even sometimes, as does *Heterodontus*, to creep backwards along the sea bed), there is a limit to reduction in fin size. Reduction in fin area is wholly beneficial (since it reduces drag), but to remain maneuverable, the shark must have pectoral fins of a certain size, and, below this, no benefit will be gained by reducing density, apart from a slight reduction in vortex drag.

We saw earlier (p. 89) that vortex drag is proportional to $1/V^2$, so that this will be most important at low swimming speeds, and unimportant at high speeds. We should expect from these considerations that sharks which swim fast would be of reduced density, but not neutrally buoyant; those that swim very slowly (for example as part of the feeding pattern) would be close to neutral buoyancy, while those living on the bottom would be very dense. Looking into the matter, this is just what we find. The huge whale-shark and basking shark which sieve plankton while swimming very slowly, are close to neutral buoyancy; the fast pelagic tiger and blue sharks are of reduced density, but are not so close to neutral buoyancy; and the bottom-living dogfish and rays of shallow water have no special arrangements for static lift.

Since teleosts have differently designed fins, which can vary their geometry, the arguments above do not apply, and we should not expect to find any teleosts using lipid to reduce their density to a constant figure not close to neutral buoyancy; so far, none has been found.

4.4 Gas as a Source of Static Lift

Fish swimbladders obey Boyle's law nearly perfectly, (Figure 4.1A) changing volume in proportion to the ambient (hydrostatic) pressure, but often retaining a slight positive internal pressure. To be at neutral buoyancy, freshwater fish need a swimbladder occupying about 7% of the body volume while marine fish (in denser seawater), need a swimbladder occupying 5% of the body volume. By no means do all fish keep their swimbladders at a volume to make them neutrally buoyant. For example, as Cavadias and Gee (1987) showed, the buoyancy of the freshwater *Percina* depended on its environment. In the still waters of lakes, the fish were close to neutral buoyancy, while in flowing rivers and off exposed beaches, where there is wind-generated turbulence, the fish were negatively buoyant and many had no gas in the swimbladder. Since the ambient pressure increases by 1 atmosphere (101.3 kPa) for every 10 m depth, the volume of the swimbladder changes as fish move up and down, especially near the surface. Sinking from the surface (1 atm) to 10 m (2 atm) causes the swimbladder to halve in volume. Readers can figure out for themselves how much swimbladdder volume would change for a fish at 200 m when it moves up or down 10m, and see that midwater or deep-dwelling fish do not suffer large volume changes when changing depth by tens of m, as surface dwellers would do. It is hardly surprising that many surface-dwelling pelagic fish such as tunas and scombroids have either lost the swimbladder altogether, for example the English mackerel (*Scomber*), or reduced its importance as in the Spanish mackerel (*Scomberomorus*). Only those fish that are strictly limited to the surface layers, such as flying fish (*Cypsilurus*; *Exocoetus*) and half-beaks (*Hemirhamphus*) have swimbladders. Similarly, many bottom-dwelling fishes have lost their swimbladders and those that make extensive vertical migrations, as we have seen earlier, sometimes abandon gas and fill the swimbladder with lipid. The distribution of fishes with gas-filled swimbladders was examined by Marshall (1960). As Figure 4.7 shows, they occur down to the greatest depths. Although the effect of depth changes is much less significant at great depths, the problems of secreting and retaining gas are formidable. Yet the macrourid *Nematonurus armatus* has been caught at depths of 4000–4500 m where the pressure is about 450 atm (45585 kPa).

Swimbladder structure

Swimbladders vary in shape and in their connections with other internal organs partly because swimbladders perform other functions in hearing and sound production (p. 296). They share certain special structural features (Figure 4.8). The connections with the blood system are complicated; there are sometimes diaphragms or sphincters isolating one region of the swimbladder from the gas inside; and parts of the lining epithelium are specially modified.

In ontogeny the swimbladder arises from a diverticulum in the roof of the foregut. *Physostomatous* teleosts, (most are freshwater) retain the connection with the gut as the pneumatic duct so that the swimbladder lumen remains in contact with the environment and gas can be obtained by swallowing air at the surface and passing it down the gut (or burping it in the reverse direction). Nevertheless, some physostomes such as charr (*Salvelinus alpinus*) and coregonids share with physoclists gas-secretion and gas-resorption

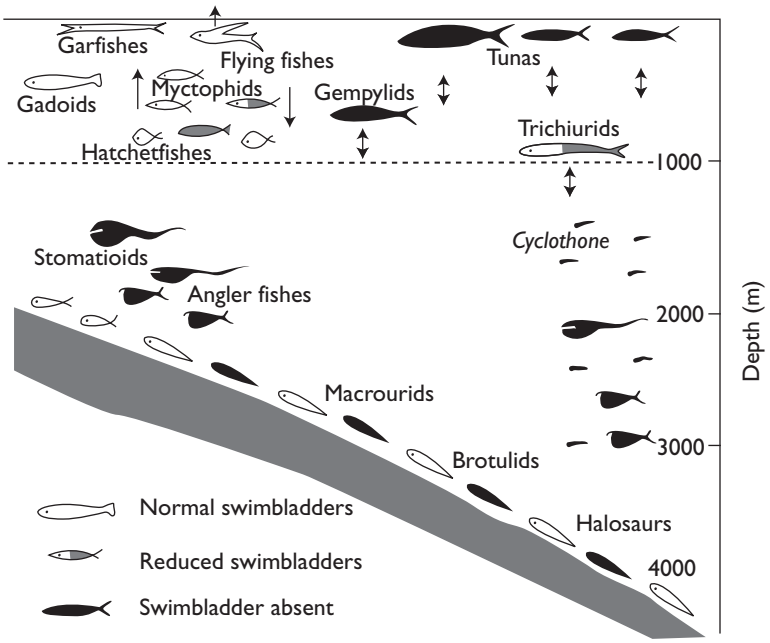

Figure 4.7 Distribution of fishes with and without swimbladders in the oceans. After Marshall (1960).

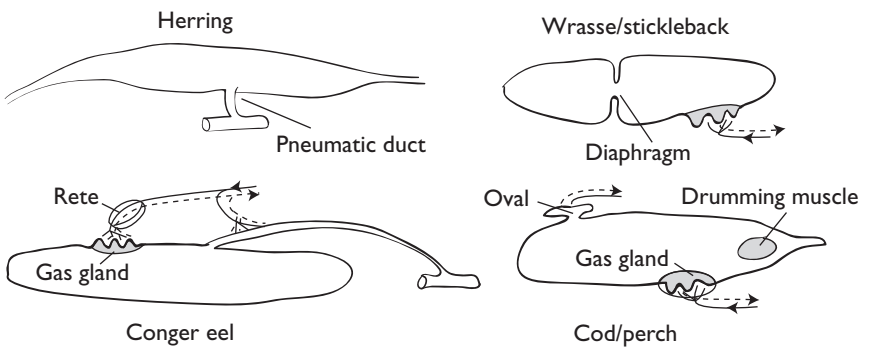

Figure 4.8 Swimbladder structure. Left: in two physostomes. Right: in two physoclists. Note that the conger eel is a physostome with a gas gland and rete. After Denton (1961).

mechanisms. In the mainly marine *physoclistous* teleosts, the swimbladder is open for a brief time in ontogeny, which allows the larvae to swallow air at the surface and fill the swimbladder for the first time (Figure 4.9). Thereafter in the adult it is closed, relying on intrinsic gas-secretion and absorption mechanisms The physostomatous state is the primitive condition and is found in the primitive elopomorphs such as eels and tarpons, in clupeids, and in freshwater coregonids. The selective advantage that has made the majority of fish physoclistous is not evident. Anyone who has been on deck when a deep-sea trawl or long line has been hauled aboard will have been

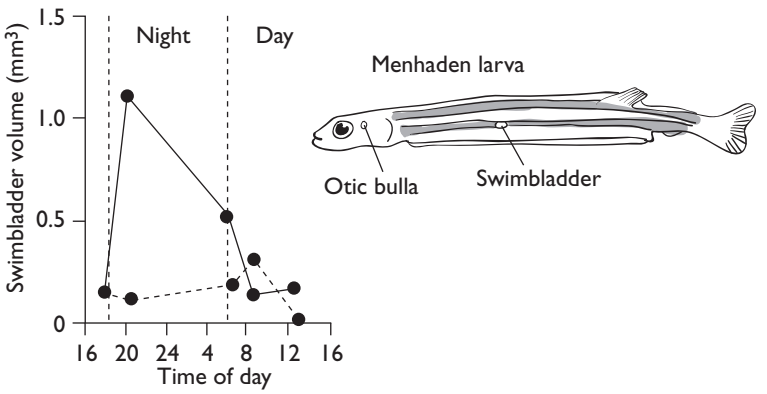

Figure 4.9 Refilling the swimbladder at night. Left: the swimbaldder volume of menhaden larvae by night and day with access to water surface (continuous line) and denied access to the surface (dashed line). Right: a 15 mm long menhaden larva showing the swimbladder and otic bulla. After Hoss and Phonlor (1984).

much struck by the piteous state of the fish, with their viscera protruding from the mouth by the hugely enlarged and often ruptured swimbladder. But fish are not designed to be fished.

Gas in the swimbladder

The swimbladder gas is mainly O_2 when freshly secreted, as it is in the swimbladders of deep-sea fish. Studies of the swimbladder gas in deep-sea fish literally began with an explosion. The polymathic French physicist J.-B. Biot took gas from deep-sea fish caught by long-lining, and put it (with excess hydrogen) into a glass eudiometer tube. A violent explosion (which broke his instrument) resulted when he passed a spark, and he realized at once that the swimbladder gas had more oxygen than in air. Of Biot, a contemporary remarked he "was endowed to the highest degree with all the qualities of curiosity, finesse, penetration, precision, ingenious analysis, method, clarity, in short with all the essential and secondary qualities, bar one, genius, in the sense of originality and invention." No doubt a just, but, nonetheless, a sad assessment.

With a new instrument, he continued his studies, to find that in the swimbladders of fish caught near the surface there was less oxygen than in air, while in those caught at depth, considerably more (Biot, 1807). Much later experiments with $O_2{}^{18}$ showed that the oxygen in the swimbladder does not come from chemical processes in the fish tissues: it is derived from oxygen dissolved in the surrounding water which passes into the swimbladder from the blood. So a fish using a gas-filled swimbladder has to overcome three different problems:

1. How to drive gas across the opposing partial pressure gradient from blood to swimbladder lumen.
2. How to retain gas within the swimbladder and prevent it diffusing out.
3. How gas may be sometimes permitted under controlled conditions to pass from the swimbladder to the blood or directly to the water.

Gas release

Obviously enough, the last of these is the simplest problem to solve. Physostomes simply have to open the pneumatic duct from the swimbladder to the gut and allow gas to bubble out of the mouth or anus. As herrings rise in the water column, they release bubbles, as do freshwater white fish (*Coregonus*) seen with echosounder transducers (Figure 4.10, after Knudsen and Gjelland, 2004). Recently there has been the unexpected finding that herrings may signal to other members of their school by noisily farting bubbles out of the anus (Wilson *et al.*, 2003). In the conger eel the pneumatic duct is first expanded and gas comes into contact with the systemic circulation, before further expanding to release gas into the esophagus via a valved opening (Figure 4.11). The duct is innervated by the vagus and vagal stimulation or

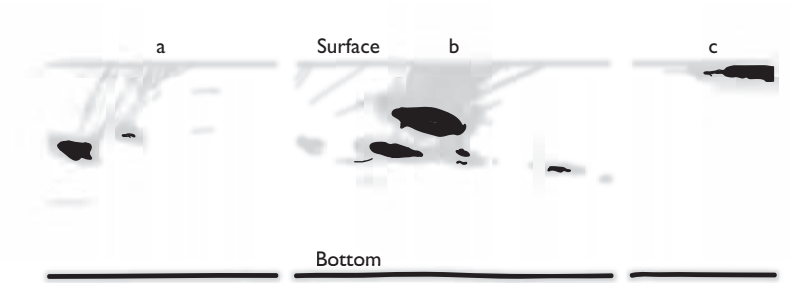

Figure 4.10 Sequence of three echograms of small schools of Coregonids in Lake Skrukkebukta (Norway), rising from midwater to the surface and releasing gas from the swimbladder as night falls. The straight lines are gas being released. Left: while still light, mid: at dusk, right: fully dark. From Knudsen and Gjelland (2004).

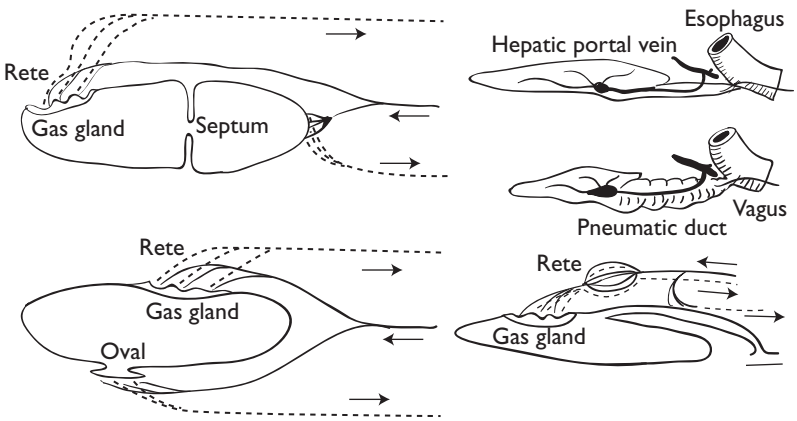

Figure 4.11 Structure of physoclistous (left) and physostomous swimbladders. Upper left: divided swimbladder (as in wrasse) – direct connection to systemic circulation on right. Lower left: gadoid swimbladder with direct connection to systemic circulation at oval. Lower right: eel swimbladder with direct connection to systemic circulation at pneumatic duct. Upper right: appearance of *Conger* swimbladder before (above) and after 5 minutes vagal stimulation, showing gas loss through enlarged pneumatic duct. After Denton (1961) and Fänge (1966).

injection of sympatheticomimetic drugs such as adrenaline (p. 376) causes gas release.

Physoclists simply need a capillary system to bring systemic blood in contact with the swimbladder wall. Owing to the partial pressure gradient, gases will rapidly diffuse from the swimbladder into the blood, and thence across the gills and into the water. If it is possible to occlude this connection with the systemic circulation at will, then the problem of controlled loss of gas is solved. In gadoids and perciformes, a sphincter closes off an oval area of the swimbladder wall in contact with the systemic circulation; in wrasse (labrids) a similar area is occluded by an adjustable diaphragm across the swimbladder.

Gas retention

This is a much more difficult design problem. The swimbladder wall must be impermeable to gas, yet it is thin and has to be elastic. Krogh (1919) found that if its wall was simply ordinary connective tissue, it would be about 100 times as permeable as it actually is. Denton and his colleagues (Denton *et al.*, 1972) found by ingenious experiments with *Conger* swimbladders that their strikingly low permeability resulted from the investment of the swimbladder by a cellular layer containing sheets of guanine crystals some 3 μm thick. The long tortuous diffusion pathways around the overlapping guanine sheets confer impermeability, as was shown by cautiously removing the silvery guanine layer without damaging the underlying layers, when permeability rose forty-fold. As we would expect, the guanine content of deep-sea fish swimbladders is much higher than that of surface-living fish. *Synaphobranchus* and *Halosaurus* have 15 times the amount of guanine that eels have and five times that of a herring. We can only admire the ingenuity of the way that fish swimbladders have evolved impermeability while still permitting elasticity and change in volume, using a material that elsewhere is used for an entirely different purpose (p. 327).

Gas enters the swimbladder via blood capillaries that run into a modified area of the inner wall: the gas gland. This has a glandular cap, which may be extensively folded to increase its surface area, and in actively secreting swimbladders, the surface of the gland is covered with a foamy mucus. The link with the systemic circulation is an obvious potential gas leak, avoided by the arrangement of the capillaries in a counter-current *rete mirabile* similar to the systems used for heat conservation in tunas and sharks (p. 81). The rete consists of thousands of alternately opposed and parallel afferent and efferent capillaries. We can well understand how the rete operates to prevent loss of gas from the swimbladder. Blood leaving the swimbladder flows through the rete in venous capillaries closely apposed to the entering arterial capillaries. This venous blood will have been in contact with the swimbladder gas and so the partial pressure of oxygen (P_{O2}) within it will be greater than that in the adjacent systemic arterial capillaries. The deeper the fish lives, the greater the partial pressure difference between the swimbladder gas, and gases in the surrounding water and systemic circulation. Not surprisingly, the longest retia known (25 mm) are from the abyssal ophidiid *Bassozetus taenia* caught between 4575 and 5610 m. In the unipolar rete, typical of most physoclists, the capillaries have many hair-pin loops embedded in the glandular cap itself.

Much of the work on gas secretion has been done on the eel, *Anguilla vulgaris*, which is a physostome with a functional pneumatic duct but also secretes gas. In the bipolar rete of the eel, the afferent and efferent capillaries are apposed in a bundle a short distance from the glandular cap making it possible to cannulate the blood supply entering and leaving the bundle (Figure 4.12), a helpful design for physiologists (see Kobayashi *et al.*, 1990).

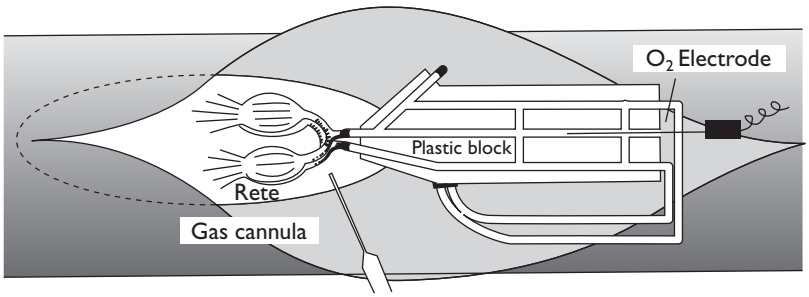

Figure 4.12 Experimental arrangement to determine changes in blood properties during gas secretion by the eel *Anguilla*. After Berg and Steen (1968).

Gas secretion

If it is easy to see how the rete operates to prevent gas leaving the swimbladder when the fish is at constant depth and in equilibrium, how does it work when the fish has descended in the water, and needs to secrete gas into the swimbladder? This long remained a most challenging mystery, and exercised the minds of eminent physiologists for many years. What was baffling, was that the arrangement of the rete seemed very well adapted to *retain* gas in the swimbladder, but also to *prevent* any gas from entering! Even now, some aspects still remain to be studied.

What is evidently needed is some change or changes in blood properties that reduces the amount of gas in the venous (efferent) capillaries leaving the rete, compared with the arterial (afferent) capillaries entering the rete. This mechanism must be ingenious, for if P_{O2} in the venous capillaries of the rete is lower than in the arterial capillaries, O_2 might be expected to diffuse from the arterial to the venous capillaries, and the secretory process would grind (or rather diffuse) to a halt. Changes in the properties of the blood, as it passes into the gas gland, are needed to raise the P_{O2} but decrease the actual oxygen carrying capacity of the blood. O_2 will then pass into the lumen of the swimbladder and at the same time diffuse from the venous to the arterial capillaries within the rete. Elegant cannulation experiments on eels show that the change is that the blood increases in acidity as it passes through the gas gland, which is made up of highly specialized cells.

These cells are functionally and structurally bipolar. They secrete surfactant into the swimbladder (Daniels *et al.*, 2004) by exocytosis, and at the basal pole, acid metabolites into the blood. Different kinds of ATPase found at each pole drive these processes. The cells are rich in glycogen, carbonic anhydrase, and lactate dehydrogenase, and when gas secretion is required, release lactic acid into the venous capillaries (Figure 4.13). They do this, unusually, almost

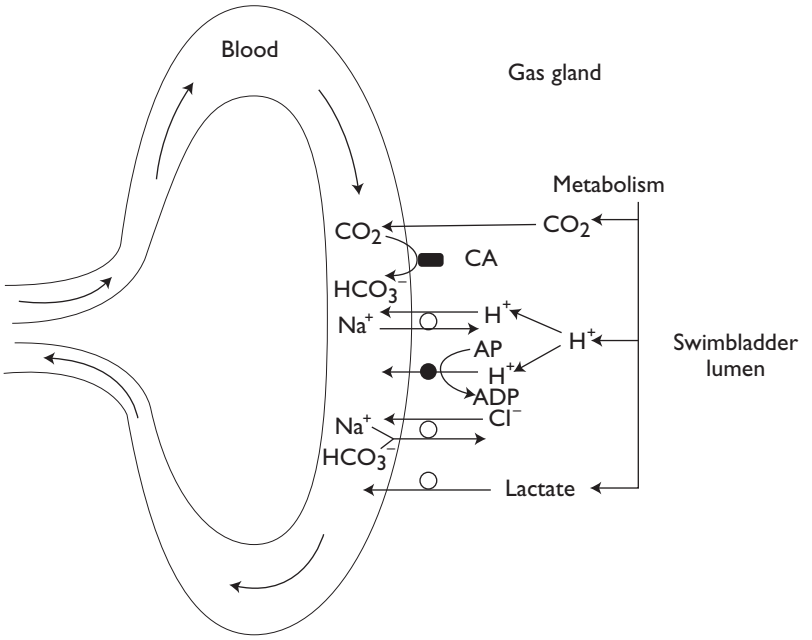

Figure 4.13 Mechanisms of acidification of swimbladder retial vessels at the gas gland. From Pelster (2004).

entirely by *anaerobic* glycolysis and there is no Pasteur effect preventing anaerobic glycolysis in the presence of O_2. CO_2 is also produced, but by decarboxylation in the pentose phosphate shunt rather than by glucose oxidation. So the gas gland cells have a high P_{CO2} and CO_2 rapidly diffuses across into the red blood cells to begin the Root effect (Figure 4.14).

The Root effect (Root and Irving, 1943) is the reduction in the O_2-carrying capacity for hemoglobin as pH decreases, even when P_{O2} is very high, so differing from the Bohr effect, which simply reduces its affinity for O_2. A change of one pH unit can unload 50% of the oxygen even against considerable oxygen partial pressures. Actively secreting gas gland cells can reduce blood pH down to pH 6.5. Lactic acid from the gas gland cells not only produces the Bohr and Root effects, but the increased solute concentration reduces the solubility of O_2 in the blood plasma, so salting-out further O_2 as gas. Carbonic anhydrase also has a role; it apparently accelerates the formation of bicarbonate from excess CO_2 which would otherwise buffer the protons released by the dissociation of lactic acid.

Although these mechanisms explain how the partial pressure of oxygen is raised to a modest extent in the rete by the changes in blood chemistry, they do not explain how very high partial pressures are achieved. Figure 4.15 shows how a countercurrent multiplication system operates. The parallel and unbranched arrangement of arterial and venous capillaries provides a maximum area for countercurrent diffusion. It is thought that transretial diffusion of lactate is enhanced by the endothelium of the capillaries being very thin. Multiplication of P_{O2} takes place across the rete as, with a continuous process

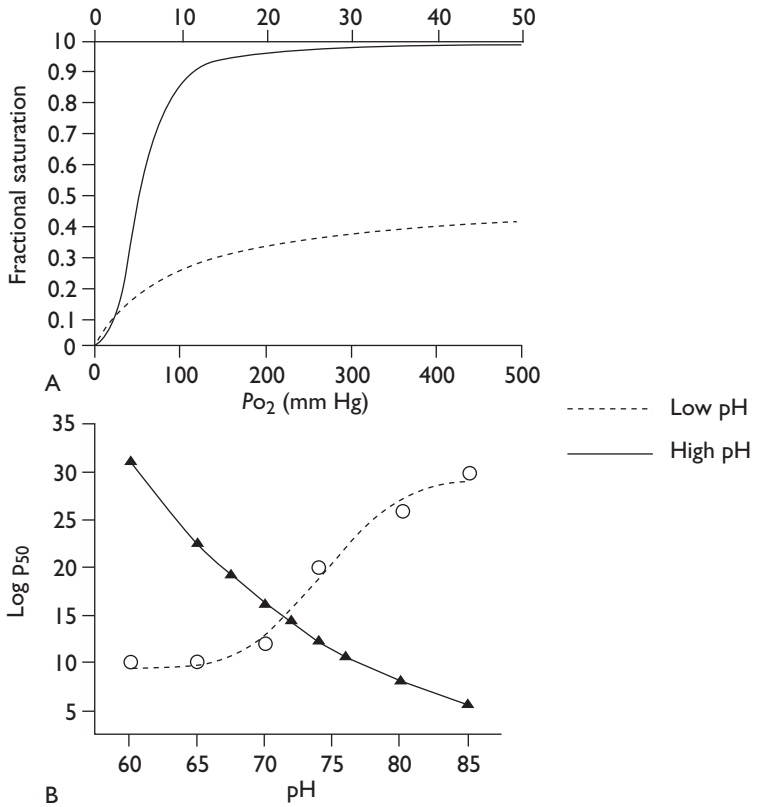

Figure 4.14 Root effect graph. Oxygen-binding curves for typical Root effect at low and high pH. Topscale O_2 concentration relates to binding curve at high pH. In the bottom graph the effect of pH on overall O_2 binding (δ) is seen. After Brittain (2005).

of lactate secretion, excess O_2 in the efferent capillaries will be added to O_2 already present from previous oxygen enrichment of the afferent capillaries.

It is not surprising that the length and complexity of the retial system increases with the problem of secreting gas against high concentration gradients. Other gases are released by salting-out. Nitrogen is secreted, but very slowly, and in some fish, for example the freshwater *Coregonus*, the main component of the gas is N_2. CO_2 is secreted and mopped up by bicarbonate but also diffuses out of the rete, or indeed through the swimbladder wall, very fast. The transfer of gas from the blood to the swimbladder lumen via the glandular cap may be enhanced by the presence of CO_2 which helps to initiate bubble formation, as do phospholipids which are secreted by the epithelium. Some part of the N_2 and of noble gases such as argon may pass into the bubbles of O_2 in the blood which are destined for the swimbladder. Experiments on eels show that CO_2 diffuses 40 times faster than O_2, and O_2 twice as fast as N_2. In physostomes, which swallow air, the swimbladder gas will have less than 21% oxygen (its proportion in air) unless the fish has recently visited the surface. The percentage of nitrogen may thus be used to calculate the time of the last visit. Diffusion of gases is reduced by the presence of guanine, the silvery pigment so characteristic

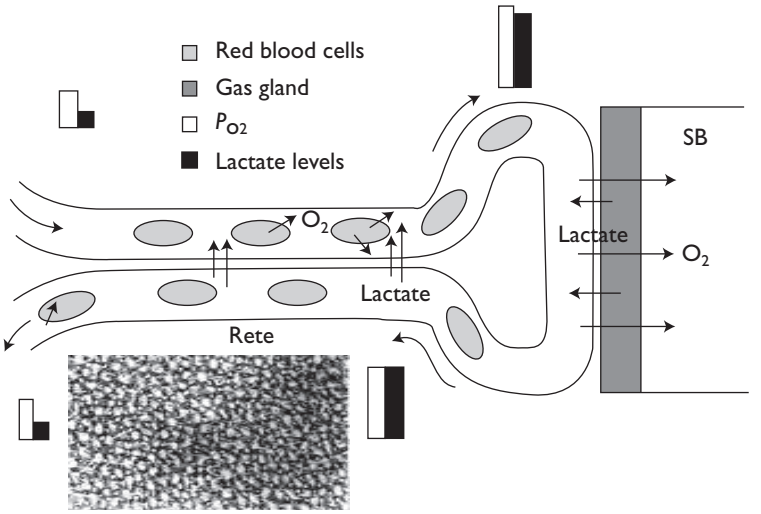

Figure 4.15 Diagram showing how the rete operates during gas secretion into swimbladder (SB). Inset shows part of the rete of *Conger* in transverse section (arterial capillaries smaller than venous).

of swimbladders, which is distributed in overlapping platelets that increase the path length for gases diffusing through the swimbladder wall. As gas is lost, the swimbladder contracts and the wall thickens, so increasing the path length for diffusion. The epithelium of the gas gland is also impervious to outward diffusion and acts as an additional barrier; it may cover as much as 50% of the internal surface of the swimbladder. Larval fish have little guanine in the swimbladder which has a high surface:volume ratio, both of which will increase gas loss. Frequent sampling of larval anchovy and menhaden at sea shows that they make daily visits to the surface to replenish their swimbladders (Figure 4.9).

4.5 The Swimbladder and Vertical Migration

If physoclists are placed under increased hydrostatic pressure they secrete gas into their swimbladders, conversely if the pressure is reduced they resorb gas. O_2 is the main component of the swimbladder gas of physoclists and, in general, the greater the depth, the higher the percentage of oxygen and the lower the percentage of nitrogen. Clearly, the O_2 has to be secreted into the swimbladder against the concentration gradient; P_{O2} may be several tens or even hundreds of atmospheres, compared with 0.2 atm in the tissues and possibly even less in the ambient water.

How fishes monitor the degree of distension in the swimbladder to know whether to secrete or absorb gas is unknown: stretch receptors have been reported in the swimbladder wall of roach (*Leuciscus rutilus*) and rudd (*Scardinius erythrophthalmus*) but have not been identified histologically: probably they are simple branching nerve endings. Experiments involving cardiac conditioning (p. 120) and observing spontaneous pressure changes show that fishes can respond to very small changes of pressure even without a

swimbladder (Table 4.1). Such pressure sensitivity is expressed as the percentage change perceived because this best expresses the degree of change of volume of the swimbladder. A threshold of 0.1% near the surface means that a fish can perceive a change of depth of 1 cm or at 90 m a depth change of 10 cm. The swimbladder can act as a depth gauge in the short term but in the long term it can never act as an absolute depth gauge because the gas secretion/resorption mechanisms act to retain neutral buoyancy. Fishes not only move up and down short distances as part of their normal daily routine of feeding and avoiding predators, they also make diel vertical migrations (p. 114), generally moving toward the surface at dusk and toward the bottom at dawn. Can such fish maintain neutral buoyancy during these migrations? Our guess that the answer is negative is almost certainly correct! Cod (*Gadus morrhua*) make diel vertical migrations, and can secrete gas at a sufficient rate to allow them to move down at about 1 m h^{-1} (dependent on the ambient temperature), or can resorb gas to move up at 2.4 m h^{-1} and still remain at neutral buoyancy (Table 4.2). Strand *et al.* (2005) used a set of vertical positions for an individual cod (obtained from a year-long data tag) to test the predictions of their buoyancy model. The conclusion was that cod would be negatively or neutrally buoyant, with the swimbladder being as much as 40% smaller than the optimum volume. The perch (*Perca fluviatilis*), a freshwater vertical migrant, requires 24 hours to adapt to an increase of pressure from 1 to 2 atm and about 9 hours to adapt to a decrease from 2 to 1 atm. In these species the speed of vertical migration far exceeds the possibility of remaining at neutral buoyancy during the ascent, let alone the descent. What almost certainly happens is that their buoyancy always lags behind the optimum. They may reach neutral buoyancy after a long spell near the surface during the night (remember that gas resorption is faster than secretion). As they move down at dawn to the daytime depth, they will become negatively buoyant and may not reach neutral buoyancy by the following dusk. This means that they can move up a greater distance without bursting the swimbladder than if they had reached neutral buoyancy at the daytime depth. Tanaka *et al.* (2002) monitored body attitude, depth, swimming speed, and tail beat

Table 4.1 Pressure sensitivity thresholds of various species determined experimentally and expressed as the minimum percentage pressure change perceived (from various sources summarized by Blaxter and Tytler, 1978)

Species	Threshold (%)	Experimental technique
Minnow	0.05–0.1	Operant conditioning
Minnow	0.5	Spontaneous behavior
Perch	0.1–0.2	Spontaneous behavior
Pinfish	0.02	Yawning behavior
Cod, saithe	0.5	Cardiac conditioning
Plaice*, dab*	1–2	Cardiac conditioning

*Flatfish with no swimbladders.

Table 4.2 Gas secretion and resorption rates (from various authors summarized by Blaxter and Tytler, 1978)

Species	Secretion rate*	Resorption rate*	Temperature (°C)
Physoclists			
Sunfish	1.36–1.60		12–32
Saithe	1.67–2.50	7.80	9–13
Cod	1.08–6.42	12–36**	0–15
Physostomes			
Eel	0.28	–	18–20
Goldfish	0.18–0.48	–	29

*Expressed in cm^3 (STP) kg^{-1} h^{-1}.

**Resorption rate pressure dependent, not temperature dependent.

frequency of free-swimming Pacific salmon (*Oncorhynchus keta*) as they returned to their home river using small data loggers. The loggers were later recovered from fishermen and scrutinized, showing that the salmon made considerable depth excursions in the water column and used more energy ascending than descending. Their observations clearly indicated that the salmon could not maintain neutral buoyancy at depth. Strand *et al.* (2004) have modeled swimbladder changes with respect to depth.

4.6 The Swimbladder as a Dynamic Organ: Its Other Functions

Because the swimbladder changes its volume (and no doubt sometimes its internal gas pressure) its other functions must be affected. The volume influences the resonance frequency and so its role both in hearing and in sound production (p. 297). The swimbladder returns the echoes from commercial echo-sounders, depending on its size and the attitude of the fish with respect to the echo-sounder. The "target strength" of fish changes as they move vertically, and this has all sorts of implications for the estimation of fish biomass by acoustic methods. It was not until a decade after it was first discovered, that Marshall (1951) correctly identified the swimbladders of deep-sea fish as the source of the mysterious deep scattering layer worldwide in the oceans.

Mass kills of freshwater teleosts resulting from accidental run-off of farm chemicals, have sadly made it obvious to almost all of us that dead teleosts float bottom up, and this is because the center of volume of the swimbladder is below the dense vertebral skeleton (Figure 1. 22), or in other words, the center of gravity lies below the center of buoyancy. You might suppose that this is a design fault, because for the fish to remain upright in the water, constant "trim" movements of the fins have to be made. However, this arrangement confers maneuverability. Stability in position without adjustments is exactly

what is *not* needed, just as in 1914 it was soon found that stability (although safer) was the last requirement for an effective fighter aircraft.

4.7 Other Sources of Static Lift

Because body fluids of marine teleosts are more dilute than seawater, they provide a small amount of lift, and in some deep-sea fishes, such as *Bathylagus*, there are fluid-filled spaces under the skin which increase the volume of lift from dilute fluid. The situation in elasmobranchs is somewhat different, because they contain urea and trimethylamine oxide. Withers *et al*. (1994) have pointed out that as well as being balancing osmolytes (Chapter 6) these organic solutes contribute static lift. They concluded from measurements on a black whaler shark *Furgaleus ventralis* that these compounds contributed some 8.45 g $l^{-1}$of static lift.

Envoi

Fish buoyancy is a satisfying topic, for not only are the mechanisms involved fairly well understood, but we can compare quite different solutions to the problem of achieving weightlessness in water. As we have come to expect with any kind of solution to problems in our own lives, each has snags as well as benefits. By no means are all fish designed to be neutrally buoyant, but those that are show adaptations astonishing in their ingenuity.

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5 Gas Exchange, Blood, and the Circulatory System

Fishes show an interesting diversity of approach to the problem of acquiring oxygen and transporting it to the tissues. Even though most fishes obtain oxygen from the water, they do so using gills (and sometimes other surfaces) of varied designs, causing water to flow over them in a variety of ways. There are also air-breathing species in no less than 70 freshwater genera; some of these have rather curious methods of aerial gas exchange, involving unexpected structures such as the body scales and the hindgut. As well as lungfish, many phyletically ancient freshwater fishes breathe air, such as *Amia*, *Megalops*, *Polypterus*, and *Lepisosteus*, testifying to its adaptive advantage in some freshwater environments which contain little oxygen or are at risk of drying up.

Compared with air, water contains relatively little oxygen. At the sea surface, for example, air-saturated water at 20°C contains only around 3% of the oxygen in the same volume of air. Since oxygen only crosses cell surfaces by diffusion, this means that the gas exchanger to acquire oxygen from the water must have a large area. In the active menhaden (*Brevoortia*), for example, the gill area is over 18 times that of the body surface excluding the fins. Also, because water is dense and viscous, quite a large expenditure of energy is needed to force water to flow around the gas exchanger. Unlike most terrestrial animals (including ourselves), which breathe air in and out of a lung in tidal fashion, water flow over the fish gill is almost invariably unidirectional, whether it is pumped over the gills or simply flows over the gill via the open mouth as the fish swims forward.

Adult lampreys are the only fishes where flow over the gills is tidal. We can easily see why unidirectional flow of water is advantageous, for it means that the blood flow in the gill can be arranged in the opposite direction. If blood flow through the gas exchanger was in the same direction as the water flow, the maximum partial pressure (P_{O2}) of oxygen in the blood of the exchanger would be the same as that in the exhalent water stream. On the other hand, if the blood flows in the opposite direction to the water, the maximum P_{O2} of the blood leaving the exchanger could be very close to that of the incoming (ambient) water, and above that of the exhaled water. Similar counter current systems (between arterial and venous blood vessels) in the muscles of warm fish and in the swimbladder rete are described in Chapters 3 and 4. In

gnathostome fishes, 70–80% of the oxygen in the water flowing into the gill chambers can be extracted, a remarkable degree of efficiency.

Fish obtaining oxygen from the water have to surmount a real problem. The blood in the gas exchanger is almost always very different in osmolarity from the ambient water, and always different in ion content, but it must be in intimate contact with the water to make the diffusion pathway short. So we should expect to find special arrangements to circumvent (or at least to cope with) water and ion fluxes across the exchanger. Some possible mechanisms will be considered later in this chapter on page 133. An excellent review of the complex multi-functional nature of the fish gill has recently been provided by Evans *et al.* (2005).

5.1 The Origin of Respiratory Gills

In ascidian and doliolid tunicates, and in amphioxus, the gills are ciliated food-collecting devices, trapping particles on mucous nets produced by the endostyle; blood flowing through them is probably de-oxygenated since the ciliary tracts of the gill bars must use more oxygen than is provided by the water flowing through them. The respiratory gills of larger and more complex chordates are likely to have been derived from a filtering arrangement of the kind seen in amphioxus, the significant step being the change from ciliary to muscular movement of water through the gill. This change, which led to the arrangement seen today in the lamprey ammocoete larva, presumably came about as a consequence of the demand for a higher filtering rate than cilia alone could provide. When this more efficient filtering system allowed increase in body size beyond that where simple diffusion across epithelial surfaces sufficed for gas exchange, respiratory gills became specialized. In lampreys, a significant proportion of the oxygen needed is still gained across the skin, despite the development of respiratory gills. Cutaneous respiration is the only source of oxygen for many larval fishes, and is important for some adult teleosts, for example, the Antarctic icefishes lacking hemoglobin (see Section 5.6).

5.2 Respiration of Fish Larvae

Elasmobranchs, and teleosts with large eggs, such as salmon, hatch with functional gills, a well-developed circulatory system, and blood cells containing hemoglobin. Most teleosts, however, hatch as much smaller larvae depending on cutaneous respiration across the body surface. Since many of these small transparent larvae live a pelagic existence, where oxygen is plentiful, cutaneous respiration suffices, so hemoglobin is not needed and might make them conspicuous to predators. The leptocephalus larvae of elopomorph teleosts, for example, may be quite surprisingly large (some notoacanth leptocephali are 2 m long) but all are laterally compressed so that diffusion distances are small), and, lacking hemoglobin, are exceptionally transparent.

As larvae increase in size, two changes take place that affect respiration profoundly: the surface-to-volume ratio becomes smaller, so that the surface for cutaneous respiration becomes relatively smaller, and the pathways for the diffusion of gases and metabolites become longer (Figure 5.1). A size is reached when gill respiration (vastly increasing the area for diffusion)

becomes essential, especially in very active fishes. The development of hemoglobin about the same time, or a little later, increases the oxygen-carrying capacity of the blood. The chemical form of hemoglobin then changes with age, as judged by electrophoretic banding patterns.

Larvae living in hypoxic environments may generate convective water flow along the body, for example in the lungfish *Neoceratodus forsteri* by means of cilia or by movement of the pectoral fins in *Monopterus albus* (Figure 5.2). In

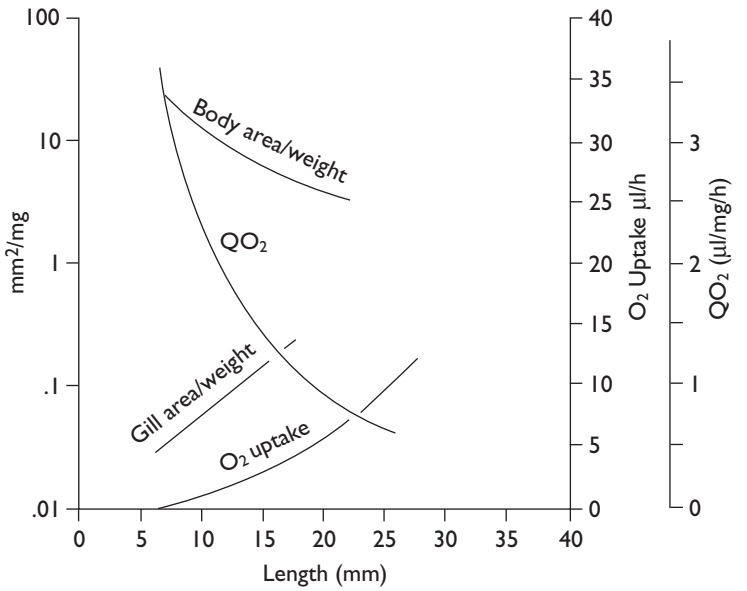


Figure 5.1 Graphs showing changes in the respiratory characteristics during the development of a plaice larva. As the body area falls in relation to body weight, the cutaneous area for respiration also falls but gill area per unit weight increases. Although the total oxygen uptake increases with length, as would be expected, the QO_2 (oxygen uptake per unit weight) falls, a phenomenon found in all animals.

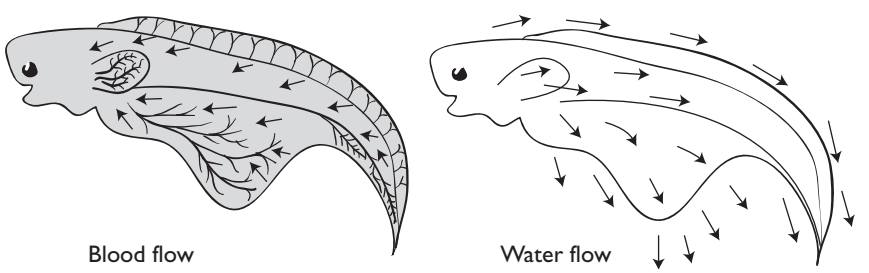


Figure 5.2 Countercurrent water and blood flow in the larva of the freshwater symbranchiform teleost *Monopterus*. The larvae live in oxygen-poor water and use the whole body surface to gain oxygen; the current of water flowing over the body surface produced by the movement of the pectoral fins, is countercurrent to skin blood flow. After Liem (1981).

Atlantic salmon alevins, pectoral fin movements seem to draw water over the gills. Alternatively, they may undertake regular short bouts of swimming to move them out of the water they have de-oxygenated.

5.3 Respiration in Hagfish, and Lampreys

Hagfish

In hagfishes, unidirectional water flow through the serial muscular gill pouches is chiefly brought about by rolling and unrolling of velar folds (Figure 5.3). These lie in a chamber developed from the naso-hypophyseal tract and are operated by a complex set of muscles inserting onto cartilages of the neurocranium. Peristaltic contractions of the gill pouches and their ducts assist in producing the flow. In *Myxine*, the gill pouches open by a common duct, while in *Eptatretus* 5–16 gill pouches (according to species) open directly to the exterior. Since hagfishes feed half-buried in their prey, and since they survive well after the nostrils have been blocked to interrupt gill ventilation, it is clear that cutaneous respiration is important. Indeed, this is indicated by the vast subcutaneous blood sinuses found in hagfishes; the skin is like a loosely fitting sock and if one holds up a living hagfish blood at once flows down to swell the lower end. Blood volume at 180 ml kg^{-1} is over twice that of gnathostome fishes, and much greater than that of lampreys. Ingenious measurements of resting oxygen consumption by hagfish on the sea bed at 1230 m, using a respirometer mounted on a remotely controlled vehicle, gave average values of only 3.1 μg g^{-1} h^{-1}. So cutaneous respiration may well suffice for hagfishes at rest, in *Myxine* the water pumped by the velar folds bypassing the gills.

Lampreys

In the ammocoete larva (Figure 5.4), the pharynx is undivided, and, unlike the adult, the larva filter-feeds and respires from the same unidirectional inhalent water flow. This is driven partly by the action of the anterior muscular velum,

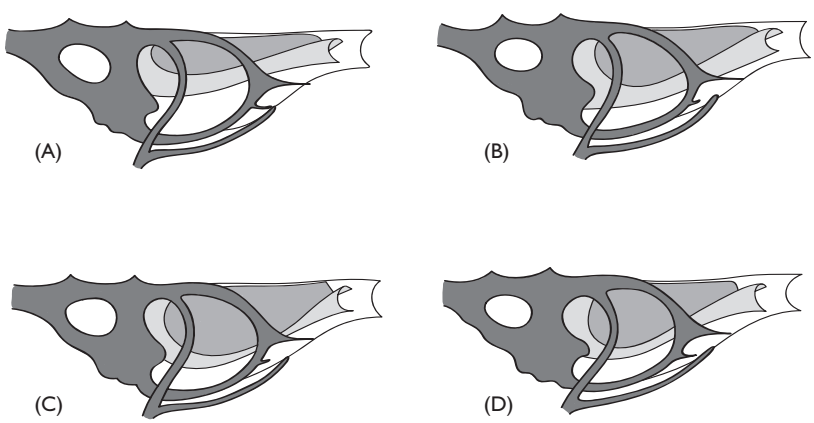


Figure 5.3 Velar folds of the hagfish *Myxine* showing stages (A–D) in the pumping cycle. Cranial cartilages (dark shading), inner side of velum (light shading). After Strahan (1963).

and partly by contractions of the branchial basket brought about by gill muscles. The branchial basket is a continuous cartilaginous meshwork, rather than being jointed, and expands by its elasticity. Valves at the entrance and exit of the gill pouches ensure unidirectional flow during the rhythmic movements of the branchial basket. The possibility for countercurrent flow exists in the ammocoete gill, but this has not yet been demonstrated experimentally, although oxygen extraction rates are about double those of adults where the water flow is tidal.

In the adult, where the mouth and sucker are involved in feeding, the velum is not involved in pumping water (it remains after metamorphosis as a small flap valve), and contraction and expansion of the branchial skeleton pumps water in and out of the gill pouches. The direction of flow is controlled by valves, and is tidal. It obviously has to be tidal when the lamprey is feeding, or moving pebbles with its sucker as it makes its redd to spawn, but it always seems to be tidal even if the lamprey is free-swimming and could inhale through the mouth.

We should expect, therefore, that oxygen extraction would be relatively inefficient in adult lampreys, and, instead of achieving gnathostome fish values, *Entosphenus* can only manage to extract between 10 and 28% of the oxygen in the inhaled water. Another way of looking at respiratory efficiency is to consider it as the ratio between the amount of oxygen acquired at the gas exchanger available for metabolic purposes, and that used by the respiratory muscles themselves.

Lampreys have a relatively large amount of branchial musculature, so here again, respiratory efficiency seems likely to be low.

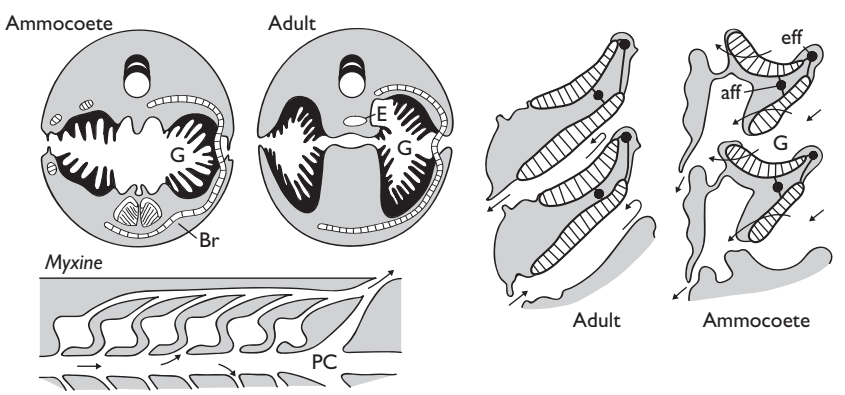


Figure 5.4 The arrangement of the gills in hagfish and lampreys. Upper left: transverse sections of branchial region of ammocoete and adult lamprey. Right: horizontal sections of left side of branchial region in adult and ammocoete, showing tidal water flow in the adult gill sac. Br: branchial skeleton; G: gill sac; N: notochord; E: esophagus. In the ammocoete, the efferent (eff) and afferent (aff) vessels are arranged to permit counter current flow. Bottom left: horizontal section of right gill sacs in *Myxine* (anterior to left) showing pharyngo-cutaneous duct (PC) and common outflow. After Alcock (1898), Sterba (1966) and Goodrich (1909).

5.4 Gnathostome Fishes

Gill design

Gill structure is essentially similar in all gnathostome fishes. Certainly elasmobranch gills differ from most teleost gills in the way that the gill filaments remain attached along their length (hence their name, see Chapter 1), but the basic design is the same. There are usually four branchial arches bearing gills in teleosts (the number is reduced in air-breathing fishes such as *Anabas* or *Amphipnous*), but in elasmobranchiomorpha and chondrosteans the hyoid arch bears a posterior respiratory hemibranch, and there are thus usually five gill-bearing arches in sharks, and up to seven in the shark *Heptranchias*. Each arch bears a series of regular comb-like gill filaments, supported by skeletal gill bars, and on each of these there are closely ranged primary gill lamellae. These in turn bear a number of stacks of smaller secondary lamellae set parallel to the water flow through the branchial chamber: these are the sites of gas exchange. Figures 5.5 and 5.6 show the arrangement in elasmobranchs and teleosts, and the way water flows through the gills.

The numbers and dimensions of the secondary lamellae vary between different species according to their activity; typically, in a fish weighing 1 kg there may be up to 18 000 cm^2 of secondary lamellae, and in very active fishes

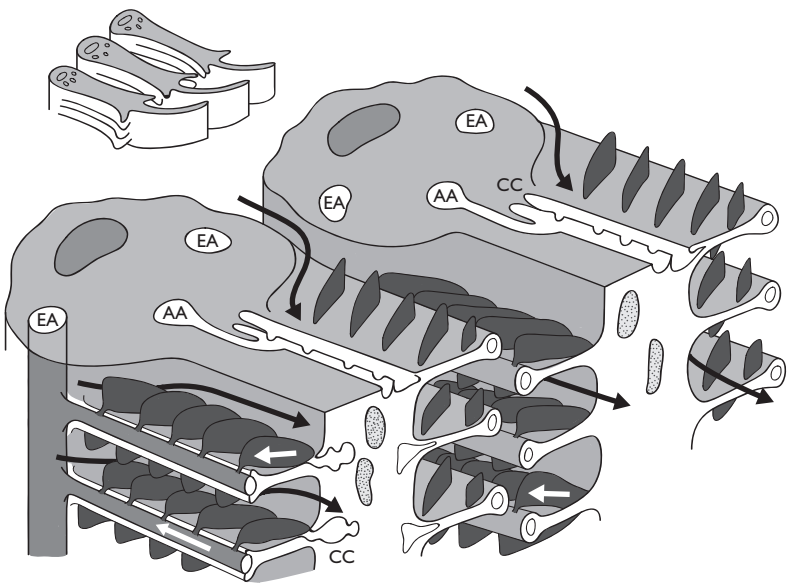


Figure 5.5 Design of the elasmobranch gill as seen in the dogfish *Scyliorhinus*. Upper left: general view of three gills and their flaps. Main diagram: part of two adjacent gill arches showing alternation of secondary lamellae (dark stipple) on adjacent gill filaments. Afferent blood from the afferent branchial arteries (AA) passes along afferent arterioles to the corpora cavernosa (CC) and thence to the secondary lamellae and to the efferent branchial artery (EA). Note that the direction of water flow (black arrows) is counter to the flow of blood in the secondary lamellae. After Wright (1973).

such as tunas, there may be more than 5 million secondary lamellae. This huge area is needed partly because the oxygen content of water is low, and partly because rates of oxygen diffusion are relatively low in animal tissues. In connective tissue, for example, the oxygen diffusion rate is only 10^{-6} that in

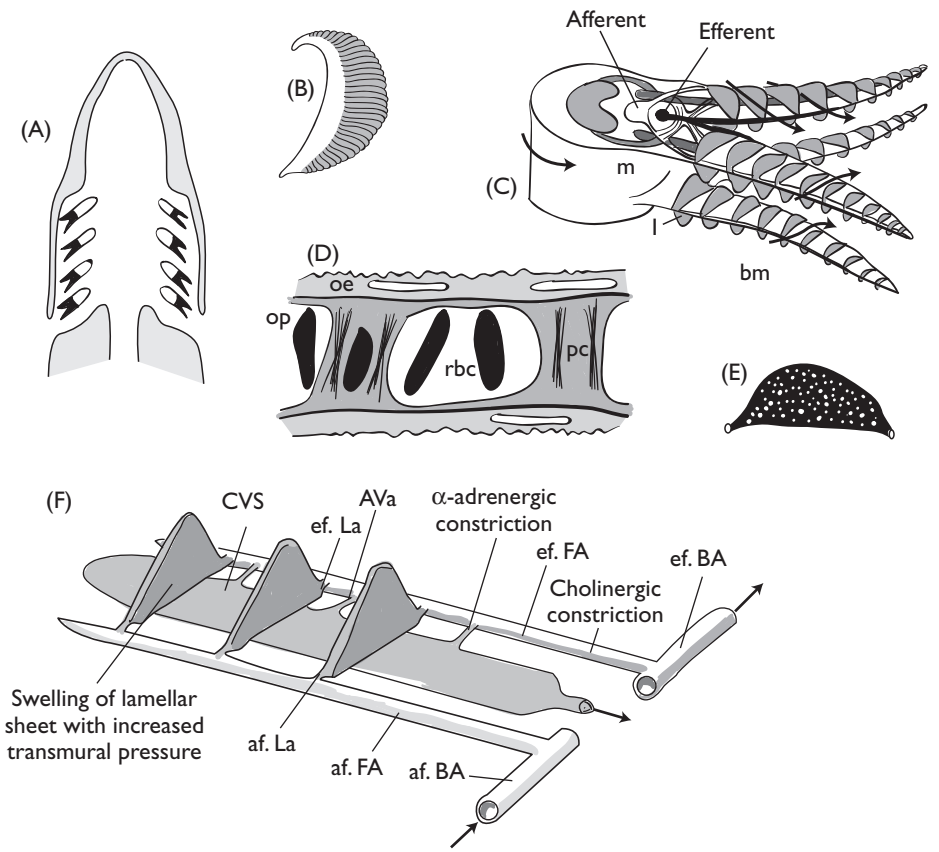


Figure 5.6 Design of the teleost gill. (A) Horizontal section showing disposition of gill filaments (black) on gill arches; (B) single hemibranch; (C) arrangement of gill filaments with secondary lamellae (light stipple) supplied by efferent and afferent vessels. Water flow (arrows) is *countercurrent* to blood flow within the lamellae. The bony gill arch and gill rays (dark stipple) are linked by intrinsic muscles (m) which can change the apposition of the gill filaments (unlike the elasmobranch gill design seen in Figure 5.5); (D) section across secondary lamella showing pillar cells (pc), red blood cells (rbc) in blood space, and basement membrane (bm) separating the pillar cells from the outer epithelium (oe) which has spaces linked to the secondary circulation; (E) cast of vascular spaces in secondary lamella, the white dots are where pillar cells interrupt the space; (F) semi-schematic diagram showing major vascular paths in teleost gill filament, and some of their regulating mechanisms. Decrease in gill vascular resistance with β-adrenergic vasodilation may result from relaxation of either (or both) of the sphincters to and from the lamellae (ef.La and af.LA). af.BA and ef.BA: afferent and efferent filament arteries; AVa: arteriovenous anastamoses; CVS: central venous sinus. From Munshi and Singh (1968); Hughes and Grimstone (1965); and Olson (1996).

air. It is no surprise, then, that the secondary lamellae have very thin walls to minimize the diffusion distance between the water and the blood within them. In fact, they are essentially thin-walled sacs filled with blood flowing around the pillar cell posts that separate the lamellar walls (Figure 5.6E). Blood in the secondary lamella therefore flows through an interrupted sinus, rather than through capillaries. In some fishes, such as tunas and the bowfin *Amia*, the pillar cells are not distributed polygonally but in discrete rows, so that, although the secondary lamella is a sinus, the pillar cell array effectively divides the interior into a series of parallel channels. As we have seen earlier, to enable efficient oxygen extraction, the flow within the secondary lamellae is countercurrent to that of the water passing them, and the tuna arrangement ensures that the flow of blood is exactly parallel to the water flow.

The lining of the sinus is formed by flanges from the pillar cells, so that, to reach the blood in the lamella, oxygen in the water has first to pass across an epithelial cell layer, then across the basement membrane of the epithelial cells, and, lastly, across the pillar cell flanges lining the lamella. This diffusion barrier differs in thickness in different fishes, and, as we might expect, it is thinnest in those fish requiring the greatest rate of oxygen uptake (Table 5.1). Apart from the specialized pillar cells (which are already present in hagfish and lamprey gills), the arrangement is rather similar to that in lungfish or tetrapod lungs, but there are complications arising from the intimate proximity of the blood to the water flowing by the secondary lamellae.

The large area of the gas exchanger, and the difference in composition between the blood and the water, mean that the fish possesses a structure that will inevitably not only act as a gas exchanger, but also as an efficient heat, ion, and water exchanger. Thermal diffusion is much more rapid than gaseous diffusion, and so fishes can only retain metabolic heat by organizing special countercurrent heat exchangers near the organs that are to be kept warm (Chapter 3).

Readers who have indulged in ice-fishing will immediately ponder how fish manage to cope with gas exchange when the ambient water is close to or

Table 5.1 Diffusion distances between water and blood in the secondary lamellae of different fishes. Distances in μm. Note minimum distances in the active pelagic skipjack and mackerel, as compared with the (other) benthic fishes. From Hughes and Morgan (1973)

Fish	Epithelium	Basement membrane	Pillar cell flange	Total water–blood (mean)
Dogfish (*S. canicula*)	2.38–18.48	0.3–0.95	0.37–0.71	11.27
Squalus	3.0–22.5	0.3–0.6	0.12–0.6	10.14
Raja clavata	0.5–11.5	0.13–0.63	0.03–1.13	5.99
Microstomus kitt	0.21–16.7	0.1–0.69	0.1–0.13	3.23
Skipjack (*Katsuwonus*)	0.013–0.625	0.075–1.875	0.017–0.375	0.598
Mackerel (*S. scombrus*)	0.165–1.875	0.066–1.0	0.033–1.75	1.215

below the colligative freezing point of their blood and tissues. This topic is considered in "Anti-freeze proteins," later in this chapter.

What about osmotic and ion exchange? The mechanisms for ion and water exchange in the gills are considered in Chapter 6; here we are concerned with the possibility that ion and water exchange might be minimized when oxygen demand is low, by avoiding as far as possible the intimate blood/water contact in the gills, that is by reducing functional gill area.

Functional gill area

In principle, functional gill area could be reduced without much difficulty by re-routing blood flow in the gills to non-lamellar pathways, by reducing and re-routing flow within the secondary lamellae themselves and, by altering the water flow past them. Such mechanisms would seem sensible, since they would avoid the cost of running the branchial ion and water pumps at maximum levels when oxygen demand is low, that is when the fish is at rest or swimming very slowly. What evidence is there for changes in functional gill area, and how significant might these changes be? Exercise (i.e. increased oxygen demand) in trout is followed by increased urine production to get rid of the increased entry of water across the gills, and, in lampreys, activity is well correlated with urine production. This certainly suggests that functional gill area is related to oxygen demand, but other explanations are possible. In fishes such as the eel or dogfish (*Scyliorhinus*) there are anatomical connections between the efferent and afferent arteries in the gill filaments, and other links between the afferent arteries and the central venous space of the gill filaments. Thus, in principle, some proportion of the blood could be shunted via these links to a "non-respiratory" route, bypassing the "respiratory" route through the secondary lamellae when oxygen demand is low. But considerations of the dimensions of these links, and calculations of the pressures in the different vascular spaces, have made it unclear whether fishes actually have such a switchable double circulation in the gills. However, direct evidence that, in eels at least, this is the case, was provided by ingenious experiments (Figure 5.7) where cardiac output (O) was measured directly by a flowmeter in an external extension fitted to the ventral aorta, and compared to the output calculated by the Fick principle: $Qr = Vo_2 / (Cao_2 - Cvo_2)$ that is cardiac output (Qp) = oxygen uptake at the gills (Vo_2) divided by oxygen content difference between arterial and venous mixed blood ($Cao_2 - Cvo_2$). The oxygen uptake at the gills was calculated from measurements of ventilatory water flow and the inspired–expired Po_2 difference. The result of this experiment was that $Qp/Q = 0.72$, indicating that, in the resting eel, about 30% of the mixed venous blood afferent to the gills returns directly to the heart, bypassing the lamellar "respiratory" route. After injection of adrenaline, Qp/Q changed to near unity, so it seems that the vascular shunts of the eel gill have adrenergic sphincters, and that circulating catecholamines such as adrenaline will fully "open" the "respiratory" route as the sphincters are closed. In eels, injection of adrenaline increases arterial P_{O2}, as expected. Figure 5.8 gives some idea of the complexity of the control systems in the gills of teleosts. However, it is not yet clear that this result applies to all fishes. An interesting and rather different possibility for changing the functional surface area of the secondary lamellae is raised by the structure of the pillar cells in the lamellae themselves. These have a ring of connective-tissue

supporting columns (Figure 5.9), but also contain arrays of myosin and actin filaments around them (Mistry *et al.*, 2004). The sinuous shapes of the connective-tissue columns in electron micrographs of fixed gills suggest that pillar cells are contractile, shortening to reduce or re-route blood flow through the lamellae, as first suggested in 1895. Recent work by Stenslokken *et al.* (2006) has shown directly that the pillar cell columns contract under the influence of endothelin B, (so increasing gill vascular resistance) as do the linings of small arterioles and that the pillar cells bear endothelin receptors. They seem to form part of an auto-regulative system in the lamellae, responding to rapid increases of blood pressure by increasing tonus to prevent lamellar swelling and rupture. Their cytoplasm contains the enzyme carbonic anhydrase in quantity; hence they are perhaps concerned in CO_2 excretion (see p. 156).

In most teleosts, but not in elasmobranchs, intrinsic muscles in the gill filaments can change the angles adopted by the gill filaments on each arch, and so alter the ventilation pattern of the secondary lamellae, but (as might be expected) it is hard to discover whether such changes in gill geometry are significant in changing functional gill area in normal fishes. We have to conclude

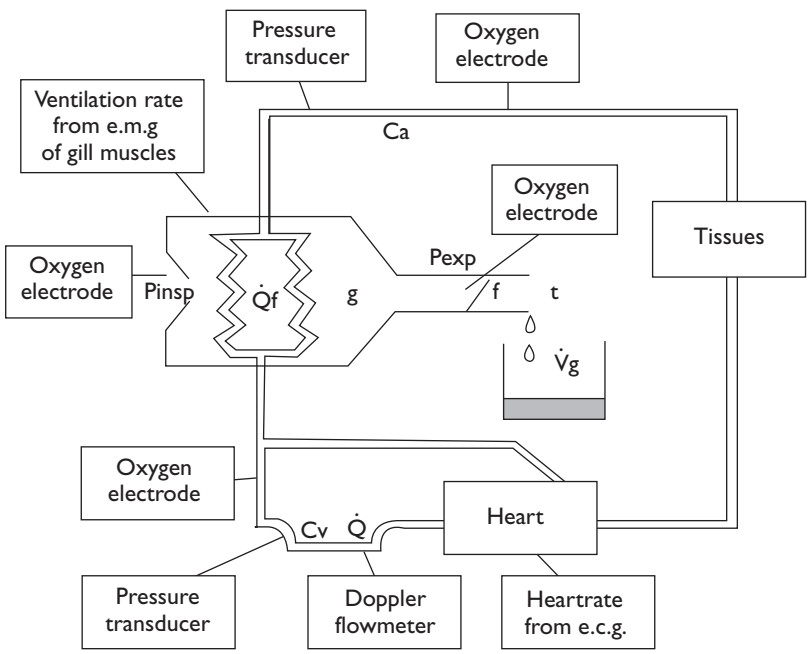


Figure 5.7 Diagrammatic scheme of the complex experimental arrangement required to study respiratory "shunting" in the eel *Anguilla*. Water flowing over the gills (g) was collected from tubes (t) in the round opercular openings, which were provided with rubber flaps (f) to mimic the normal opercular valves. The ventral aortic circulation was extended outside the body to permit blood flow velocity from the heart to be measured accurately by a Doppler flowmeter. Heart rate was monitored by ECG electrodes, and other electrodes near the opercular monitored ventilation frequency. Pressure transducers monitored dorsal and ventral aorta blood pressures, and oxygen electrodes Po_2 in these vessels and in the inspired and expired water flowing over the gills. After Hughes *et al.* (1982).

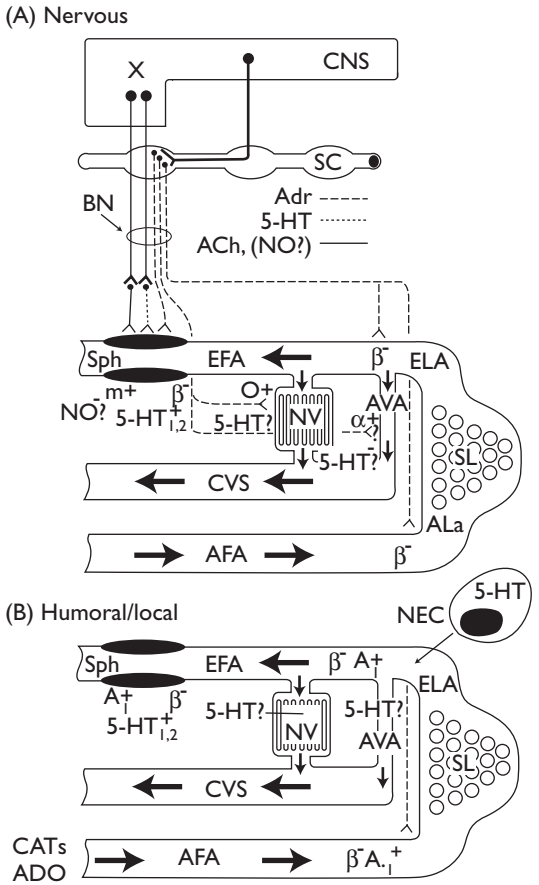


Figure 5.8 The complexities of nervous control of the teleost gill blood pathways. Nilsson and Sundin (1998).

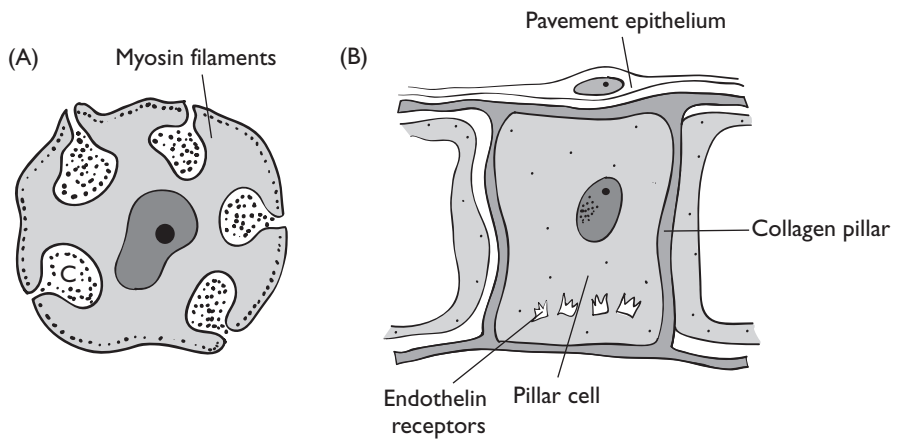


Figure 5.9 Pillar cell structure. (A) Transverse section of pillar cell showing infolded collagen pillars; (B) vertical section of pillar cell with two pillars in section. After Laurent (1984), and Stenslokken *et al.* (2006).

that although there is evidence for several kinds of mechanism that could change the functional area of fish gills in response to oxygen demand, it is still not known how these are interrelated, or, indeed, how important they may be in the life of the fish. We have considered them in relation to possible limitation of ionic and osmotic exchange, and a significant part of the energy budget of the fish has to be devoted to the operation of pumps to cope with the exchange problem (Chapter 6). But fish gills offer a significant resistance to blood flow, and the changes in blood and water flow pathways in the gills we have been considering as mechanisms for reducing ion and water exchange, may be equally important in reducing the load on the heart when oxygen demand is low.

Branchial pumps

In both teleost and elasmobranchs, the gills are ventilated by water driven across the gill chamber by double pumps: a pressure pump upstream to the gill resistance, and a suction pump downstream. In fishes which swim continually, such as scombroids or lamnid sharks, the pumps exist but they are not in use above a certain swimming speed, since forward motion provides sufficient ram gill ventilation. It is, of course, difficult to see how these pumps operate directly (although one can see their result as water is expelled from the branchial chamber), but by a combination of pressure records from strategically-placed cannulae, strain gauge records of the movements of mouth and gill openings, and emg's (Chapter 3) from different muscles, it is possible to glean a fairly complete picture of the way that the pumps operate. With these techniques, Hughes and his colleagues (see, e.g., Hughes and Ballantijn, 1965) have examined various teleosts and elasmobranchs; their work on dogfish is an example of the approach. Figure 5.10 shows the main muscles and skeletal structures involved, while Figure 5.11 shows the relationships of the pressures measured during the three phases of the cycle, with the activity of the different muscles. First, when most of the respiratory muscles are active, the volume of the orobranchial chamber is reduced, and pressure within it rises. After an

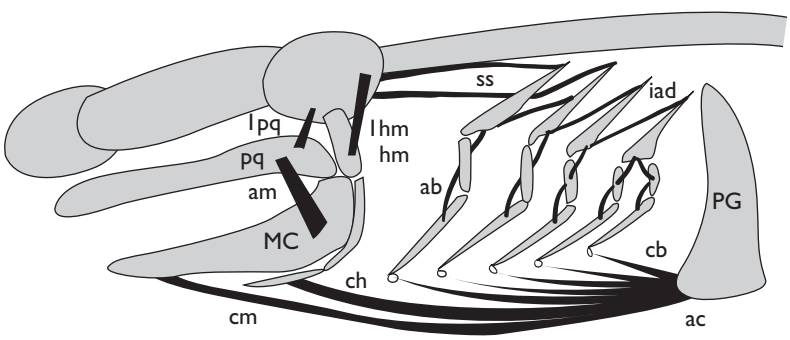


Figure 5.10 The skeletal structures and muscles involved in the branchial pumps of *Scyliorhinus*. ab: adductor branchialis; am: adductor mandibulae; ch: coraco-hyoideus; cm: coraco-mandibularis; hm: hyomandibular; iad: interarcualis dorsalis; lhm: levator palatoquadrati; MC: Meckel's cartilage; PG: pectoral girdle; pq: palatoquadrate. After Hughes and Ballantijn (1965).

initial increase (as water flows out of the orobranchial cavity) the parabranchial cavities also decrease in volume, and pressure there also rises. Next, pressure within the orobranchial cavity drops as it passively expands, owing to the elasticity of the skeletal and ligamentous elements compressed during inspiration; no muscles are active in this phase. Finally, there is a short pause, before the cycle begins again, and this may be preceded by a more rapid expansion of the orobranchial cavity at the end of the second phase, during which the hypobranchial musculature may be active.

As we should expect from the anatomical arrangements shown diagrammatically in Figure 5.10, there are many interactions between the different parts of the system, and the two pumps are not completely separate. For example, the muscles driving the orobranchial pump also affect the parabranchial pump. Figure 5.11 illustrates diagrammatically a model incorporating some of these interactions. The most important feature is that, by using the interaction between an upstream pressure pump and a downstream suction pump, the dogfish can maintain unidirectional flow over the gills to enable countercurrent water and blood flow in the secondary lamellae.

In teleosts, this dual pump is essentially similar to that in dogfish, but since the skin is stiffer and there is normally a rigid operculum, coupling of the two pumps is closer than in dogfish and both expansion and contraction phases of the pumps are active. The relative contributions of the pressure and suction pumps to gill ventilation differ in different teleosts. In bottom-living teleosts such as plaice and sole the opercular suction pump is most important. Increased gill ventilation when ambient Po_2 falls or when oxygen demand rises can be brought about by increasing cycle frequency or by increasing the stroke volume for each cycle; fishes may do either or both. In trout, for example, cycle

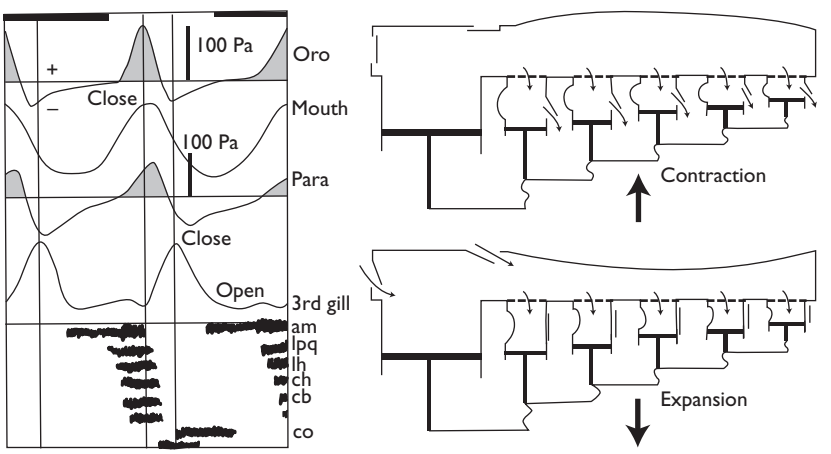


Figure 5.11 The branchial pumps of *Scyliorhinus*. Left: upper: orobranchial (oro) and parabranchial (para) pressures (above ambient stippled) and the movements of the mouth and third gill slit. Below: the periods of activity (EMG records) of the different muscles driving the pumps during the respirator cycle. Right: model of the branchial pumps in expiratory (upper) and intake phases. After Hughes and Ballantijn (1965).

frequency alters little, but the volume of water pumped may increase up to five times. An interesting puzzle is provided by the way that the respiratory muscles are controlled, for, although it is known physiologically (in trout) that receptors detecting low ambient P_{O2} are located on the dorsal part of the first gill arch, P_{O2} receptors are also present in the pseudobranch. Neither has been identified histologically, nor have the length and tension receptors been seen, known by physiological experiment to be associated with the muscles themselves.

Many fishes cease respiratory pumping as soon as they are swimming fast enough to ventilate the gills simply by keeping their mouths open, allowing water to flow into the gill chambers. The change takes place when the pressure difference across the gills reaches 2 kPa. Some, like the larger fast-cruising scombroids, can only respire by this ram-jet method, and in skipjack tuna (*Katsuwonus*) the dynamic pressure difference required is around 80 Pa. This is similar to the pressures (50–100 Pa) developed by resting fishes using the branchial pumps to ventilate the gills, but ram-jet ventilation is less energetically costly. For example, a striped bass (*Morone saxatilis*) swimming in a respirometer at 30 cm s^{-1} (1.35 body lengths s^{-1}) used 322 mg O_2 h^{-1}, and used its branchial pumps to respire. At 55 cm s^{-1} (2.47 body lengths s^{-1}) when it had switched to ram ventilation, it used 360 mg O_2 h^{-1}. Although swimming speed had increased over 80%, the O_2 used only increased by just under 12%! Estimates for the cost of ram ventilation (where the fish does not have to accelerate and decelerate volumes of dense water) vs. branchial pumping suggest that ram ventilation requires about 9% of the total energy budget, whereas branchial pumping requires nearly double this, about 15%. However, these estimates did not take into account a second advantage of ram ventilation. Not only does it avoid the cost of branchial pumping, but the steady exit of water from the opercula during ram ventilation also helps to maintain the boundary layer (Chapter 3), producing a better flow regime with less drag. It is partly this additional advantage that accounts for the unexpectedly small increase in oxygen consumption as the bass were forced to swim more rapidly. In the special case of remoras (echeneids) which live attached by the dorsal sucker to larger fishes, ram ventilation is free! Obviously, when using ram ventilation, a greater flow rate over the gills can easily be obtained by increasing mouth gape, but this will greatly increase drag. Probably pseudobranch baroreceptors monitor the entry pressure over the gills in order to keep mouth gape as small as possible at different swimming speeds.

Tuna and swordfish gills do not look like those shown in Figure 5.6, because the gill filaments are linked by a series of bridges and the filaments may be fused at their edges (Figure 5.12). A somewhat similar design is seen in the holostean bowfin *Amia. Amia* is a lurking predator in freshwaters of north-east America. It would hardly be possible to think of a fish more different in taxonomic position and lifestyle from tunas and swordfish! Tuna and swordfish gills often show damaged and regenerating areas, and it seems clear that the fused design here is to strengthen the gill sieve against damage by floating objects in the rapid inhalent flow. In the sluggish air-breathing *Amia*, on the other hand, fusion may have evolved to keep the gill sieve patent in air to assist the swimbladder in gas exchange.

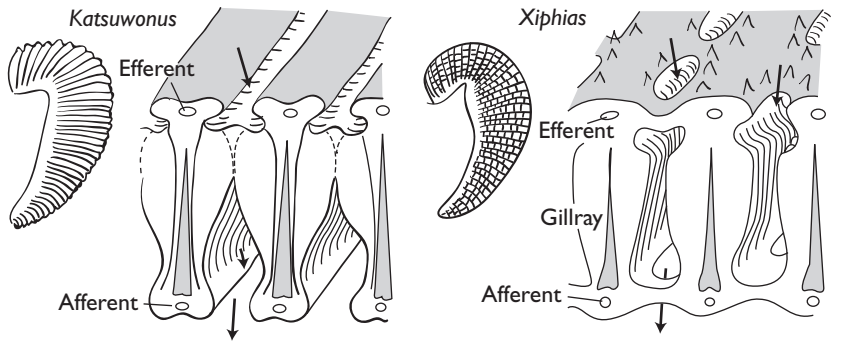


Figure 5.12 The gill filaments of two fast swimming ram-ventilating teleosts, the skipjack tuna (*Katsuwonus*; left), and the swordfish (*Xiphias*; right). Arrows show water flow. Hemibranchs of first gill arches inset. After Muir and Kendall (1968).

5.5 Air-breathing Fishes

Lungs and gills

Amia is unusual in being an air-breather living in temperate waters, for most of the extremely fascinating variety of air-breathing fishes live in tropical swamps, where a combination of stagnant water, high temperature, and abundant microorganisms make the water very acid, with high Pco_2 and low P_{O2}. These unfavorable conditions for aquatic respiration have led to extraordinary adaptations for acquiring oxygen, also found in fishes which normally live out of the water. In tropical swamps, some fish manage by ventilating the gills with water from just below the surface, where it is oxygenated, but most have to use accessory respiratory organs of various kinds. These are essentially hollow spaces with richly vascularized walls, which can be ventilated periodically. Many air-breathing fishes use the swimbladder as a lung, as well as for buoyancy, and sometimes for sound production (Chapters 4 and 10), and its surface area is much increased by septation (see Figure 5.17 below).

Polypterus and the African (*Protopterus*) and South American (*Lepidosiren*) lungfishes all have single or double "lungs," opening ventrally to the pharynx (Figure 5.13) they are obligate air-breathers and drown if denied access to the surface. The Australian lungfish (*Neoceratodus*) only breathes air if stressed, and has a single "lung" much less septated than those of other lungfishes. With the exception of the tarpon *Megalops*, a very active euryhaline tropical fish whose juveniles live in fresh and brackish water, all teleosts with respiratory swimbladders live in fresh water. Remarkable modifications are found in osteoglossomorphs such as the obligate air-breather *Pantodon*, where extensions of the swimbladder penetrate the transverse processes of the vertebrae and in drums (sciaenids) where there are varied finger-like extensions (Figure 5.14). Air is exchanged in such respiratory swimbladders and lungs by a variety of methods. The most curious is that in *Polypterus* where the elastic recoil of the scales deformed by the decrease in lung volume caused by intrinsic muscles, provides positive "recoil aspiration." Another hollow space commonly used by air-breathing fishes is the gill chamber itself where there are

often accessory respiratory organs, as in the climbing perch (*Anabas*) or the walking catfish (C*larias batrachus*), which ventilates its respiratory trees in the gill chambers during synchronized trips to the surface. The water boils with a mass of fishes for a few moments, and then is undisturbed until the catfish take their next gulps of air, a strategy which apparently confuses predators. Oddly, although at least six of the 31 catfish families breathe air, only the obligate air-breather *Pangasias* uses the swimbladder; other catfishes use the stomach, intestine, or (like *Clarias*) the opercular chambers. In other fish, other regions of the gut are used to absorb oxygen, for example, the loach *Misgurnus* uses the rectum, and the Alaskan blackfish *Dallia* uses the esophagus. Such fish ventilate these parts of the gut by gulping air via the mouth and exhaling by burping through the mouth, or farting via the anus. Mudskippers (periophthalmids), perhaps the most terrestrial of all fishes, acquire oxygen via highly vascularized opercular cavities, and can spend extended periods out of water, periodically refilling the enlarged opercular chamber with air (and lying on their sides to dampen the skin).

Fish that breathe water perfuse the gills with systemic venous blood, which is oxygenated and sent direct to the systemic arterial system. In air-breathers,

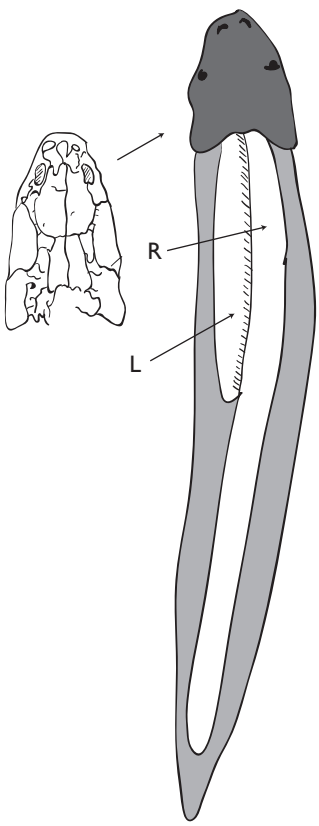


Figure 5.13 Diagram showing the lungs of the bichir (*Polypterus*) seen from above; the right lung is longer than the left. After Humphries (2003).

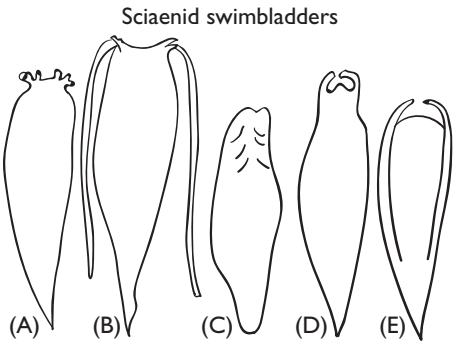


Figure 5.14 Varied *Sciaenid* swimbladders. Note anterior extensions to the vicinity of inner ear, and in *Pachypops*, underlying the lateral line. After Cassati (2002).

the circulatory arrangements are necessarily more complicated (Figure 5.15). When the swimbladder is used to acquire O_2, afferent vessels from the gill circulation supply it, and oxygenated blood leaves to enter the venous circulation before the heart. In lungfish, oxygenated blood passes from the lung direct to the heart via a pulmonary vein (as it does in ourselves). Some air-breathers have accessory respiratory organs in the buccal and opercular cavities in parallel with the gills, and their blood supply is linked to the gills in such a way that oxygenated blood from the accessory organs joins that from the gills and passes to the systemic arterial circulation. But in other air-breathers, such as the electric eel *Electrophorus*, the arrangement is less efficient, since the oxygenated blood from the gas exchanger simply enters the systemic venous circulation anterior to the heart, as it does in *Polypterus*. *Electrophorus* is an obligate air-breather, the buccal mucosa which is papillated and highly vascular (Figure 5.16) provides a respiratory surface around 15% of the body surface. It seems extraordinary that a fish which feeds on living prey can use a delicate respiratory surface in this position, until we recall that it stuns its prey with a powerful electric shock (Chapter 10) and swallows it whole.

Even obligate air-breathing fishes retain the gills (although they may be reduced to avoid loss of oxygen at the gills in waters of low Po_2), because together with the skin, they still act as the site of CO_2 excretion, and some oxygen uptake. Since CO_2 diffuses much more rapidly than O_2, loss of O_2 at the gills can be diminished not only by reducing gill area, but also by increasing the diffusion distance, which will have little effect on CO_2 excretion. Thus, in the climbing perch (*Anabas*), the diffusion distance is 15 μm, compared to the 1–3 μm found in the gills of fish respiring in water (Table 5.1). Sacca and Burggren (1982) examined relative oxygen uptake in *Polypterus* from the lungs, skin and gills, showing the importance of the lungs during (voluntary) air exposure, but that skin and gills contributed when the fish was in water.

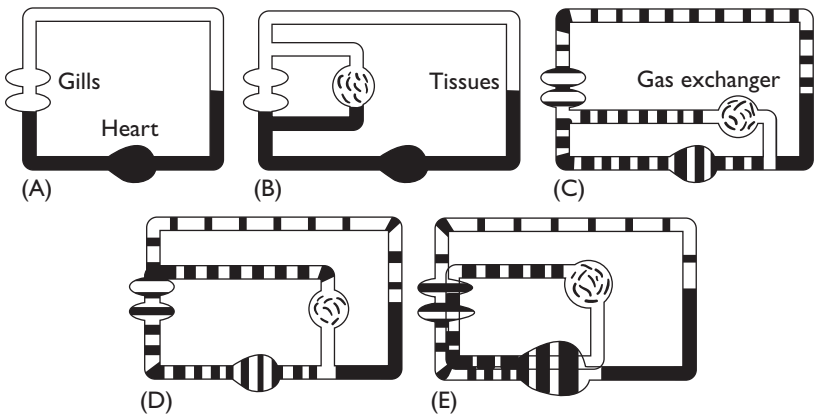


Figure 5.15 Different circulatory patterns in various air-breathing fishes. Black: low blood O_2 content; white: high O_2 content. (A) Normal fish gaining oxygen from gills in water; the gills are in series with the tissues of the systemic bed. (B) Fishes using the opercular chambers or buccal mucosa as airbreathing organs (*Clarias, Saccobranchus*). (C) Fish using the opercular or pharyngeal mucosa as the air-breathing organ (*Electrophorus, Anabas, Periophthalamus*). (D) Swimbladder used for respiration (holosteans). (E) Lung-like swimbladder, partial division between pulmonary and branchial circulation (lungfishes). After Johansen (1970).

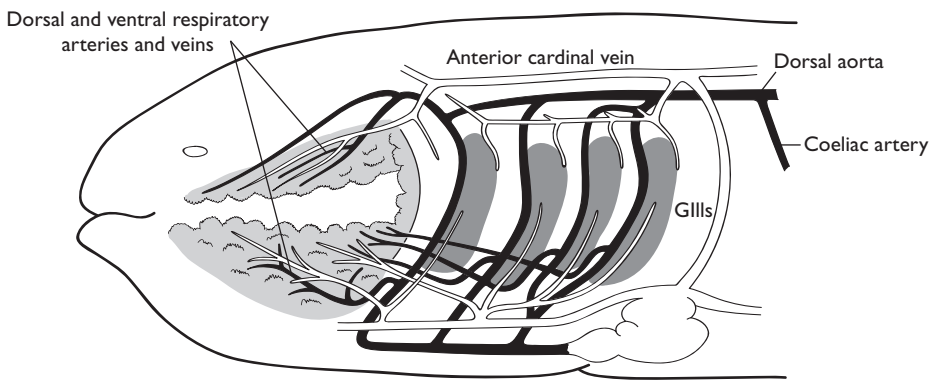


Figure 5.16 The blood supply to the respiratory buccal mucosa of the electric eel *Electrophorus*. Compare with schematic diagram of Figure 5.12. After Johansen *et al.* (1968).

Remmers *et al.* (2001) pointed out that while CO_2 excretion is simple in water where it is 20 times more soluble than O_2, in air the relative solubilities are the same, so the problem of trying to avoid O_2 loss) is more difficult. Perhaps as a direct result, terrestrial air breathers such as birds and mammals show ventilatory responses to CO_2 mediated in part by a central chemoreceptor. Remmers and his colleagues have shown that the isolated *Lepisosteus* brainstem preparation shows intrinsic rhythmic patterns in response to CO_2, and suggest that this implies that air-breathing and the consequent need for a

central chemoreceptor arose early in phylogeny, before the separation of lobe-finned and ray-finned fish.

Lungfishes

Lungfishes are unlike most air-breathing fishes, because they can respire with their lungs and gills simultaneously, as also does *Megalops*; just as in many amphibians there is both lung and cutaneous respiration. To do this, they can adjust the circulation to favor gas exchange by one or other route, according to external conditions, as Johansen and his colleagues showed in the African lungfish, *Protopterus*, and in the normally water-breathing Australian *Neoceratodus*. They measured blood Po_2 at different points (Figure 5.17) and converted the values obtained to O_2 content (to account for the O_2 combined with hemoglobin). From the O_2 content at these different points, they were able to estimate the degree to which blood was selectively passed through different circulatory routes, and the relative importance of the gills and lungs in O_2 uptake. Table 5.2 shows the results they obtained.

In well-oxygenated water, *Neoceratodus* does not ventilate its single lung, which has no respiratory function. Nevertheless, rather surprisingly, the fish sends about the same amount of blood to the heart from the pulmonary vein as from the vena cava. But when ambient water Po_2 is lowered to 5.3–10.6 kPa, the fish begins to breathe air, and, as Table 5.2 shows, by far the most important site of O_2 uptake is the lung. Blood in the anterior branchial arteries (which supply the systemic circulation) is now made up of about 5 parts of pulmonary vein blood to 1 part of blood from the vena cava. So *Neoceratodus* is able partially to separate the blood leaving the heart into streams flowing to the anterior and posterior branchial arches. This it can do because there is a rudimentary spiral valve in the sinus and conus of the heart (Figure 5.18), foreshadowing that of amphibians. *Protopterus* (an obligate air-breather) has more efficient separation of the two streams of blood, and blood in the anterior branchial artery contains about 10 parts of pulmonary vein blood to 1 part of vena cava blood. Lungfishes breathe through the mouth, gulping air into the expanded buccal cavity (previously emptied of water). They then deflate the lung with intrinsic muscles and force air into it by closing the mouth and operecula and raising the floor of the buccal cavity. In water, *Protopterus* breathes every 5–7 minutes, but if kept out of water (which, like *Polypterus*, they do not seem to mind greatly), they breathe every 1–3 minutes.

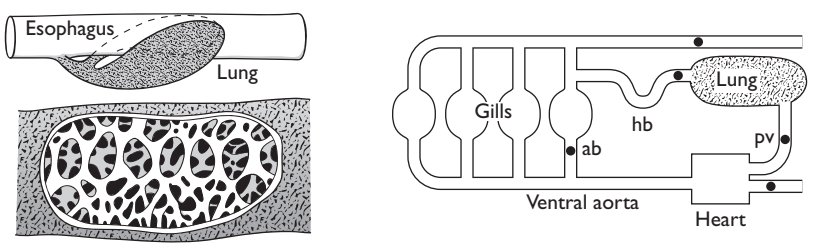


Figure 5.17 Left: lung of *Protopterus* showing septated structure. Right: sampling sites (spots) in the experiments of Johansen *et al.* (see Table 5.2). ab: anterior branchial; hb: hemibranch; pv: pulmonary vein. After Spencer (1898) and Johansen *et al.* (1968).

Table 5.2 Blood O_2 content in two lungfishes under different conditions. From Johansen *et al.* (1968)

Species	Condition	O_2 content (vol%)				Pulmonary venous blood/vena cava blood	
		Pulmonary artery	Pulmonary vein	Anterior branchial	Vena cava	Anterior branchial	Pulmonary artery
Neoceratodus	In aerated water	7.3	7.25	5.0	3.4	5/4	
	In hypoxic water	6.0	7.9	6.75	0.8	5/1	3/1
Protopterus	In aerated water	4.3	6.05	5.5	0.15	10/1	7/3

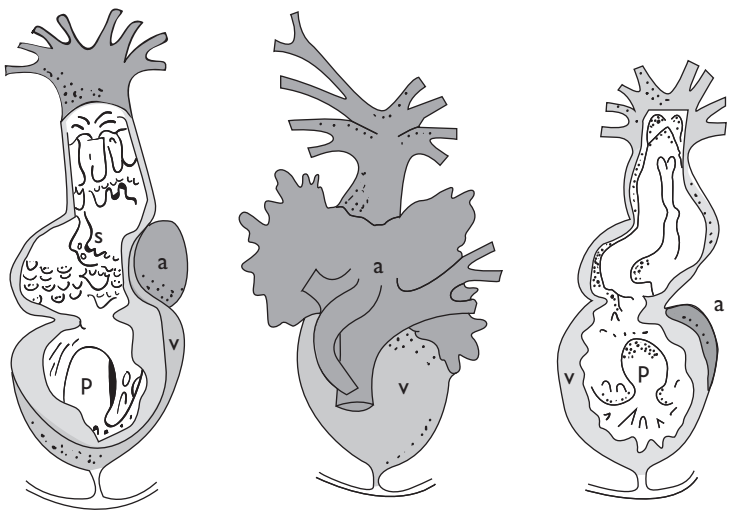


Figure 5.18 Lungfish hearts. Left: ventral view of opened heart of *Neoceratodus*. Note that the conus is partially separated by a row of especially large valves (s). Middle and right *Protopterus*, where separation is more complete. a: auricle; v: ventricle, and p: plug in atrio-ventricular junction. After Goodrich (1930).

Estivation

Neoceratodus live in deep pools of permanent rivers where ambient water Po_2 is high, but both the other lungfishes can not only live in waters of low Po_2, and possibly flounder across from a drying pool to another where water remains, but they can also survive a prolonged dry season by estivating. As the water dries up and becomes more and more muddy, the fish burrow into the mud and become torpid, reducing their metabolic rate and oxygen demand until re-awakened by the first rains. *Protopterus* makes a bottle-shaped burrow lined by mucus secreted by the skin to form a cocoon; the nares are plugged with mucus, and the fish breathes air through its mouth once an hour or so, via the tube leading to the surface. In nature, estivation lasts 4–6 months, but

estivating fish taken into the laboratory have survived in their cocoons for several years. In water, *Protopterus* excretes nitrogen as ammonia across the gills, but when estivating this is no longer possible, and nitrogen (from the muscle proteins metabolized) is converted in the liver to non-toxic urea which reaches high levels in the blood. When water enters the tube to the surface and reaches the mouth, the fish makes breathing movements and after a series of convulsive jerks, swims out of its burrow. Estivation is not peculiar to lungfish. The enigmatic little Western Australian *Lepidogalaxias salamandroides* (it is not a galaxeid, but does look much like a salamander, Figure 5.19), which was discovered in 1961, estivates in the mud of dried stream beds curled up in a pear-shaped burrow connected to the surface by a thin tube. More spectacular are the New Zealand mudminnows (*Neochanna*) which estivate up to 2 m below the ground for 1–2 months in summer and autumn. This habit gave rise to the early comment that "the colonists obtained a bounteous harvest of potatoes and fish at one digging."

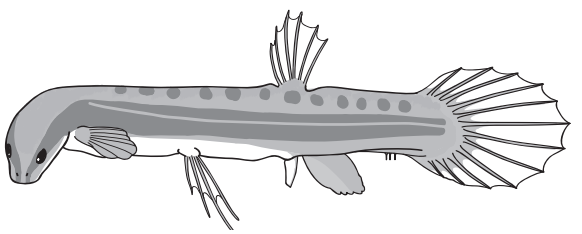


Figure 5.19 The salamander fish of Western Australia (*Lepidogalaxias salamandroides*), showing its remarkable ability to bend its neck. After Berra (1997).

5.6 The Circulatory System

Primary and secondary circulations

The oxygen acquired (from water or air), and the carbon dioxide excreted at the gills, have to be transported around the body by the circulation of the blood. In fishes using the gills as a gas exchanger, the primary circulation is single, blood leaves the heart to pass first through the gill capillary bed, thence to the systemic capillaries, and back to the heart. Usually the gills account for approximately 30% of the total resistance to blood flow, the remainder being in the visceral and somatic vasculature, but in tunas, which have high oxygen requirements and large gill areas, the gills account for up to 57% of the total resistance. In most fishes, blood pressures in the primary circulation are relatively low (very low in hagfishes), although once again tunas are the exception, and pressures in the ventral aorta in resting tunas reach 87 mmHg (10.58 kPa), and in exercising fish 90 mmHg (12.4 kPa). In the venous system, pressures are very low, and may even be sub-ambient; venous return is assisted by an unusual variety of pumps including accessory hearts. Hagfishes, for example, have no fewer than five "hearts" in addition to the usual one (Figure 5.20).

As well as the primary circulation, many kinds of fish, such as lampreys, elasmobranchs, and euteleosts, have an unusual secondary circulation,

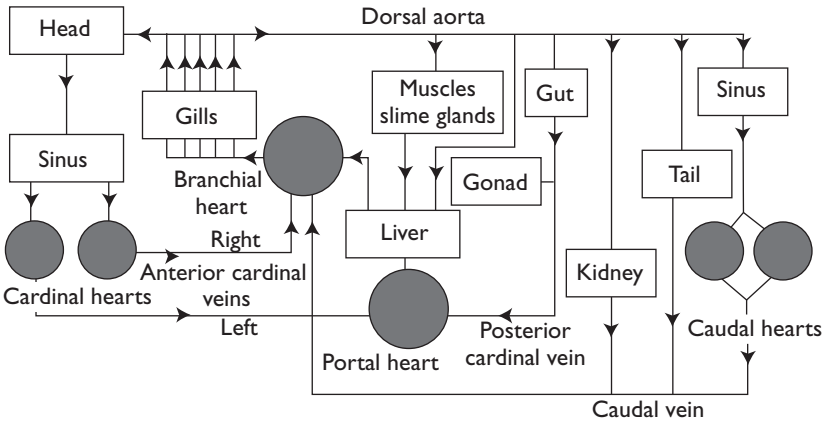


Figure 5.20A Schematic diagram of the complicated circulation in hagfish, with no less than five accessory hearts. After Satchell (1992).

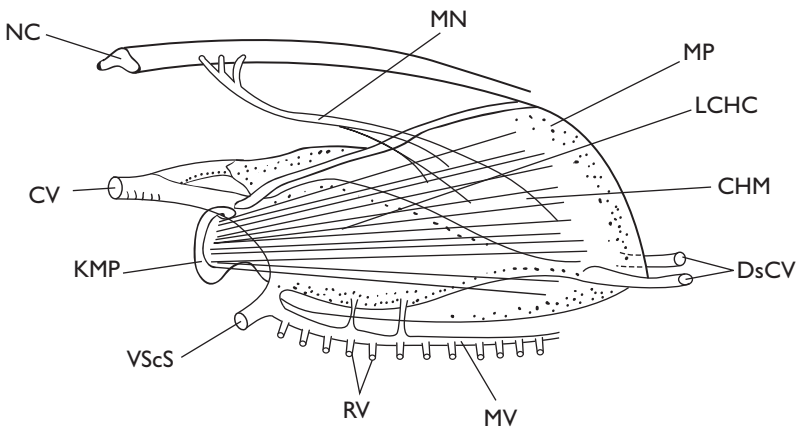


Figure 5.20B Lateral view of caudal heart of *Myxine glutinosa*. CHM: caudal heart muscle; CV: caudal vein: DsCV: distal caudal vein; KMP: knob on moveable portion of the median plate (MP); LCHC: left caudal heart chamber; MN: motor nerves from spinal cord (NC; VScS: vein from subcutaneous sinus; RV radial veins. After Kampmeier (1969) from Satchell (1992).

(Figure 5.21) connected to the primary circulation by fine-bore coiled arterio-arterial anastamoses. These were first discovered in 1929, but not until Vogel and Claviez (1981) examined corrosion casts by scanning microscopy (which gave superb pictures of minute vessels and their connections) was it accepted that these were *not* lymphatics (so regarded even in some recent texts), but a separate secondary circulation. After receiving blood from the primary circulation via small arterioles whose connections are guarded by fingers from endothelial cells, the vessels of the secondary circulation form their own capillary beds in the fins, gills, mouth, skin, and peritoneum (Figure 5.22), then forming secondary vessels which join the primary circulation at caudal and cutaneous veins. While not easy to make unambiguous measurements, it

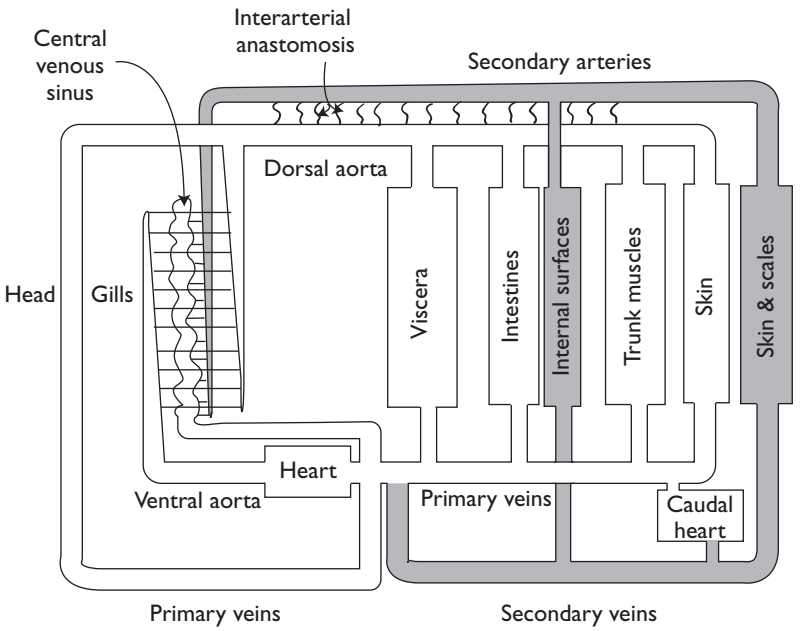


Figure 5.21 Block diagram of the teleost primary and secondary circulations. After Olson (1996).

seems that the serum in the secondary circulation is similar to that in the primary circulation, although hematocrit levels are much lower, and while volume is much larger (perhaps 1.5 times that of the primary circulation), flow rates are much lower.

So there are two rather different *parallel* circulations in fish, what might be the function of the secondary? One possibility is that because the secondary circulation capillaries seem directed to exposed epithelia near the ambient water, they may function in controlling volume, ion levels, or in immunoregulation. But more experiments are needed, and, although it is tempting to guess a nutritive function, it is probably more sensible to await more data. Only in lungfish is there a lymphatic system and the secondary circulation is absent.

In general, the teleost circulatory system is more efficient than that of elasmobranchs, blood volume is lower, as is cardiac output, and narrower veins occur instead of venous sinuses. There are interesting exceptions to some of these generalizations, which will be considered later, for example, in the Antarctic icefish (*Chaenocephalus aceratus*), which lacks hemoglobin, Q is exceptionally high.

The heart

Fish hearts are S-shaped and four-chambered with, from behind forwards, sinus venosus, atrium, ventricle and either a bulbus or conus leading to the ventral aorta (Figures 5.18 and 5.23). We have already seen that some air-breathing fishes have a double circulation (the Japanese mudfish, *Channa argus*, even having a double ventral aorta), but only lungfishes have a

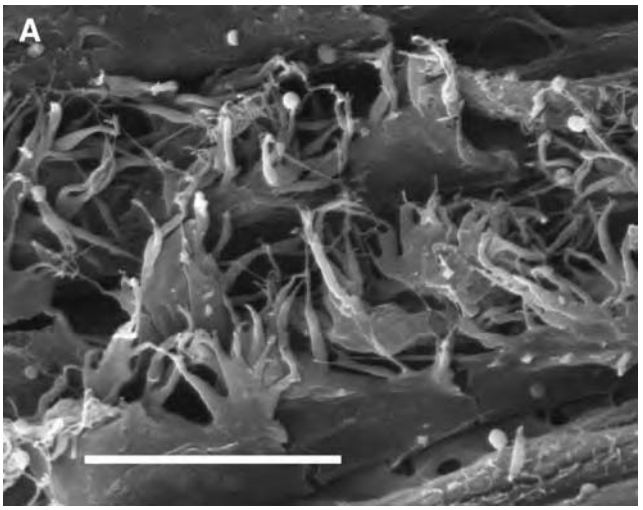


Figure 5.22A Scanning electron micrograph (SEM) showing characteristic endothelial cell processes near origin of secondary arterioles. From Skov and Bennett (2004).

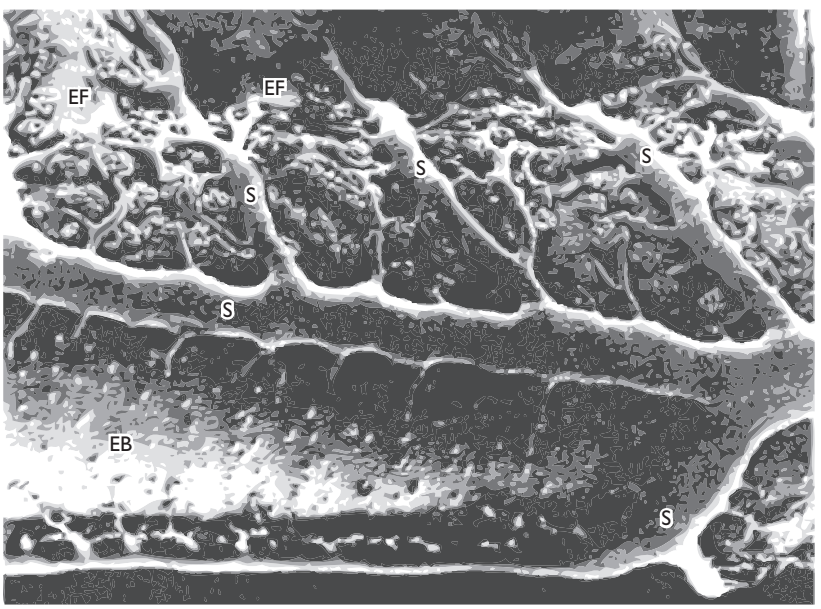


Figure 5.22B SEM showing many tortuous small arterioles arising from efferent filamental (EF) and efferent branchial (EB) arteries, anastamosing to form the secondary circulation (S) of the gill in the climbing perch (*Anabas scandens*). From Olson (1996).

morphologically partially divided heart. The teleost bulbus is an elastic reservoir passively enlarged by blood driven forwards out of the ventricle, but the equivalent conus (in elasmobranchs, *Amia, Lepisosteus* and *Polypterus*) is contractile, contracting in sequence with the rest of the heart. Valves at the junctions between the different regions assure unidirectional flow, and pocket valves are also found along the conus (Figure 5.23), sometimes in large numbers; *Lepisosteus* has no less than 72 valves in eight rows. Heart mass in most fishes scales as body mass (as in other vertebrates), but the size of the ventricle differs a good deal, being largest in tunas and icefishes where Q is greatest.

The cardiac cycle consists of systole, when the ventricle is emptied, and diastole when it is refilled; it is accompanied by a progression of electrical events along the heart resulting from the depolarization and repolarization of the cardiac muscle cells. The sum of these cardiac action potentials, which are relatively easy to record *in situ* with electrodes which need not be in or on the heart itself, are electrocardiograms (ECGs). Fish ECGs are essentially the same as those of mammals: atrial contraction produces the P wave, ventricular contraction the QRS complex, and ventricular relaxation the T wave. But fish heart ECGs can be more complex than in mammals. Contraction of the conus in elasmobranches adds a small B wave, and contraction of the sinus venosus adds a V wave, prominent in the hagfish (*Eptatretus*) and in the eel. Contractions of the atrium and ventricle have to be coordinated in such a way that there is a delay between atrial and ventricular contraction, and a rapid synchronous contraction of the ventricle. The specially modified conduction pathways (His-Purkinje fibers) found in higher vertebrates seemed to be lacking in fish hearts, and it is only recently that Sedmera *et al.* (2002) recognized their equivalent in zebra fish. Ventricular contractions naturally lead to cyclic variations in pressure and flow in the ventral aorta from which blood flows through the serial capillary resistances of the gill and systemic capillary beds

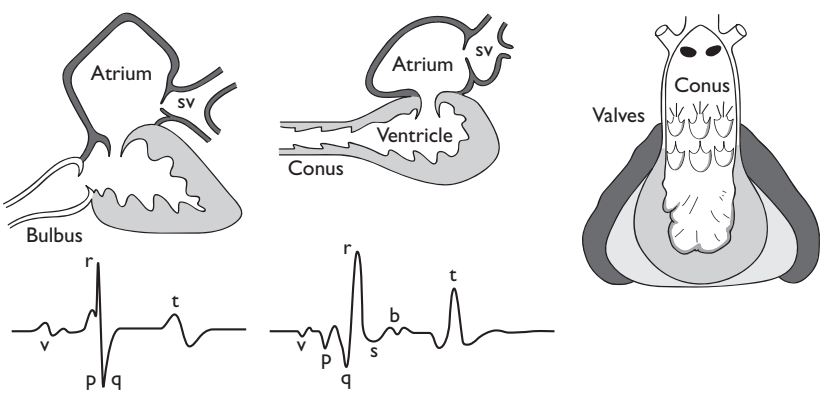


Figure 5.23 Fish hearts. Above, left: teleost; mid: shark; right: valves in the conus of the rapidly-swimming mako shark (*Isurus*). sv: sinus venosus. Below: electrocardiograms of trout (left) and Port Jackson shark (*Heterodontus*). v, p, q, r, s, t, and b: depolarizations associated with different regions of the heart (see text). After Daniel (1922); Randall (1970) and Satchell (1991).

(Figure 5.24). Such oscillations are damped in teleosts by the elastic bulbus (constant flow is what is optimal for the gill gas exchanger), but it does not seem that the elasmobranch conus can act in this way, although it has been claimed to do so; conus contraction is too slow to do other than make the pocket valves close together to prevent backflow, and pressures oscillate much more in the ventral aorta than in teleosts.

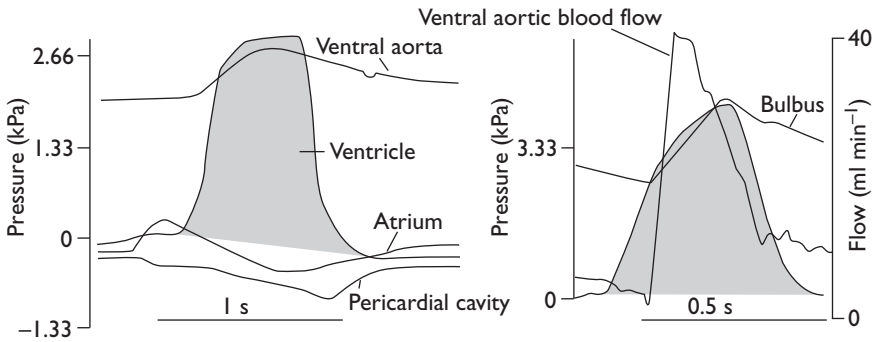


Figure 5.24 Pressures recorded in different regions of the elasmobranch and teleost heart. Left: the small shark *Mustelus* (note sub-ambient pressures in pericardial activity); right: in the lingcod (*Ophidion*). After Sudak (1965) and Randall (1970).

White hearts

As we should expect, fish hearts are usually red owing to the myoglobin content of the auricle and (particularly) ventricle, but, astonishingly, some sluggish benthic fishes of the North Atlantic, such as wolf fish (*Anarhichas*), angler fish (*Lophius*), and lumpfish (*Cyclopterus*) all have pale hearts lacking myoglobin (Grove and Sidell, 2002). Their oxidative "red" cruising muscle (if any) also lacks myoglobin. The reader may care to figure out without assistance why this is so remarkable.

Obviously enough, the amount of blood entering the ventral aorta is determined (1) by the volume ejected from the ventricle at each stroke, and (2) by heart rate. In mammals, increase in heart rate is the most significant response to a demand for increased cardiac output, but, in many fishes, stroke volume changes are more important (as in sharks, for example). The ventricle is filled by the contraction of the atrium (which is itself filled both by expanding as the ventricle contracts and pericardial pressure decreases, known as force from in front, *vis a fronte*, and by force from behind, *vis a tergo*, from the pressure in the venous return to the sinus venosus). The relative importance of these two mechanisms is presently unclear. In benthic teleosts, only the *vis a tergo* mechanism seems to be used but in active teleosts such as tunas, the *vis a fronte* mechanism seems important, at least at high stroke volumes. In order for *vis a fronte* to operate, the pericardium clearly has to be rigid (as it is in tunas), for, if not, as the ventricle contracted, the pericardium would simply follow ventricular contraction and blood would not be sucked into the atrium by sub-ambient pericardial pressures. Recent work on sea bass (*Dicentrarchus labrax*) has

shown that relative cardiac output (Q) during swimming may depend upon increased venous return, compensating for reduced cardiac filling time.

It is always interesting when new work upsets long-held dogmas, and this seems to be the case for the operation of the shark heart. In sharks, the pericardium is thick and rigid, and, in almost all texts, it is stated that pericardial pressures are sub-ambient, and that the atrium is filled by the *vis a fronte* mechanism. Recent work (Lai *et al*., 1990) on leopard sharks (*Triakis semifasciatus*) set up to swim in a respirometer and appropriately cannulated to measure blood and pericardial pressures, has shown that when swimming, as stroke volume increases, pericardial pressures rise and the pericardio-peritoneal canal opens to reduce pericardial volume. In accord with this, puncturing the pericardium does not reduce stroke volume. So, at least when they swim, sharks seem to fill their hearts by the *vis a tergo* mechanism, rather than mainly by the *vis a fronte* mechanism.

The more blood enters the atrium and ventricle, the more are their muscle fibers stretched, and the more powerfully they contract in accord with the Frank–Starling mechanism of the heart which states that "the energy of contraction is a function of the length of the muscle fiber." So stroke volume changes depend on the atrial-ventricular blood flow, and are controlled by circulating catecholamines, and by vagal and autonomic nerve supply as the heart rate. The American physiologist Greene discovered in 1902 that hagfishes are unique in completely lacking any heart innervation, when he set up a class experiment to demonstrate vagal control of the heart using *Bdellostoma*. Fortunately he had been conscientious enough to try the experiment himself before giving it to his students.

Intrinsic or resting heart rate varies from around 15 beats min^{-1} (bpm) in hagfishes to 30–50 bpm in most elasmobranchs and teleosts, but in skipjack tuna (*Katsuwonus pelamis*), intrinsic heart rate is around 120 bpm, while in swimming skipjack, rates up to 240 bpm have been recorded. During long-sustained aerobic swimming, cardiac output naturally rises to meet increased tissue oxygen demand (the so-called scope for activity, Chapter 3). In trout it triples, and in tuna doubles from a basal level of 132 ml min^{-1} kg^{-1} (at 26°C) which is about half that of a mammal at 37°C. Contrary to what might be expected, perhaps, heart rate and cardiac output decline during (anaerobically driven) burst exercise.

Accessory pumps

A remarkable variety of accessory pumps occurs in the venous circulation of different fishes. These range from the portal heart of hagfishes behind the liver (Figure 5.20) which has cardiac-type muscle and resembles the atrium of the main heart, with an ECG with P and T waves; to the caudal hearts of elasmobranchs and teleosts. These fish also have hemal arch and fin pumps. Apart from the hagfish portal heart, all of these interesting devices are driven by skeletal muscles. For example, in the shark hemal arch pump (Figure 5.25) venous blood from the myotomes is driven into the caudal vein past ostial valves as the myotomes contract and compress the vascular bed, and so when the fish swims there are cyclical pressure pulses in the caudal vein, and venous blood flow increases (just when cardiac output increases and a higher venous return is required).

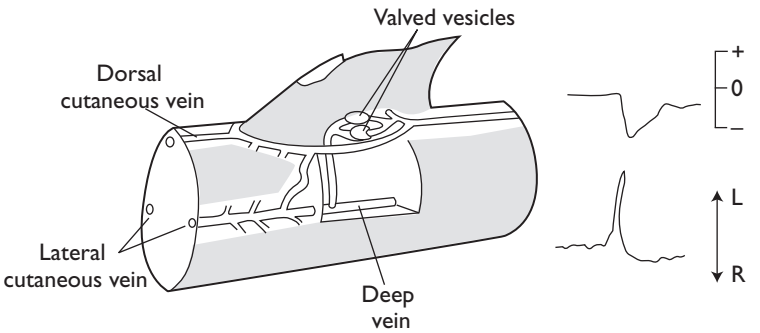


Figure 5.25 The hemal arch pump of the shark *Heterodontus*. After Satchell (1992).

5.7 Fish Blood and Gas Transport

Blood properties

Different fishes have very different lifestyles, so it is not surprising that the properties of their blood vary according to metabolic demands, and the way that the fish acquires O_2 and excretes CO_2. For example, blood in active fishes such as scombroids must have a much higher O_2 capacity than in sluggish fishes such as angler fish; in obligate air-breathers it must be less sensitive to CO_2 content than it is in water-breathing fishes. Table 5.3 gives the O_2-capacity values of whole blood in different fishes. Note that the O_2 capacity of whole blood comprises O_2 in solution in the blood plus O_2 combined with hemoglobin; this red cell respiratory pigment raising the O_2-capacity up to 40 times. However, high oxygen capacity with a high hematocrit incurs costs in increased blood viscosity, (see, e.g., Sadler *et al.*, 2000) and these are not

Table 5.3 Blood O_2 capacity and gill areas in fish of different habit. After Steen (1971)

Species	O_2 capacity (vol%)	Gill area (mm^2 g body wt^{-1})	Habit
Bonito (*Sarda*)	18.0	595	
Mackerel (*Scomber*)	19.6	1158	Very active
Menhaden (*Brevoortia*)	16.2	1773	
Butterfish (*Pholis*)	10.7	598	
Sea-robin (*Prionotus*)	9.3	360	Active
Eel (*Anguilla*)	8.0	302	
Goosefish (*Lophius*)	5.7	196	
Toadfish (*Opsanus*)	5.3	200	Sluggish
Sand-dab (*Hippoglossoides*)	4.6	188	

trivial since O_2-carrying capacity increases linearly with hematocrit, while the linked increase in viscosity is exponential. Fish can reduce blood viscosity when stressed, since the adrenergic stress response releases circulating catecholamines which make red cells swollen, but also more deformable presumably by acting on their surface receptors.

Remarkably, in several Antarctic icefishes in the family Chaenichthyidae, blood hemoglobin is much reduced or totally lacking, and there are no red blood cells. All the O_2 reaching the tissues must do so in solution in the blood, which has the same O_2 carrying capacity as seawater, viz. around 0.7 vol%, compared with around 8 vol% in many normal fishes with hemoglobin. To overcome the low O_2-capacity of the blood, icefishes have relatively large gills, well-vascularized skin for cutaneous respiration, large hearts with large cardiac output and large-diameter blood vessels; to reduce O_2 demand they have reduced their red aerobic myotomal musculature. Unexpectedly, as O'Brien *et al.* (2002) have shown, the large red myotomal fibers have almost double the density of mitochondria seen in the fibers of notothenioids with hemoglobin. Since this cannot mean enhanced aerobic capacity, the suggestion is that the mitochondria aid in intracellular oxygen transport as O_2 is more soluble in lipid than in aqueous cytoplasm. The resting O_2 uptake of icefishes is from one-half to two-thirds that of fishes in the same habitat that possess hemoglobin, and they survive by a combination of such adaptations, low metabolic rate, high cardiac output, and living at low temperatures (when blood O_2 capacity is high). It remains a mystery why they should have lost hemoglobin. The early larvae of many teleosts also lack hemoglobin (although, of course, it appears later in development), and so do the long-lived leptocephali larvae of eels, where it is reasonable to guess that it is lacking to complete their glassy transparency. But even in fish such as trout, pike (*Esox*) and goldfish, O_2 dissolved in the blood suffices for resting metabolism. When these fishes are poisoned with CO (so that the hemoglobin cannot combine with O_2), they survive well until exercise increases O_2 demand, when they perish.

Anti-freeze proteins

The colligative effects of solutes in seawater (around 450 mM) depresses seawater freezing point to –1.9°C , while ordinary teleost blood (much more dilute, p. 162) freezes at –0.4°C. So to avoid the blood and all tissues freezing solid, marine coldwater teleosts produce small anti-freeze proteins in the scales, skin, fins, and gills, and in the liver. There are five kinds of anti-freeze molecules, four proteins and one glycoprotein, (Figure 5.26) and they are as chemically diverse as they are in shape. The structure of a single unit of an anti-freeze glycoprotein, with a flat surface of hydrogen bonding residues attached to the surface of an ice crystal is seen on the right of Figure 5.26. The anti-freeze proteins are produced in the liver and in the skin, while the glycoproteins come from the exocrine pancreas and enter the gut via the pancreatic duct to prevent the gut contents freezing (Cheng *et al.*, 2006). As so often happens, these substances were first discovered in fish, and later found in insects and plants. They recognize ice crystal surfaces and bind to them, seemingly modifying the ice surface, or inserting themselves along steps in the lattice, but it is still not entirely clear how these interesting substances work (see Ewart *et al.*, 1999; Fletcher *et al.*, 2001). The latter authors suggest that the

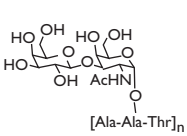
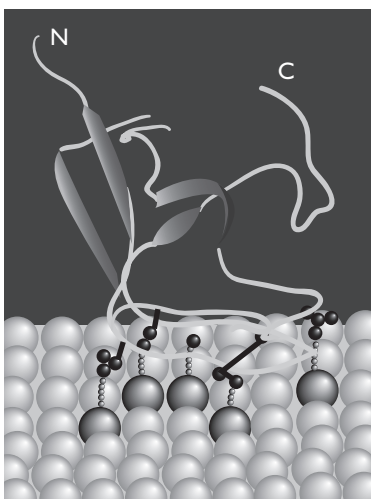
Characteristic	AFGP	Type I AFP	Type II AFP	Type III AFP	Type IV AFP
Mass (Da)	2600–33 000	3300–4500	11 000–24 000	6500	12 000
Key properties	AAT repeat; disaccharide	alanine-rich α-helix	disulfide bonded	β-sandwich	alanine rich; helical bundle
Representative structure					
Natural source	Antarctic notothenioids; northern cods	Right-eyed flounders; sculpins	Sea raven; smelt; herring	Ocean pout; wolf fish; eel pout	Longhorn sculpin

Figure 5.26A Types of anti-freeze glycoprotein (AFGP) and anti-freeze proteins (AFPs) found in different teleosts. From Harding *et al.* (2003).



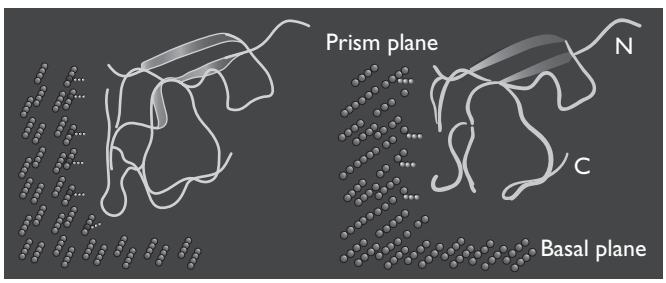


Figure 5.26B Left: ice-docking model of a globular AFP linked to oxygen atoms in the ice-crystal lattice by hydrogen bonding residues (dotted). Right: stereodiagram showing flat portion of AFP interacting with the first layer of water of the ice step. From Jia *et al.* (1996).

diversity of anti-freeze types and their curious phylogenetic distribution indicate that they evolved recently, in response to sea level glaciation some 1–2 mya in the N hemisphere and 10–30 mya around Antarctica. The rate of loss of these small molecules in the kidney in the Antarctic fish is minimized because the kidneys are aglomerular (Eastman, 1993).

The little rainbow smelt (*Osmerus mordax*) living in the sea under the winter ice around Newfoundland at –1.8°C, show no evidence of death by freezing (as Driedzic and Ewart (2004) remark, perhaps thinking of rigor mortis). This fish uses an anti-freeze protein, but also uses the same cryoprotectant that various invertebrates use glycerol. As any stock-farming reader will know, we use glycerol ourselves in the cryopreservation of sperm straws. Naturally,

glycerol diffuses out of the fish by the gills and so in winter has to be produced continually.

Fish hemoglobins and oxygen transport

Apart from the special case of the channichthyid icefishes, most oxygen in the blood is carried by red cell hemoglobin, oxygenated when Po_2 is high and deoxygenated when it falls, according to the oxygen dissociation curve (blood Po_2 plotted against the amount of O_2 bound to the hemoglobin). Oxygen dissociation curves are always non-linear, and in active fishes, usually sigmoid (Figure 5.27), this shape representing a compromise between high O_2 affinity needed for loading at the gills and lower affinity for unloading at the tissues. The normal working range in the fish usually lies on the steep part of the curve so that much O_2 can be unloaded for small changes in Po_2. The slope of the dissociation curve can be changed by changes in pH; with increase in acidity, the curve is usually shifted markedly to the right. This is the Bohr shift (defined as the shift or change in log 50% O_2 saturation divided by the pH change causing it; see Figure 5.27 and also Figure 4.15 in Chapter 4). It results from pH-dependent configurational changes in the hemoglobin molecules which inhibit O_2 binding; what it means in practice is that O_2 is unloaded at sites where Pco_2 is high, just where it is needed by the fish. In many fishes, increase in Pco_2 not only shifts the dissociation curve to the right, but it also prevents complete oxygenation of the hemoglobin, thus depressing the curve. The lowering of blood O_2-carrying capacity (rather than O_2 affinity) in this way is the Root shift (as we saw in Chapter 4 the Root shift is used by the fish to drive O_2 into the swimbladder from the rete). The Root shift is really a very extreme case of the Bohr shift, and it is found in the blood of fishes with swimbladders or

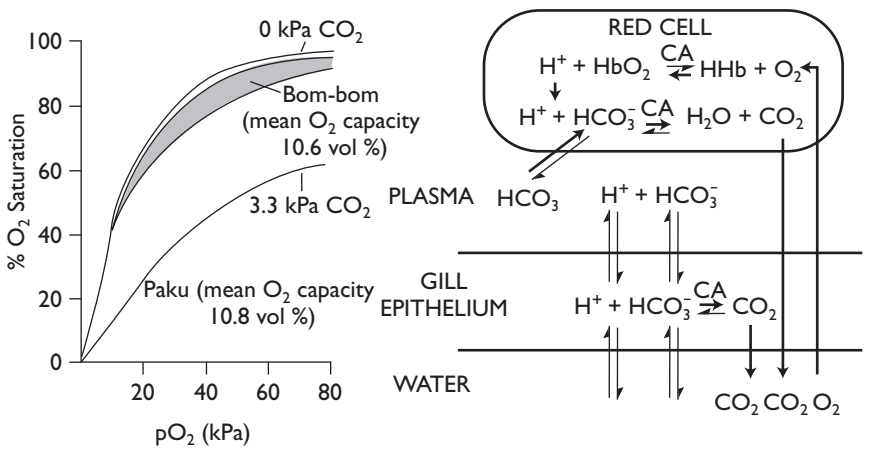


Figure 5.27 Left: O_2 dissociation curves of blood from Paku (*Pterodoras granulosus*), which lives in well-oxygenated water, and Bom-bom (*Myleus setiger*), which lives in oxygen-poor water. The blood of the latter has a much smaller Root effect. See also Figure 4.14 in Chapter 4. Right: schematic diagram to show CO_2 and H^+ movements between plasma, red cells, the gill epithelium and the ambient water. After Willmer (1934), Randall and Daxboeck (1984).

other O_2-concentrating retia (like those of the choroid plexus of the eye) but is absent from elasmobranchs that do not possess such retia. Air-breathing fishes such as lungfish or the electric eel (*Electrophorus*) have hemoglobins that are rather insensitive to Pco_2, and they need to have this reduced Bohr shift, because Pco_2 at the gas exchanger and in the blood will be higher than in water-breathing fishes. Hagfishes and lampreys have monomeric hemoglobins, but in all other fishes the hemoglobins are tetrameric (as they are in mammals), and polymorphic. Several different hemoglobins may occur in one fish, perhaps to adapt the gas-transport system to changing conditions, as in the American eel (*Anguilla rostrata*), where one type has a high O_2 affinity in seawater, the other in freshwater. As Figure 5.26 shows, a large Root effect is seen in fish living in oxygen-rich water, a small effect in oxygen-poor water.

CO_2 transport

Only a small proportion of the CO_2 diffusing into the blood at the tissues remains dissolved in the plasma; most is hydrated to the bicarbonate ion (about 95% of CO_2 in the venous blood is plasma HCO_3^-), and so CO_2 from the tissues is transported in the blood mainly as HCO_3^-. Rehydration of CO_2 to HCO_3^- is slow in the veins, taking place after the venous blood has left the respiring tissue, but is rapidly catalyzed by carbonic anhydrase in the red cells, where O_2 is driven off the hemoglobin in the respiring tissues as it binds the resulting protons. HCO_3^- entry in the red cells is accompanied by water entry (to rehydrate the HCO_3^-) and by Cl^- (to maintain electro-neutrality). This chloride shift increases the osmolarity of the red cells, which therefore swell slightly so that their volume becomes 2–3% greater in venous than in arterial blood. The right part of Figure 5.27 shows the situation schematically. At the gills (Figure 5.27) total blood CO_2 is reduced by 10–20%, mainly because HCO_3^- falls by 20% in the plasma. One scheme by which this could occur is shown in the lower half of Figure 5.20, where carbonic anhydrase-catalyzed CO_2 produced in the red cell from HCO_3^- diffuses away across the plasma and gill epithelium to the water flowing over the gills. Carbonic anhydrase is present in the gill epithelium, but does not appear to play a role in CO_2 excretion.

Oxygen and carbon dioxide transport are complementary, and combine to make a system efficient enough to satisfy the gas transport demands of such active fishes as tunas. Probably it is generally true (however see p. 361) that fishes use most oxygen in the red muscle, driving cruising swimming (Chapter 3), and in the fast-swimming scombroids this tissue is extremely well vascularized. Capillary fiber ratios up to 4.5:1 and external diffusion distances of around 10μm are seen in skipjack (*Katsuwonus*) red muscle and compare very favorably with those of mammalian muscle.

Envoi

Fish gills are remarkably efficient in gas uptake and excretion, but they are involved in much more than just gas exchange. The complexity of gill design and the striking variety of control mechanisms and blood pathways not only enable fish to "fine-tune" gas transfer with the water, but also permit variable extra renal pathways for ionic and osmotic exchange. There is a large variety of

blood properties interestingly linked to such constraints as secretion of swim-bladder gas or the operation of the retina, and even, in several instances, to abandoning hemoglobin.

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6 Osmoregulation and Ion Balance

6.1 The Osmotic Problem: What Fish Have to Cope With

Different fishes live in waters from almost distilled purity, to hypersaline ponds where they have difficulty keeping below the surface. The ability to live in such different waters is remarkable enough for stenohaline fish which do not move from a single environment, but, perhaps more striking, there are a good many euryhaline species able to move (sometimes rapidly) from freshwater to the sea and back again. It is obvious that freshwater fish cannot be isosmotic with the external medium, but, less obviously, very few marine fish are isosmotic with seawater. Although fish skin is on the whole rather impermeable (puzzlingly so in those fish where cutaneous respiration is important), the problem is that fish have large areas of permeable epithelium in close contact with the water. Not only is there the vast area of the gill epithelium, but there are the narial and oral mucosae, and some water will inevitably be swallowed as the fish feeds. Since very few fishes are isosmotic with the external medium, there will be osmotic gradients across these permeable surfaces, and fish have to devote 25–50% of their total energy output to cope with this. In marine teleosts, these are of the order of 600–800 mosmol kg^{-1}, and water will tend to be lost and ions gained, while, in freshwater fish, water will tend to be gained and ions lost (Figure 6.1). It may seem surprising that the osmoregulatory mechanisms for coping with these opposite problems in seawater and freshwater can be switched by euryhaline fish as they move from one to the other. Changes in body fluids indeed occur as euryhaline fish are confronted with salinity changes: thus in eels, salmon, and flounders (*Platichthys flesus*) plasma osmolarity rises by 20% or so (mainly due to increase in plasma Na^+) when they adapt from freshwater to seawater. The little killifish, *Fundulus*, can surmount the greatest osmotic challenge, living in nature both in freshwater, and in southern California in hypersaline pools (128‰ salinity). Naturally, it has been the subject of much experiment, and, as shown in Figure 6.2, can regulate body water and plasma salt concentration between 0 and 60% NaCl. Higher salinities are tolerated by accepting 5% tissue water loss, and an increase in blood osmolarity of around 30%. Recent work has indicated that when killifish enter waters of different salinity, the switch from Cl^- secretion on entering low salinity water to Cl^- absorption and vice versa is a direct effect on

the osmo-sensing chloride cells themselves (p. 168), causing changes in gene expression. When challenged by transfer from freshwater to seawater, numbers of chloride cells increase, as has been shown by laser scanning cytometry (Figure 6.3). Such short term changes following transfer to different salinities may be followed by longer-term changes in gene expression (Scott *et al.*, 2004). In eels, which can cope with rapid changes from freshwater to seawater, natriouretic peptides and the renin-angiotensin hormones (see Chapter 9) are involved (Takei and Hirose, 2002)

The reader will surely already have realized that the large areas of permeable epithelium in contact with the surrounding water imply that extra renal routes of excretion and ion exchange can be exploited, in contrast to the renal routes of most terrestrial forms, but before considering the mechanisms involved (recently reviewed by Evans *et al.*, 2005), we may first examine the situation in hagfish, unique not only in being isosmotic with seawater, but also in having blood Na^+ and Cl^- levels similar to seawater.

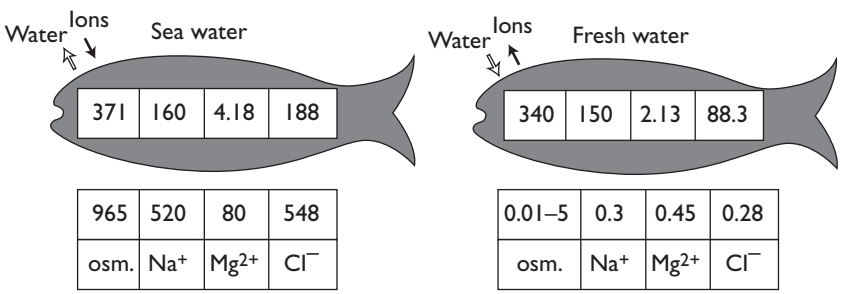


Figure 6.1 The plasma and surrounding water osmolarity and molarity of major ions in representative marine and freshwater teleost fishes, showing the opposite tendencies of water loss and ion gain in the former, and water gain and ion loss in the latter. After Pang *et al.* (1977).

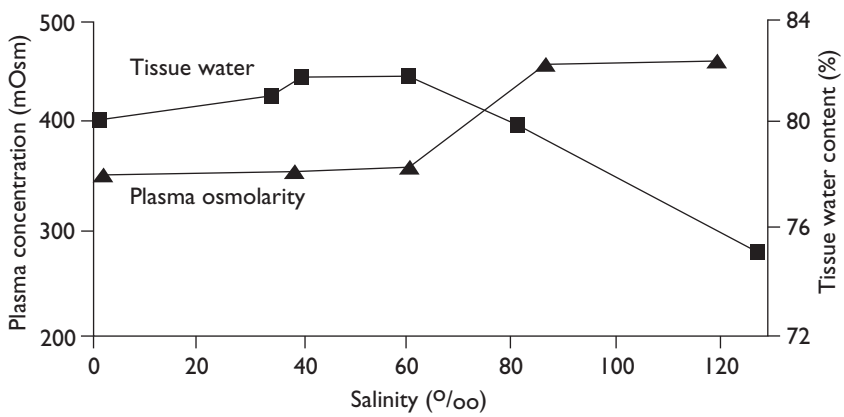


Figure 6.2 Tissue water content and plasma osmolarity in killifishes (*Fundulus*) adapted to different salinities. After Feldmeth and Wagoner (1972).

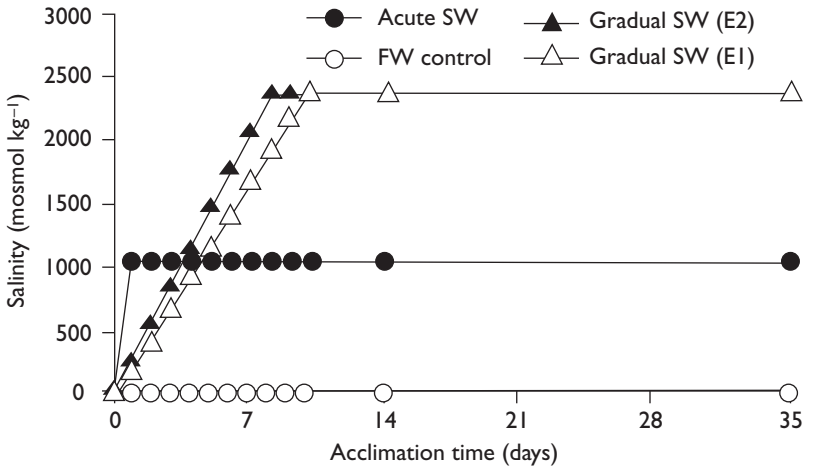


Figure 6.3 Changes in Cl^- cell number after killifish (*Fundulus*) were transferred from freshwater to various salinities. Open circles: control transfer to freshwater; filled circles: abrupt transfer to seawater; triangles: transfer to 2.4 × seawater (2400 mosmol kg^{-1}) at different rates of change, more (filled) or less rapidly (open triangles). From Lima and Kültz (2004).

6.2 Hagfish, Lampreys, and the Origins of the Glomerular Kidney

Hagfish live close to the seabed, in seawater of unchanging salinity, yet *Eptatretus* tolerates gradual experimental changes in salinity within certain limits, swelling and shrinking like a perfect osmometer since it is perfectly permeable to water. Blood volume is large, and it is very nearly isosmotic with seawater (Table 6.1). There is therefore little or no *osmotic* exchange of water, although tritiated water experiments have shown very high water exchange rates (2287 ml kg^{-1} hr^{-1}). Na^+ and Cl^- concentrations in the blood are similar to those in seawater, but higher than in the tissues; internal osmotic balance is maintained by intracellular amino acids which can be regulated to some degree when hagfish are exposed to osmotic stress. The kidney is essentially of the usual vertebrate mesonephric type, although since the very large glomeruli (30–35 in each kidney) lie very close to the longitudinal archinephric duct (Figure 6.4) it is sometimes termed an atubular kidney. There is also a persistent pronephros, but this plays no role in urine production as the pronephric tubules do not retain their original connection with the kidney duct. The large glomeruli perhaps account for high filtration rates, but there remain problems in understanding urine formation. The longitudinal archinephric duct itself, functionally analogous with the proximal tubule I of gnathostomes, secretes urea, K^+, Mg^{2-}, SO_4^{2-} and PO_4^{3-}. None of the water filtered is resorbed. The kidney does not seem to control body osmolality nor Na^+ content, the urine is close to the osmolality of seawater (Table 6.1). There are specialized mitochondria-rich chloride cells (p. 168) in the gills, and analysis of the copious slime hagfish secrete suggests that this other extrarenal route may be the main route of secretion of Ca^{2+}.

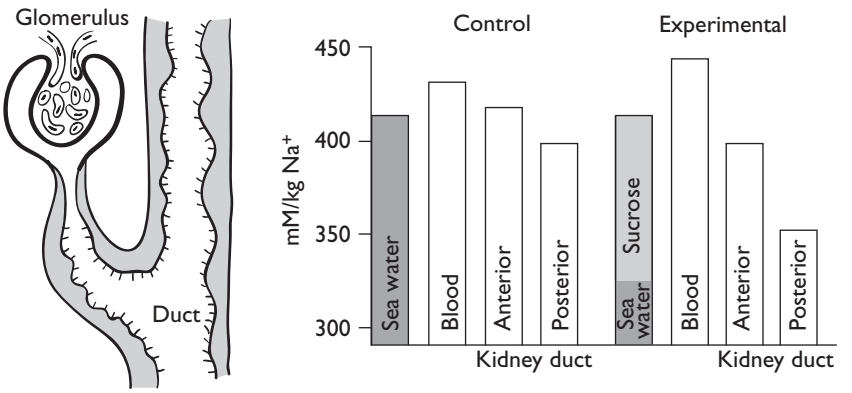


Figure 6.4 The hagfish kidney. Left: single kidney tubule of *Myxine*. Right: sodium levels in control and experimental *Eptatretus* (see text). After McInerney (1974).

Macallum long ago (1910, 1926) made the famous suggestion that the present-day ionic composition and osmotic level of vertebrate blood reflected their past history, and that of the environment their ancestors lived in. This seductively attractive (but possibly incorrect!) idea naturally made it seem reasonable to suppose that hagfish (all marine today) were from their origin a marine group. Yet careful experiments by McInerney (1974) on *Eptatretus*, where blood and kidney filtrates were sampled after placing the hagfish in seawater diluted with sucrose (to maintain osmolarity but diminish Na^+ content) have shown that the hagfish responded to low external Na^+ by increasing renal Na^+ uptake (Figure 6.4). Re-absorption of Na^+ from the glomerular filtrate is a necessary trick for any fish living in freshwater, and it is hard to see how this "surplus" capacity of the Na^+ recovery mechanism could be useful in seawater. If it were a left-over relic from an original freshwater ancestry, this would fit with Homer Smith's equally celebrated idea that the glomerular kidney first arose in freshwater as a device for excreting water (Marshall and Smith, 1930). But it now seems more likely that the glomerular kidney first arose in seawater as a device to regulate ions by producing a filtrate that could be selectively altered by tubular secretion and absorption. Divalent cations like Mg^{2+} and Ca^{2+} are at much lower levels in hagfish plasma and urine than they are in seawater (Table 6.1), but Mg^{2+} is apparently mainly excreted by the liver via the gall bladder. The low systemic blood pressure in hagfish (5–7 mm Hg) and counteracting plasma osmotic pressure due to organic solutes means that glomerular ultrafiltration is hardly possible, at least when the hagfish is quiescent. Perhaps this may mean that the hagfish kidney is an on/off ion-regulating device, filtering only when blood pressure rises during activity, and that it does not act as a water-regulating device. Notice that this view of the origin of the glomerular kidney means that the common ancestor of hagfish and of all other fishes was marine and "pre-adapted" for entry into freshwater.

How do lampreys fit into this scheme? In freshwater, like all other freshwater fish, both adult and ammocoete larvae tend to gain water and lose salts: they excrete large quantities of dilute (20–30 mosmol) urine. The mesonephric kidney (see Figure 6.12) has much longer and more complex tubules than

Table 6.1 Composition of plasma and urine (mM) in some marine fishes. Values for seawater representative. From Pang *et al.* (1977) and Griffith and Pang (1979)

		Agnatha			Elasmobranchiomorpha			Coelacanth		Teleostei			
		Myxine	*Eptatretus*	*Chimaera*	*Hydrolagus*	*Squalus*		*Latimeria*		*Fundulus*	*Muraena*	*Paralichthys*	*Lophius*
Ion	Sea water	blood	urine	blood	urine	blood	urine	blood	urine	blood		urine	
Na^+	470	487	553	338	162	296	240	197	184	183	212	59	11
K^+	10	8.4	11	11.7	7.8	7.2	2.0	5.8	9	4.8	2.0	3.4	2.0
Ca^{2+}	10	4.8	4	4.3	17	3.0	3.0	4.8	2.0	2.3	3.9	11	7
Mg^{2+}	54	9.3	15	6.1	69	3.5	40	5.3	30	2.1	2.4	78	137
Cl^-	548	500	548	353	268	276	240	187	15	146	188	124	132
HCO_3^-	–	7.2	–	2.6	–	–	–	9.6	–	13.3	–	–	–
PO_4^{3-}	–	0.4	9	–	25	2.4	33	5.1	38	5.3	–	11	2.0
SO_4^{2-}	28	3.7	7	5.2	26	3.1	70	4.8	104	–	5.7	28	42
Urea	–	2.8	9	332	52	308	100	377	384	4	9.1	–	0.6
TMAO	–	–	–	0.0	–	72	10	122	94	–	–	–	13
Amino acids	–	–	–	–	–	11.6	–	16	–	8.5	–	–	–
Osmolarity (mosmol)	1011	969	–	1046	820	998	800	932	962	363	–	295	406

those of hagfish, and micropuncture experiments have shown the gradual dilution of the filtrate as Na^+, Cl^- and (less efficiently) K^+ are absorbed from the filtrate as it passes along towards the collecting duct. But tubular absorption cannot prevent significant loss of these ions, and so, in freshwater, lampreys have special ion uptake chloride cells (p. 173) in the gill epithelia, as do freshwater teleosts, to make good the loss in the urine. This extra-renal uptake is remarkably efficient, as was shown by isotope studies in the ammocoete larva, where even in solutions containing only 30 μmol Na^+ l^{-1}, they were able to maintain stable blood composition. Recent work on lampreys and ammocoetes in freshwater using immunocytochemistry to examine transport mechanisms for salt uptake cells will be discussed after considering freshwater teleosts (p. 171).

In the sea, lampreys face the reverse problem of losing water, just as do teleosts. Unfortunately, adult lampreys are rarely caught at sea, and although the large sea lampreys (*Petromyzon marinus*) can usually be seen attached to any basking shark encountered, they drop off if the shark is caught or stranded. A recent study where two sea lampreys were detached from basking sharks by divers and then sampled, showed (not surprisingly) that they were well able to excrete urea and de-aminate amino-acids. Both this species and smaller lampreys such as the southern pouched lamprey (*Geotria australis*) and *Lampetra fluviatilis*, which enter rivers to spawn, have already begun to change their marine osmoregulatory mechanisms. So experiments on lamprey osmoregulation in seawater have only been done on partially freshwater-adapted fish, which are then adapted to various salinities in the laboratory. In seawater, such fish swallow water which is absorbed in the anterior intestine by the active uptake of Na^+ and Cl^- followed passively by water along the osmotic gradient across the gut wall. These monovalent ions are excreted by chloride cells in the gills, whereas divalent ions are mostly excreted rectally, although some are excreted via the kidney, where there is a recently discovered renin-angiotensin system controlling blood volume and osmolarity (Brown *et al.*, 2005).

Immunocytochemical work on *G. australis* has shown that the gill chloride cells degenerate when entering freshwater and the role of ion absorption is taken over by a second cell type. Lampreys pose interesting questions about speciation (p. 19) but, today, ammocoete larvae of all species are freshwater, and those that pass down to the sea upon metamorphosis (if not landlocked) are assumed to do so to seek more abundant host fishes. The essential similarity between the kidneys of hagfish, lampreys, and gnathostomes very strongly suggests the monophyly of all living fish, *and* that they all arose in the sea.

6.3 Teleosts

Teleosts show an interesting spectrum of morphological and physiological adaptations to waters of different osmolarity and ionic composition, and, what is more, some are known to have been adapted to one environment and then secondarily and relatively recently to have entered another (such as the fishes known from freshwater that lack renal glomeruli). Osmoregulation in teleosts is an integrated combination of transport activity by the kidney, gut, and gills, and is largely under rapid hormonal control (Chapter 9).

Marine teleosts

Marine teleosts have much lower ion concentrations in their bodies than the surrounding seawater, so, like marine lampreys, they drink seawater to overcome osmotic water loss. Drinking rates vary among marine teleosts as we should expect, since relative gill areas differ according to activity levels; in *Serrranus*, for example, 12% of the body weight is drunk each day. About 75% of this water drunk is absorbed in the gut, and since urine flow is small, this can maintain water balance. Chronic esophageal perfusion experiments on silver eels in seawater (Figure 6.5), have shown that the esophagus is impermeable to water, but permeable to Na^+ and Cl^- which therefore diffuse into the blood down their concentration gradients. Thus, water entering the intestine is less concentrated than seawater, and nearly isotonic with the blood. The intestine is permeable to water, which is taken up there. But because water uptake is coupled to salt intake (as we have already seen in marine lampreys), this process replaces the osmotic problem the fish faces with an ionic problem!

The history of the gradual unraveling of the way in which marine fish solve this ionic problem is an interesting one, depending on advances in technique, and to some extent on fashions in other fields. Early experiments by Homer Smith, using rubber partitions to separate the head of the fish from the rest of the body (i.e. to separate urine outflow from water that had flowed over the gills), were the first to show that extra-renal routes of salt secretion were important, and this result was soon confirmed by gill perfusion experiments on eels (using isolated heart–gill preparations), which showed that salt levels decreased in the perfusate, and increased in the external seawater (Keys, 1931). This was the first evidence for ion transport across any epithelium. With his colleague Willmer (Keys and Willmer (1932)), Keys discovered a special mitochondria-rich cell type in the gills, which they called chloride cells, suggesting (reasonably) that they actively secreted Cl^- from the blood into the sea-

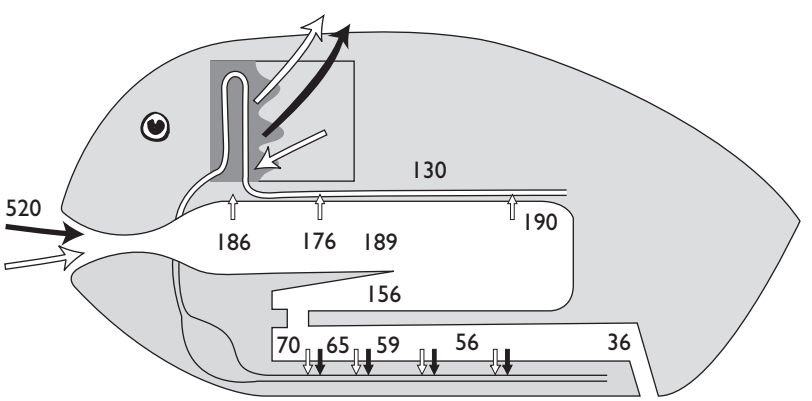


Figure 6.5 Schematic diagram showing gut osmoregulatory activity in the silver eel *Anguilla* (see text). The eel has been shortened to fit onto the page. Open arrows: water movement; solid arrows: salt movement. The numbers are milliequivalents of Cl^- per liter: note that salts are absorbed in the esophagus which is impermeable to water. Partly after Ando *et al.* (2000, 2003) and Kirsch *et al.* (1981).

water. It took nearly 20 years before any direct evidence was provided for this suggestion. At about this time, radioactive tracers such as ^{24}Na became available to biologists, and largely due to the (then young) distinguished French physiologist Jean Maetz, who persuaded the French Atomic Energy Authority to support his experiments, a series of tracer experiments was begun to monitor salt fluxes between fish and the surrounding water.

At once, the subject was revolutionized. Maetz soon found that a completely unsuspected massive salt influx took place across the gills (Figure 6.6). This was found to be 5–10 times greater than salt entry from drinking seawater, and it was obvious that salt excretion from the gills was much greater than hitherto supposed (Table 6.1). Theories of just how this salt excretion is driven have had an interesting history (see Maetz (1970), for an overview up to that time) Perhaps because in other epithelia like frog skin, active Cl^- transport was absent, attention focused on Na^+ rather than Cl^- and Na^+ transport seemed to be the main driving force for salt secretion across the gills. In the 1970s (after Maetz' untimely death in a road accident) it was concluded, by using the much less complex opercular membrane preparation instead of the gill (see caution on p. 173), that that active Cl^- transport by the chloride cells was the driving force for salt secretion by the gills, as had originally been suggested nearly 50 years before! In the 1990s, however, evidence accumulated that more probably the driving ion pumps are the classic Na^+/K^+-activated ATPase sodium pumps (salt transport is blocked by ouabain). The final direct proof that it was indeed the chloride cells of the gills and opercular membrane that are the site of Cl^- excretion was provided by the use of a vibrating probe that measured negative current peaks over the tips of secreting chloride cells visualized with a fluorescent ionophore. More recently, immunocytochemical localization of the transport proteins has proven valuable in distinguishing between different categories of ion secreting cells (as in lampreys, p. 173).

Chloride cells in marine teleosts

Chloride cells have a very particular structure (Figure 6.7). The basal (blood) and lateral sides are very extensively infolded to make a complicated system of smooth branching tubules extending almost to the apex of the cell, which is exposed to the seawater flowing over the gills. As seen in Figure 6.7, the smooth tubular system (STS) represents extra-cellular space: the cell cytoplasm is

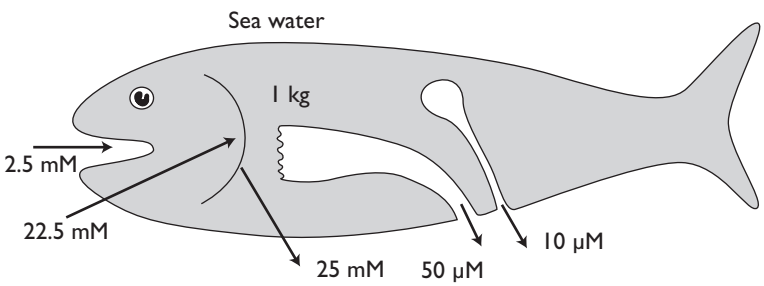


Figure 6.6 Salt balance in a marine teleost as revealed by radioactive tracer experiments. After Potts (1976).

filled with mitochondria closely packed among the STS, leaving a small apical region exposed via a crypt partially roofed by neighboring epithelial cells to which the chloride cell is linked by deep tight junctions. Chloride cells are never on their own, but are associated in groups, sometimes with accessory cells to which they are linked by leaky junctions. The STS membranes are lined with closely packed regular particle arrays, which appear to be almost solid masses of the enzyme Na^+/K^+-activated ATPase. Recent work using immunocytochemistry with heterologous antisera raised against mammalian erythrocytes has shown the existence of a Na^+ gradient from base to apex of the chloride cell, and it has been possible to isolate two types of chloride cell in physiological conditions. Figure 6.8 shows the current view of salt secretion by the chloride cell, based on work on the opercular epithelium of *Fundulus*, and the better-known rather similar Cl^--secreting cells of the rectal gland (see p. 180) in the spurdog (*Squalus acanthias*). The primary driving force for Cl^- secretion is the STS Na^+/K^+-activated ATPase sodium pump which keeps cytoplasmic Na^+ low. This pump is perhaps controlled by intracellular nitric oxide (NO) production (Ebbesson *et al.*, 2005), for the enzymes producing NO and the sodium pump were shown (by immunocytochemistry) to be co-located within the chloride cell. In the shark rectal gland cells, cytoplasmic Na^+ is 20 mM, compared to 280 mM in the body fluid. This large Na^+ gradient provides the energy for driving carrier-mediated electroneutral entry of Na^+ and Cl^-. Two Cl^- are co-transported with each Na^+ and K^+ in the shark rectal gland (as

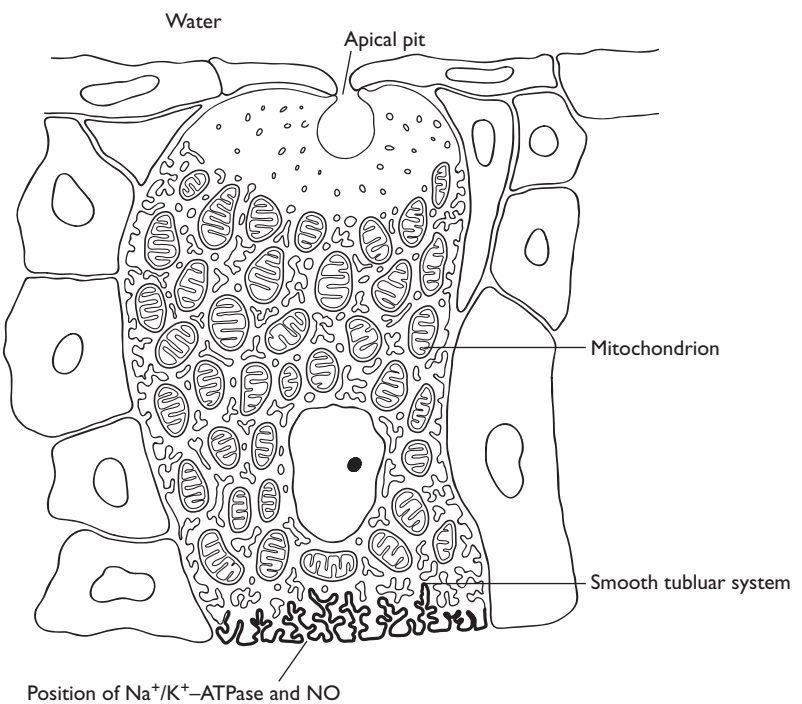


Figure 6.7 Ultrastructure of chloride cell. Modified from Degnan *et al.* (1977).

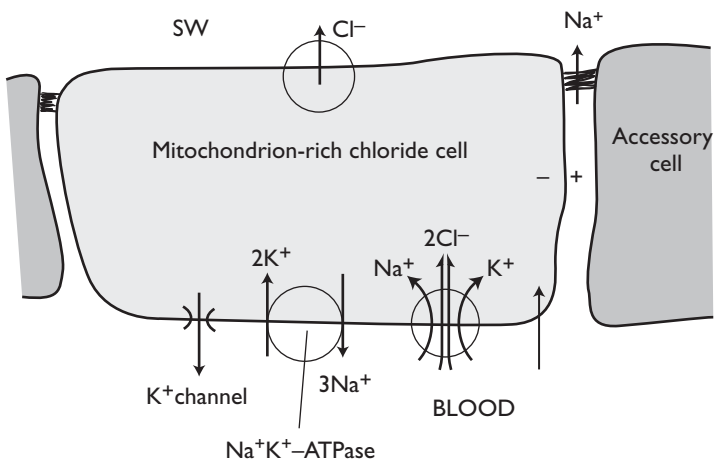


Figure 6.8 Recent model for NaCl extrusion by marine teleost gill. Na^+. K^+, and Cl^- movements are shown. The transepithelial potential across the gill epithelium (plasma side positive to seawater) drives Na^+ out of fish via leaky tight junctions between chloride and accessory cells. After Evans *et al* (2005).

in the mammalian kidney), but this remains to be conclusively proven for the teleost chloride cell. Cl^- then diffuses to the apex of the chloride cell, where it passes out via Cl^- channels. Because the membrane potential of the chloride cell is negative with respect to the seawater, any increase in cytoplasmic Cl^- will lead to corresponding Cl^- exit to the seawater. Na^+ by contrast leaves passively by the cation-selective paracellular route ending in the leaky tight junctions between chloride cells, following the transepithelial gradient established by the movement of Cl^-. The rate of movement of salt across the gills of marine teleosts brought about by the chloride cells is very high, across their apices it is continuously about the same as that across the squid axon membrane at the height of the action potential! Direct experiments on opercular membranes of teleosts adapted to seawater have shown area-specific surface current of 18 mA cm^{-2} and conductance of 580 mS cm^{-2} making them among the most actively transporting and conductive cells ever found. Experiments on *Fundulus* have shown that, in seawater, the mitochondria-rich "chloride" cells of the opercular epithelium and the gills work in the same way, to extrude Cl^- and Na^+, but in freshwater, they act differently. In the gills, they actively absorb Na^+, but not Cl^-, while in the opercular epithelium they actively absorb Cl^- but not Na^+.

However, Cl^- secretion in marine teleosts is not the only function of the chloride cell. We might well suppose that such cells would be less abundant or even absent in freshwater fishes, where this function is exactly the opposite of what is needed, and indeed they are less conspicuous in the gills of freshwater fishes, multiplying in the gills of anadromous fish such as salmon, as the smolts prepare to pass downriver to the sea. In the freshwater *Tilapia* it has been shown that chloride cells take up Ca^{2+} from solutions as dilute as 0.2 M Ca^{2+}; perhaps in freshwater they should rather be termed *calcium* cells. Interestingly, Marshall *et al.* (2005) have examined the process whereby

hypotonic shock (i.e. movement into freshwater) rapidly inhibits Cl^- secretion by chloride cells. This is a direct osmotic effect, and, as they point out, osmosensing by transporting cells themselves does not involve hormonal or nervous regulation. Recently, further work on *Tilapia* has shown that a mammalian aquaporin homolog is expressed in the basolateral region of chloride cells in seawater and in freshwater, presumably involved in volume regulation and osmoreception.

Freshwater teleosts

In freshwater, water enters across all permeable surfaces and there is a large concentration gradient favoring outward diffusion of salts across these surfaces So drinking rates are low, and water influx is met by excreting large amounts of dilute urine (between 0.1 and 1.4 ml body weight $^{-1}$ h $^{-1}$, about 10 times the values for marine teleosts). The urine is much more dilute than the plasma (see Table 6.2) because while little water is absorbed from the glomerular filtrate as it passes along the tubule, salts are very efficiently resorbed. For example, measurements on some North American freshwater fishes have shown that over 99.9% of the Na^+ and Cl^- passing into the glomerular filtrate are resorbed, the filtrate osmolarity falling from 220–230 mosmol to so low as 20–80 mosmol. Water balance can be maintained by the excretion of copious urine, but, to maintain salt balance, freshwater teleosts (such as freshwater lampreys) have to have a high affinity salt-uptake mechanism at the gills. The efficiency of this mechanism is manifested by the low rate of loss of salts in freshwater teleosts as compared with marine teleosts (Table 6.1) and by the accumulation of Na^+ from very dilute solutions (> 10^{-4} M).

Various lines of evidence indicate that Na^+ and Cl^- uptake are independent; selective blocking of one does not affect the uptake of the other. In 1939, the Danish physiologist August Krogh suggested that salt uptake in freshwater fishes was linked to acid-base metabolism, Na^+ being exchanged for NH_4^+ and Cl^- for HCO_3^-. Certainly, when fish are in a steady state with the water they are living in, the rate of NH_4^+ loss is similar to that of Na^+ uptake. But this cannot be the whole story, for in seawater marine teleosts excrete NH_4^+, and at the same time excrete Na^+ rather than absorbing it. The puzzle was in part resolved when it was found that Na^+ could also be exchanged for H^+. Figure 6.9 compares two goldfish (*Carassius*) each using a different mechanism, where the sum of the two processes is well correlated with Na^+ uptake. At present, the tentative scheme for Na^+ uptake at the gills is that a H^+-ATPase in the apical membranes of gill *epithelial* cells (not mitochondrion-rich cells) secretes protons to generate a negative potential across the membrane, so driving Na^+ inwards. In acid freshwater around pH 4, the influx of Na^+ in brown trout (*Salmo trutta*) is reduced almost to zero, while NH_4^+ excretion increases, and this is presumably because H^+/Na^+ exchange is blocked by the high H^+ concentration ratio between plasma and water. What of Cl^- ? Krogh's suggestion that Cl^- is exchanged with HCO_3^- has long withstood experimental attack. The gills are the major route of CO_2 excretion (Chapter 5), and the gill epithelium contains much carbonic anhydrase, the enzyme concerned with the conversion of CO_2 to HCO_3^-. For obvious reasons, experiments on Cl^- uptake are usually made in Na^+-free solutions, but when both ions are present in the external medium, Cl^+ uptake is facilitated.

Table 6.2 Composition of plasma and urine (mM) in lampreys and in some freshwater fishes. From Robertson (1974), Holmes and Donaldson (1969) and Hickman and Trump (1969)

		Agnatha (landlocked sea lamprey)				Chondrostei	Holostei	Teleostei			
		Blood			Urine	(sturgeon in FW)	(*Amia*)	Charr (*Salvelinus*)		Catfish (*Ameiurus*)	
Ion	Lake Huron water	ammocoete	parasitic adult	spawning adult	(adult in FW)	blood	blood	blood	urine	blood	urine
Na^+	0.02	103.0	137	136	4.8	155.8	132.5	161	17.4	122	12.2
K^+	0.05	3.4	3.3	5.1	0.99	4.3	2.0	2.8	2.5	2.7	1.61
Ca^{2+}	0.9	2.4	2.2	1.8	–	2.3	5.3	2.05	0.95	–	–
Mg^{2+}	0.25	1.6	2.0	2.7	–	1.47	0.4	0.75	0.55	–	–
Cl^-	0.05	91.0	122.0	112.0	4.7	119.7	119.5	140.6	8.1	110.0	18.0
SO_4^{2-}	2.3	0.1	0.1	0.7	–	0.7	2.2	–	–	–	–
HCO_3^-	1.75	6.0	5.0	5.2	–	–	–	–	–	3.4	0.4
Osmolarity (mosmol)	–	–	–	241.0	36.0	318.0	–	328.0	36.2	–	–

–, no data. FW = freshwater.

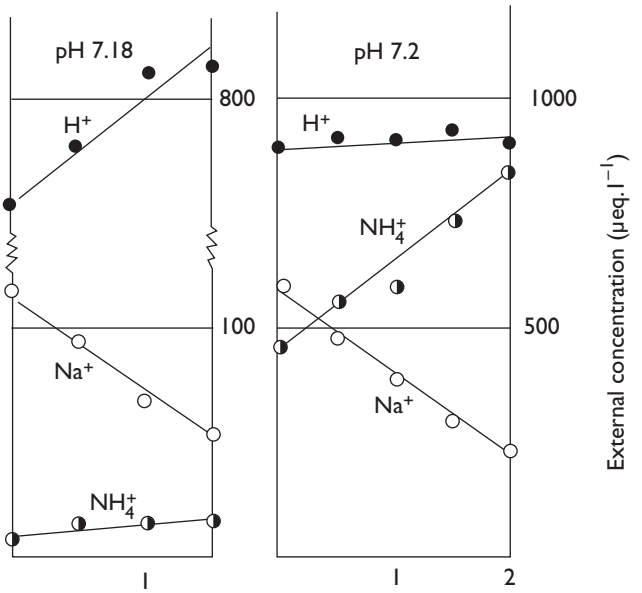


Figure 6.9 Sodium uptake in two goldfish. Measurements were made of external Na^+, titrable acidity, and total ammonium ($NH4^+$). Note that the measurements for the fish on the left show Na^+ and H^- exchange, while those for the fish on the right show Na^+–$NH4^+$ exchange. After Maetz (1974).

It is important to remember that these processes take place across the outer membrane of the respiratory epithelial cells of the secondary gill lamellae, in contrast to the mechanisms for Cl^- and Na^+ of the chloride cells in seawater. But it has also been shown that gill and opercular chloride cells of the little killifish *Fundulus* adapted to freshwater actively take up Cl^-. There is good evidence as well for two different kinds of mitochondrion-rich cells in freshwater adapted guppies (*Lebistes*), and there is certainly scope for further studies here, as well as for more work on Ca^{2+} uptake.

Freshwater lampreys and ammocoetes

Recent immunocytochemical studies (Choe *et al.*, 2004) on ammocoetes and adults of the pouched lamprey *Geotria australis* have shown that there are two types of mitochondrion-rich cells, and they propose that they function as in Figure 6.10.

6.4 The Kidney and Salt Balance

Apart from a brief foray into the Agnathan kidney, we have so far considered almost entirely *extrarenal* routes of ion balance and excretion, and the role of the kidney has hardly been mentioned. The elongate gnathostome fish kidney is mesonephric, and like those of adult lampreys, retains a segmental structure. This is most obvious in their blood supply, which is essentially venous (Figure 6.11) with a renal portal blood supply, and they operate at low systemic blood pressures not above 2.67 kPa (20 mm Hg). Fish kidneys are particularly

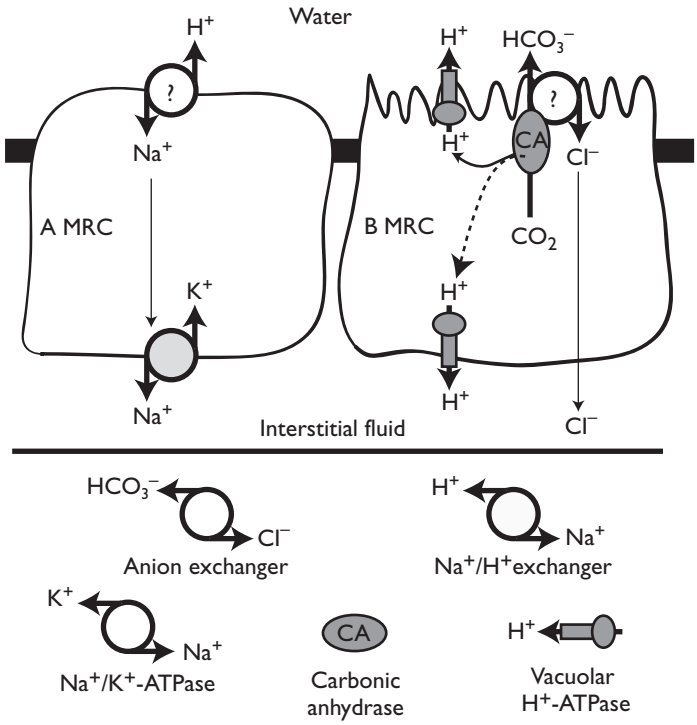


Figure 6.10 Two types of mitochondria-rich cells in freshwater lamprey gills, with different functions. From Choe *et al.* (2004).

interesting because the nephrons in different fishes are remarkably diverse and this makes it possible to infer (even without micropuncture), the functions of the different segments by comparing the nephrons of fish living in different habitats (Figure 6.12).

The most striking modification of the nephron is found in some marine fish such as the angler *Lophius* where the glomerulus is much reduced or lost completely. So far, some 30 species of marine teleosts in six families have been found to have aglomerular nephrons, and there are even some aglomerular Siamese syngnathids which have secondarily entered freshwater! As so often in physiology, the study of special cases has been rewarding, and the aglomerular kidney is no exception, for it was here that tubular secretion was first demonstrated in 1928 by the excretion of phenol red in *Lophius* and inulin was found (paradoxically!) to be the tracer of choice for glomerular filtration rate (GFR). The enormously large "giant" cottid nephrons are ideally suited for micropuncture studies, but as yet (so far as the authors are aware), remain to be investigated.

There are wide simultaneous variations in GFR and urine production in normal fishes, without change in urine osmolarity, and it seems that these involve recruitment of glomeruli under varying conditions. Since systemic blood pressure is always low, even small variations in pressure can "shut down" glomeruli or bring them into use, and coupled with linked changes in tubular absorption, can greatly change urine production without change in urine

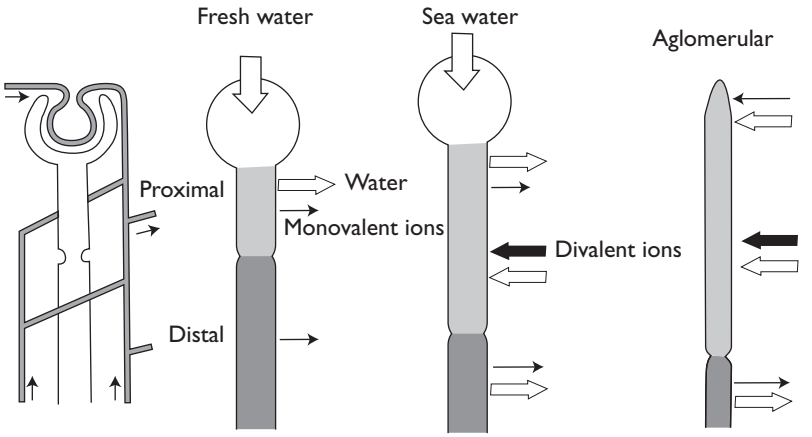


Figure 6.11 The teleost nephron. Left: blood supply (venous except to glomerulus); right: three functional types of nephron in freshwater and marine teleosts. Note development of proximal region in marine teleosts. In the aglomerular nephron, the distal region includes also the collecting ducts and bladder. After Lahlou (1981).

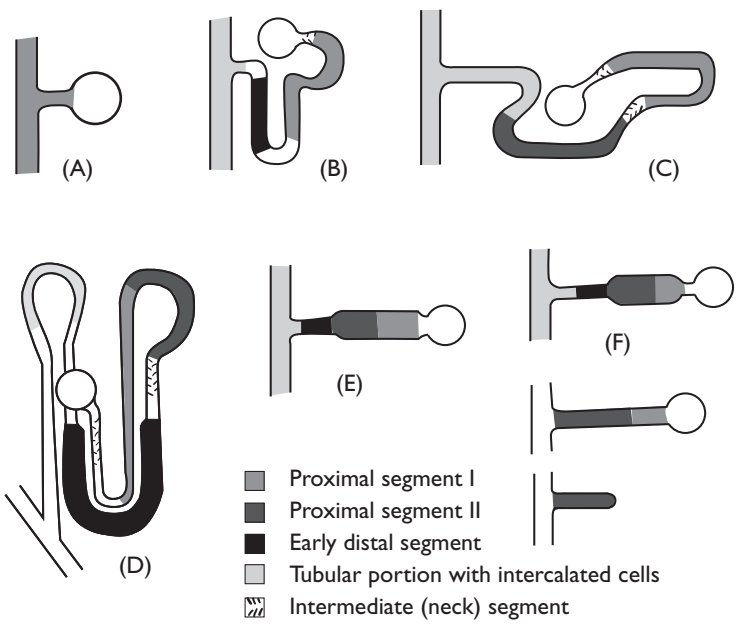


Figure 6.12 Comparison of nephron structure in different fish groups. (A) Hagfish; (B) lamprey; (C) lungfish; (D) elasmobranch; (E) sturgeon; (F) teleost (note differences between tubule structure of glomerular and aglomerular teleost nephrons). Light stipple: collecting duct. After Hentschel & Elger (1989).

osmolarity. In the trout, for example, GFR measurements in single nephrons have shown that in freshwater, around 45% of the nephrons are filtering, whereas in seawater only 5% filter. This is a flexible system, well suited to the

mainly automatic regulation of urine production, particularly so for euryhaline fishes suffering changing osmotic loads as they move to waters of different salinity. Recent work on perfused trout and also perfused dogfish kidneys have shown that, as in mammals, in addition to the systemic renin-angiotensin (RAS) system, there are local systems producing angiotensin II. Addition to the perfusate of the inhibitor captopril (which blocks the angiotensin converting enzyme), leads to profound glomerular diuresis, and to changes in salt excretion, indicating an RAS system within the kidney itself.

As we should expect, the changes in GFR leading to changes in urine production are also under the control of pituitary hormones such as prolactin, isotocin, and arginine vasotocin: hormonal release itself being controlled by a central nervous system osmoreceptor.

Tubular structure and function

Tubular structure and arrangement varies much in different fishes (Figure 6.12). Apart from the hagfish, much the simplest is that of the teleost (hardly surprising recollecting the importance of extrarenal routes that were considered a few pages back). In freshwater fishes, the proximal segment absorbs water and some monovalent ions, and the distal segment absorbs monovalent ions. In marine fishes, where the glomeruli are often much reduced, urine flow is significantly greater than GFR. The proximal tubule secretes Cl^- and Na^+ (driven by water excretion), but it also secretes divalent ions, in particular Mg^{2+} and $SO_4{}^{2+}$, which enter the plasma via the water the fish drinks. Both ions are at much higher levels in the sea (around 50 mM Mg^{2+} and 25 mM $SO_4{}^{2+}$) than in the plasma. These ions are not excreted extrarenally, and although it would scarcely do the marine teleost nephron justice to consider it purely as a magnesium sulfate pump, this is certainly one of its main roles. The distal segment (lacking in many acanthopterygians) mainly absorbs water, and although some monovalent ions are excreted, extrarenal routes are more important for these. After the distal region, collection ducts of various sizes in different species pass to the urinary bladder, where (since it is well-adapted for electrophysiology) various transport mechanisms have been figured out. In marine fish, an amiloride-sensitive electrogenic component and coupled mechanism are involved in NaCl uptake, and the bladder is permeable to water where it is resorbed. In freshwater, bladder water permeability in the starry flounder (*Platichthys stellatus*) is six times less than in seawater so that less water is resorbed in freshwater than in seawater.

6.5 Teleosts in Alkaline Saline Lakes

Interesting special cases are provided by the remarkable teleosts that live in alkaline lakes, such as the tilapia *Alcolapia grahami* (formerly *Oreochromis alkalicus*) of Lake Magadi in Kenya (pH 10) which feeds on cyanobacteria and *Chalcalburnus tarichi*, a small cyprinoid living in Lake Van in eastern Turkey (pH 9.8). Lake Van is a soda lake which has a salinity of 22‰ and it is so soapy that the locals wash their laundry in it without the need of soap! The tissue fluids of both fish are nearly isosmotic with the lake water. In *Alcolapia*, the most recent studies (Wood *et al.*, 1989, 2002b) have shown that plasma osmolarity is around 370 mosml kg^{-1} (lake water is 580 mosml kg^{-1}) and contains just over

10 $mmlr^{-1}$ urea (probably acting as an osmolyte). Remarkably enough, *Alcolapia* can withstand gradual transfer to water of twice the concentration it usually lives in, and then increases its plasma urea to around 30 $mmlr^{-1}$. Normally, urea is probably excreted, both by the kidneys and the gills via a UT-A type urea transporter (p. 178), for the typical teleost N excretion (85% as ammonia N and 15% as urea N) is impossible in Lake Magadi where the water is highly buffered at pH 10.

6.6 Teleost Eggs and Larvae

Teleost eggs and larvae are much smaller than the adults, and so have an unfavorable surface/volume ratio for osmoregulation, yet they are found in fresh water and in the sea, where many osmoregulate to maintain body-fluid ion concentrations between 11 and 14% (350–440 mosmol), that is similar to their adults. The developing oocytes are very permeable, and are protected osmotically by the mother. Before fertilization, the chorion is closely apposed to the vitelline (or plasma) membrane that surrounds the yolk, cytoplasm, and nucleus of the future embryo. Following fertilization, a perivitelline space usually appears between chorion and vitelline membrane as a result of water entry. The vitelline membrane is the site of osmoregulation, and, as we should expect, contains large numbers of chloride cells (more abundant and more active in marine fish). Within 12 hours after fertilization, plaice eggs can osmoregulate successfully in salinities between 5–50‰ (far above what they would meet in nature) the yolk osmolarity being regulated to 10–20‰.

Herring may spawn at salinities much lower than plaice and whatever the external salinity, the yolk is regulated to 12–15‰. After metamorphosis, they can tolerate salinities between 6 and 45‰. These are certainly impressive performances, and although they partly reflect the fact that embryonic and larval tissues can tolerate a wide range of ionic concentrations, there are certainly very active chloride-secreting cells in embryos and larvae. Like the adults, the larvae of plaice, herring, cod, and halibut drink seawater and water is absorbed in the gut (p. 167).

Larval fish have a functional pronephric kidney, although little is known of the functional development of the fish kidney. Newly hatched brown trout have a single pronephric nephron, as have chum salmon (*Oncorhynchus keta*) 33 days after fertilization, the first mesonephric nephron appears at hatching.

6.7 Osmoregulation in Chondrichthyes

Blood ionic composition in marine elasmobranchs and holocephalans is somewhat higher than in teleosts, but its osmolarity is very much higher, close to that of seawater (Table 6.1). This is because the blood contains, in addition to the usual ions, large amounts of low-molecular weight nitrogenous solutes, chiefly urea. First found in elasmobranchs in 1858, it is usually present at about 0.4 M. Various methylamine substances such as trimethylamine oxide (TMAO), betaine, and sarcosine, and some free amino-acids such as taurine and β-alanine are also present, in total around 0.2 M. So, over half of the osmolarity of the blood is due to these nitrogenous solutes, and, together with the inorganic ions, elasmobranch blood is osmotically close to seawater. Probably

marine elasmobranchs are always slightly hyperosmotic to seawater, as for example at Plymouth, where dogfish (*Scyliorhinus*) in the aquarium circulation seawater then at 1154 mosmol kg^{-1}, were found to have the serum at 1243 mosmol kg^{-1}. The gills are permeable to water, so under normal conditions there will always be a slight influx of water excreted as urine that is more dilute than the serum or seawater.

Urea is a small molecule (MW: 60) and very soluble in water. How do elasmobranchs manage to retain it? Almost all urea loss is across the gills, some 20–70 μmol g^{-1} h^{-1} in those species examined, where a Na^+-coupled urea transporter (UT) has been suggested in the gill, and where also, analysis of urea uptake revealed the presence of a phloretin-sensitive, Na^+-coupled urea antiporter on the basolateral membrane of gill epithelial cells, which returns urea to the blood. In addition, the low lipid partition coefficient of urea means a slow rate of diffusion across the lipid bilayers of cell membranes, and the extraordinarily high cholesterol content in the basolateral membrane (cholesterol:phospholipid molar ratio 3.68) retards passive urea loss, as Fines *et al.* (2001), pointed out. Archer *et al.* (2004) have suggested a possible link between the elasmobranch array of neurohypophysial hormones, and urea-based osmoregulation.

The kidney must also conserve urea, and it does so with excellent efficiency. The most striking feature of the elasmobranch kidney is that 95% of the urea in the glomerular filtrate is resorbed. Passive urea absorption by facilitated diffusion into intracellular space (and thence to the blood) by a countercurrent system was first suggested by Boylan (1972) and the morphological basis for it was seen in the little skate *Raja erinacea* after long and painstaking reconstruction from digitized electron micrographs. The inner thinner part of the distal tubule is applied to the proximal tubule, making a series of lateral bundles in the kidney. The end result of this folding of the tubule back upon itself is that a countercurrent system of five parallel tubular segments is formed within a peritubular sheath (Figure 6.13), seemingly effectively resorbing urea and other nitrogenous solutes as the glomerular filtrate passes down the tubule to the collecting duct. A most iconoclastic result was then more recently obtained in the kidney of the small shark *Triakis scyllia*, using the immunocytochemistry of antisera raised against a cloned cDNA for a specific UT (Hyodo *et al.*, 2004). The UT was expressed exclusively in the final segment of the lateral bundle zone, that is in the collecting tubule of the kidney, suggesting that this was the main site for urea reabsorption. Na^+/K^+-ATPase was not detected in the collecting tubule, suggesting that transport (reabsorption) of urea in the collecting tubule occurs transcellularly by facilitated diffusion. The localization of UT on both apical and basolateral membranes supported this idea. Meanwhile, other nephron segments expressing Na^+/K^+-ATPase may function as an active component of urea transport.

So far, as Hyodo *et al.* (2004) were careful to point out, there has been no physiological evidence for urea permeability through the collecting tubule, and further results are needed before we can definitely conclude that the counter-current system of the elasmobranch kidney only plays at best a subsidiary role in urea resorption. The absence of a counter-current system in truly freshwater elasmobranchs (see below) is surprising if it does not play a role in urea resorption. In contrast to the limited localization of UT, the transport enzyme Na^+/K^+-ATPase is distributed in the basolateral membrane

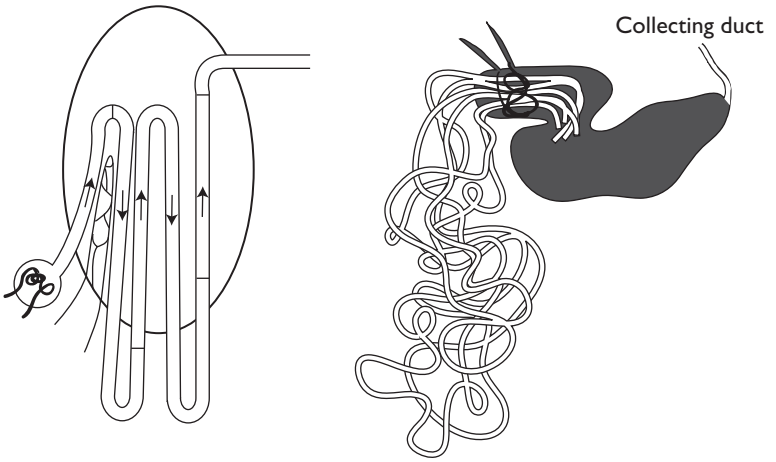


Figure 6.13 Ray nephron showing countercurrent absorption arrangement. After Lacy *et al.* (1985).

of numerous tubular segments both in the sinus zone and the bundle zone. However, in the collecting tubule, the role of the counter-current system may be to permit facilitated urea diffusion driven by Na^+/K^+-ATPase. Tubular urea resorption is tightly coupled with sodium resorption at a fixed ratio of 1.6:1 over a wide range of urine flow rates and urea resorption values, indicating the presence of a sodium-coupled urea co-transporter. The hormones involved in the blood osmotic pressure regulation of elasmobranchs are still largely unknown. It has been suggested that the great diversity of oxytocin-like hormones in elasmobranchs (see p. 267) expresses a release from an evolutionary receptor-binding constraint, so that amino-acid substitutions reflect neutral evolution. In contrast, the preservation of vasotocin suggests a selective pressure, which may be related to the regulation of renal urea transporter-recruitment mechanisms, as has been shown for vasopressin in mammals.

Urea and energy metabolism: a revision

The oxidative fuel for elasmobranchs, unlike almost all other vertebrates, is provided not by lipids but by ketone bodies, such as β-hydroxybutyrate. Until recently, this unusual metabolism seemed likely to be linked to urea-based osmoregulation. However, Speers-Roesch *et al.* (2006) have examined a freshwater potamotrygonid ray, the euryhaline stingray *Taeniura lymma*, and a marine shark (each having different amounts of urea in the blood and other tissues, see p. 181) and find that there is no correlation between urea levels and ketone bodies. As T. H. Huxley once wrote, "The great tragedy of Science is the slaying of a beautiful hypothesis by an ugly fact!" When urea is not needed as an osmolyte, as in the freshwater rays, it is deaminated rather than shunted to urea production.

Urea and proteins

We are so accustomed to making up dogfish Ringer solutions by adding urea, that it is difficult to realize what an unexpected thing this is to have to do. At

0.5 M, urea disrupts proteins in most animals, including mammalian collagen, most enzymes, and hemoglobin. Either elasmobranchs have modified their proteins in some way to resist the effects of urea (as the Na^+ channels of tetradontids have been modified so that they resist the effects of their own tetrodotoxin), or, they protect their proteins otherwise. Naturally enough, they do both! Elasmobranch hemoglobins and certain enzymes are resistant to urea denaturation and indeed the eye lens protein and the M4 lactate dehydrogenase (LDH) enzyme actually require urea to function properly. Other elasmobranch proteins are not adapted to withstand urea, and in their case, as Yancey and Somero (1980) showed at the Scripps laboratory, the destabilizing effects of urea are counteracted by the other nitrogenous solutes, especially TMAO. Several muscle enzymes, such as LDH, creatine kinase, and pyruvate kinase are protected in this way, and maximum protection in vitro was found to be given by TMAO:urea ratios of 2:1, the ratio actually found in the blood.

Extrarenal salt excretion and the rectal gland

Blood and urine values for Na^+ and Cl^- are similar (Table 6.1) and lower than in seawater, so there must be extrarenal routes for NaCl excretion. There are relatively far fewer chloride cells on the gills than in teleosts, and branchial Na^+/K^+-activated ATPase activity is relatively low, so the gills seem likely to be a site of net salt uptake rather than extrusion. This rather puzzling situation was resolved by the unexpected discovery that a rectal gland in the spur dogfish (*Squalus acanthias*) secreted a fluid which was almost pure NaCl at a concentration around 550 mM l^{-1} (twice that in the body fluids), while urea was only 10–20 mM l^{-1}. The gland opens to the rectum by a short duct just behind the spiral valve, and is made up of a mass of tubules composed of cells very like the chloride cells of teleost gills (and with the same high Na^+/K^+-activated ATPase activity). In holocephali, the gland consists of nodules in the rectal wall, while in *Latimeria* it is like those in sharks (Figure 6.14). As we have already seen ("Chloride cells in marine teleosts," above), because the Cl^--secreting cells of the rectal gland are a single population and are accessible for

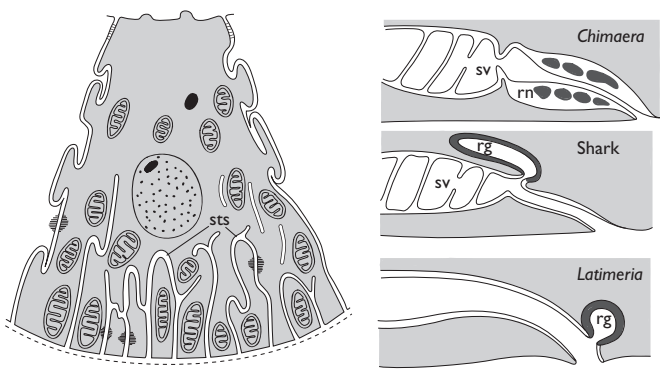
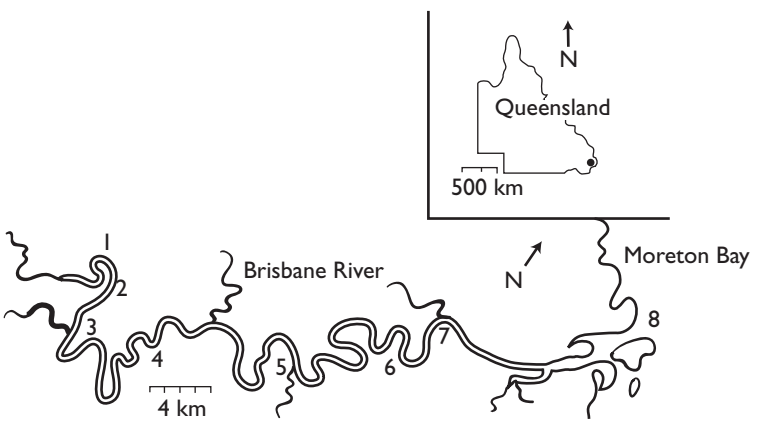


Figure 6.14 The salt-secreting rectal gland. Left: schematic ultrastructure of a rectal gland cell in *Latimeria*. sts: smooth tubular sytem. Right: rectal glands (rg) in elasmobranchiomorphs and *Latimeria*. In Holocephali, the rectal gland tissue forms nodules (rn) in the rectal wall; sv, spiral valve. After Lagios (1979).

experiment, much of what was first known about chloride cell operation has come from studies on the elasmobranch rectal gland. Since at least the small spurdog *Squalus acanthias* survives perfectly well after removal of the rectal gland, there are obviously other routes for Cl^- excretion, possibly via the kidney.

6.8 Freshwater Elasmobranchs

A small number of elasmobranchs are euryhaline, including sawfishes (*Pristis* and *Pristiurus*), stingrays (*Dasyatis*), and blacktip and bull sharks (*Carcharinus melanopterus* and *C. leucas*). They enter estuaries and are able to live for long periods in brackish or even freshwater. Bull sharks, for example, swim up the San Juan River into Lake Nicaragua, 350 km from the sea. In freshwater, they are much more hyperosmotic to the water than freshwater teleosts (Na^+: 200 mmol l^{-1}; Cl^-: 180 mmol l^{-1}; urea: 132 mOsmol l^{-1}) and survive by reducing urea and ion levels, and by secreting a copious flow of dilute urine (9–15 times more than marine species). The same species ascends the Brisbane River (Figures 6.15 and 6.16 and Table 6.2) where they have been studied by Pillans and Franklin (2004). The experiments of Morgan *et al.* (2003) on marine sting rays placed in diluted seawater have shown that the activity of renal urea transporters can be modified. Such euryhaline elasmobranchs were first studied by Homer Smith in the Perak River of Malaysia, but the most interesting freshwater elasmobranchs are the stingrays that live in the Amazon and Orinoco drainages. There are 20 species in three genera, two of which are monotypic (*Paratrygon* and *Plesiotrygon*). These stingrays live up to 4500 km from the sea, and are stenohaline, dying if exposed to more than 50% seawater. Since fossils of the same family (Potamotrygonidae) have been found in Tertiary deposits of the Parana basin, it seems probable that the family has lived in freshwater for millions of years. Lovejoy (1997; Lovejoy *et al.*, 2006) suggests that a massive marine incursion into the Amazon basin took place in



1: 3 mOsmol Kg^{-1}; 4: 113–23 mOsmol Kg^{-1}; 5: 427–565 mOsmol Kg^{-1}
7: 740–780 mOsmol Kg^{-1}, 8: 1047 mOsmol Kg^{-1}

Figure 6.15 The Brisbane River, South Queensland, showing osmolarity along its course to the sea at Moreton Bay. From Pillans and Franklin (2004).

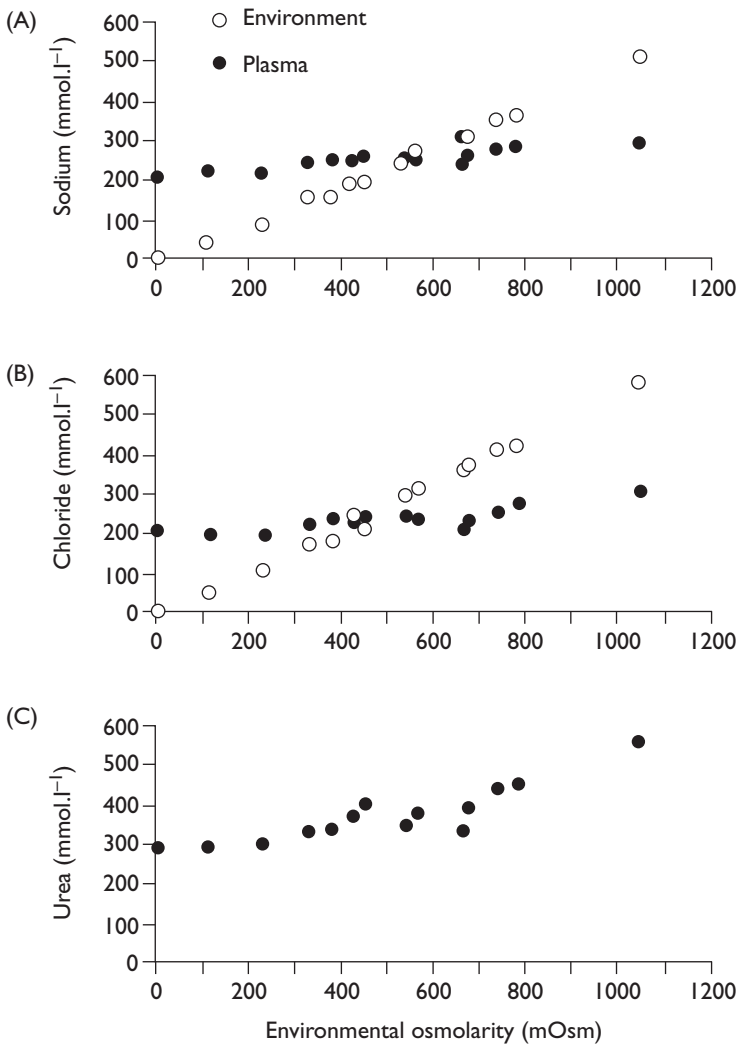


Figure 6.16 mmol.l^{-1} of Na^+, Cl^- and urea in the blood of sharks and in the river water, sampled along descent of river to the sea as its salinity increases (abscissa: mosm l^{-1}). From Pillans and Franklin (2004).

the early Miocene (15–23 mya) and that a variety of marine fish, including stingrays, were then brought into freshwater. Blood urea and ion levels in these stingrays are entirely different to those of euryhaline elasmobranchs, for *Potamotrygon* and its relatives have done just what we should have advised them to do: reduced urea to around 1 mmol l^{-1}, and reduced Na^+ and Cl^- to levels similar to those of freshwater teleosts (Table 6.3).

The rectal gland is superfluous and is vestigial, GFR is very high (suggesting copious dilute urine), but as yet urine production and possible gill uptake mechanisms await further investigation. Wood *et al.* (2002a) studied *Potamotrygon* gills for Na^+ and Cl^- uptake, but definitive proof that they are similar to those found in the euryhaline stingray, *Dasyatis sabina*, which has two types of ion

Table 6.3 Blood values for Na and Cl: mmol l^{-1}, urea, total ammonia: μmol l^{-}, osmolality mosmol kg^{-1}. Rio Negro values all μmol l^{-}. From Wood *et al.* (2002a)

	Na	Cl	Osmolality	Urea	NH_3	pH	Ca^2
Blood	178.2 ± 11	146.2 ± 11	319.6 ± 8.5	1221 ± 185	306. 6 ± 61		
Rio Negro	30	23				5.9–6.1	10

uptake cells, is still awaited. *Potamotrygon* nephrons lack the anatomical countercurrent loops which may act to conserve urea; in consequence even when transferred to 50% seawater, they cannot increase blood urea significantly.

6.9 *Latimeria*

Latimeria blood is similar in composition to that of marine elasmobranchs (Table 6.1). Unlike elasmobranchs, however, total osmolarity seems to be slightly less than seawater, so, if this is correct, *Latimeria* resembles marine teleosts in facing a slight water loss and gain in ions across the gills and other permeable surfaces. In sharks, Na^+ is at the same level in urine as in blood, (though Cl^- is lower), but what seems strange is that urea levels in blood and urine are the same. These measurements were made at the ureter, and it may be that urea is resorbed in the bladder, as it is in the urea-containing crab-eating frog, *Rana cancrivora*, which lives in estuaries. Choride cells are not abundant in the gill epithelium, but there is a rectal gland with cells of similar ultrastructure to those of the shark rectal gland. Apart from sharing urea in the blood, and a rectal gland, *Latimeria* also resembles sharks in pancreatic and pituitary histology, but the existence of urea retention in *R. cancrivora* has prevented a suggested relationship between *Latimeria* and sharks being taken seriously.

6.10 Which is the More Efficient Way of Coping with Life in Seawater: Urea Retention or NaCl Excretion?

It seems clear that urea retention is an adaptation to the marine environment, and it is natural to enquire whether this is a more, or less, energetically costly way of coping with seawater than that used by marine teleosts. Although calculations are only "order of magnitude" they suggest that in terms of mmol ATP kg^{-1} hr^{-1} used, Cl^- excretion by the teleost is about 20 times more costly than urea synthesis and limited rectal gland Cl^- excretion in the elasmobranch. Yet retaining urea requires the presence of several unusual features for fishes. For example, the ornithine-urea enzyme cycle is incomplete or inactive in lampreys and teleosts, but this is probably secondary, and it was likely present in ancestral gnathostomes. Also it was often stated in older texts that urea retention is easier for fish that are large and have internal fertilization, because the surface/volume ratio will be greatest in embryonic stages when it will be less easy to produce sufficient urea to counteract its loss across permeable surfaces. However, the egg-cases (mermaids purses) of ovoviviparous elasmobranchs such as rajids and the dogfish *Scyliorhinus* are completely permeable to urea, and the tiny developing embryos can already osmoregulate and retain urea.

Plasma ion content and the evolutionary history of different groups of fishes

Different fish groups have body fluids of characteristic ionic composition, for example lungfish and *Polypterus* are the most dilute, while hagfish are the most concentrated. In "Hagfish, lampreys, and the origins of the glomerular kidney" we saw that the isosmolarity of hagfish blood with seawater might be taken to indicate a marine origin for the group, and zoologists of a speculative bent have naturally wondered whether plasma ionic composition in other fish groups might at least hint at their evolutionary history. Figure 6.17 shows an attempt (due to Lutz, 1975) to work out how body-fluid composition (taken as the sum of Na^+ and Cl^-, the major osmotic constituents) suggests how the different groups have moved between freshwater and seawater. A good case could be made that the lowest values represented the longest history of evolution in freshwater, and that where a secondary marine phase appeared, the difference between marine and freshwater plasma concentrations was least in the groups that had most recently re-entered the sea, such as the sturgeons. Lampreys do not fit too well into this scheme since seawater values (insecurely based on fish caught in estuaries while about to enter rivers to spawn) should perhaps be even higher, yet in freshwater, blood osmolarity is nearly as low as in lungfish. Perhaps the freshwater value is the best guide to lamprey evolutionary history since it seems that migrations into the sea are of relatively recent origin.

Envoi

All fish (save hagfish) live in intimate contact with fluids very different to their body fluids. How they use different ways of coping with the problems of osmoregulation has already provided many surprises and will no doubt continue to fascinate physiologists.

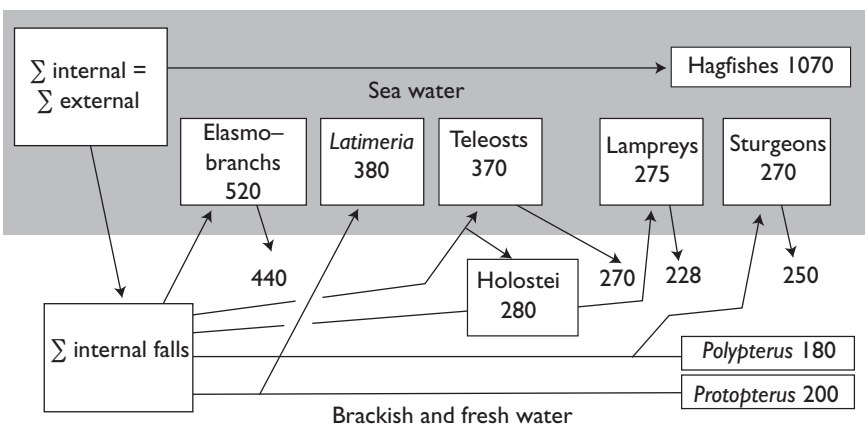


Figure 6.17 Evolutionary sequence of major fish groups, showing values of sodium and chloride in plasma (approximately equivalent to total ion content). See text. After Lutz (1975).

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7 Food and Feeding

7.1 Introduction

The great phyletic and ecological diversity of fishes discussed in earlier chapters, are reflected in the many types of food utilized by fishes as well as their many ways of acquiring food. Although most fish are classified as food generalists or opportunists, eating whatever they can which is most abundant and easily available, as does the blue shark (*Prionace*), which feeds on dead whales, fish, cephalopods, adult ascidians, gastropods, and crabs, some possess extreme specializations that permit feeding in what may seem to us to be most bizarre ways. For example, insectivores such as the archer fish (*Toxotes*) knock insects off overhanging vegetation by squirting a jet of water at them, while the much larger *Osteoglossum* leaps up to 2 m out of the water to snatch them off branches.

In a fascinating study of the cichlid fishes of the African Great Lakes, Fryer and Ilies (1972) discuss the many ways of cichlid feeding. Cichlids possess an array of morphological features, including jaw structures, mouth size, and shapes, dentition, and gill raker size and number, which allow them to occupy an even wider variety of feeding niches. Besides the typical filter, plankton picking and predatory feeding niches seen in other fishes, cichlids illustrate a number of unique feeding niches including such extraordinary specializations as head-ramming in order to dislodge scales from the sides of other fishes or even eggs or larvae that are being transported in the mouth of mouth-brooding parents, shamming dead on the bottom to decoy other fishes, and as in the Malawi eye-biter, *Haplochromis compressiceps*, sucking the eyes out of other fishes. Cichlids also provide some of the best examples of cleaning behavior among freshwater fishes (Stauffer, 1991) and scraping "aufwuchs" (an algal and detrital biolayer) from the surface of rocks (Fryer, 1959) The freshwater cichlids as well as the marine wrasses and parrot fishes (Labridae, *sensu latu*), embiotocids (surfperches), pomacentrids (damsel fishes), and odacids (Eastern Pacific butterfishes) have sometimes been classified together based on the similarity of their feeding apparatus. Although the monophyly of this grouping has been challenged by molecular evidence, these so-called labroid fishes continue to represent the epitome of feeding diversity. According to Peter Wainwright (2005) at the University of California at Davis, feeding diversity

among labrids is unparalleled among reef fish groups and is associated with high mechanical diversity in the jaws. The complex lever systems of their jaws have permitted the evolution of a wide range of physical and mechanical variation permitting a variety of feeding styles from picking parasites from the sides and gills of larger fish to capturing small invertebrates and fishes from a distance (as best illustrated by the aptly named slingjaw wrasse, *Epibulus insidiator* (Figure 7.1), in which the mouth can be thrust forward nearly 50% of the body length) to consuming relatively large prey such as heavily shelled molluscs and crabs. Within the Labridae, some parrot fishes exhibit a unique jaw apparatus which with their fundamentally different tooth morphology may explain the greater taxonomic, morphological, and mechanical diversity of parrot fishes in contrast to the wrasses *per se*. (Wainwright *et al*., 2004).

The study of the feeding apparatus and mechanics of fishes provides some of the best examples of the adage "form follows function," or as might also be said, "you are what you eat." These similarities in morphology cross phylogenetic lineages, as Norton and Brainerd found in their 1993 study – buccal and opercular pressure profiles were more similar in ecomorphologically similar feeding styles than in species that were closer related but possessed different feeding styles (e.g. predatory cichlids and basses vs. picking cichlids and sunfishes).

Rather surprisingly perhaps, although some fishes seem to have specializations that suggest a particular diet and mode of feeding, this does not prevent them from turning these adaptations to other uses. Thus, the cichlid

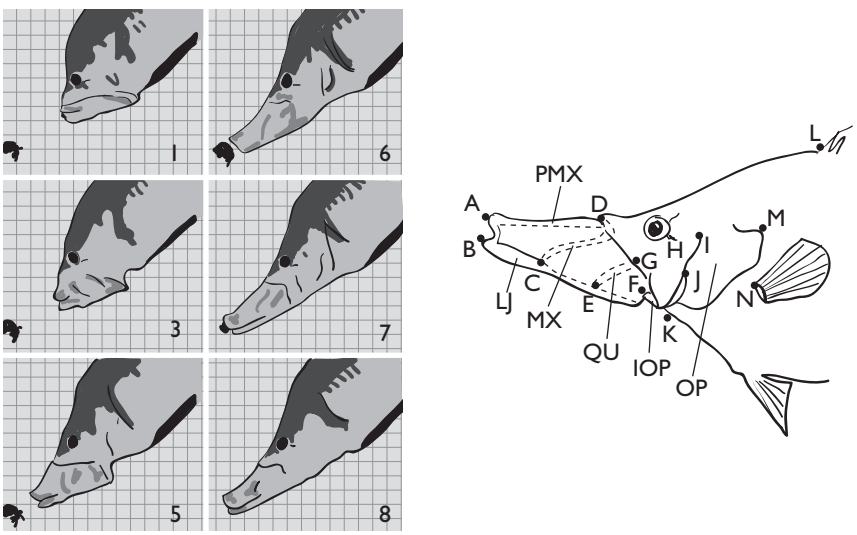

Figure 7.1 (Left) Frames 1, 3, 5, 6, 7, and 8: From a high-speed film (200 frames/sec^{-1}) of the strike of *Epibulus insidiator*. Successive frames are 0.005 sec apart. Note rotation of quadrate, maxilla, and interoperculo-mandibular ligament. Suction is apparent in frames 6, 7, and 8. See Figure 7.7 for corresponding mechanical diagrams. (Right) Diagram of a feeding *Epibulus insidiator*, with points shown (A–N) for recording kinematic variables with a computerized digitizing system. Bones of the skull are labeled as follows: LJ = lower jaw, PMX = premaxillary, MX = maxillary QU = quadrate, IOP = interopercle, OP = opercle. After Westneat and Wainwright (1989, p.139).

Petrotilapia of Lake Malawi is seemingly specialized for scraping algae off rocks with its trifid teeth, but Liem (1980) found that it could also feed in seven other ways, each involving a different pattern of jaw muscle activity. For instance, *Petrotilapia* bites scales and fins from other fishes, collects floating food, sucks invertebrates from bottom mud, and catches small fishes in mid-water (Figure 7.2). This kind of versatility in using what seems to be a morphology adapted for a single purpose may be uncommon, but it is a salutary warning to anyone attempting to interpret function from morphology alone!

7.2 Techniques for Studying Food Habits and Feeding

While most early studies of fish feeding involved capture and subsequent dissection to examine stomach contents, killing fish may be undesirable because of ethical considerations and possible population impacts (Kamler and Pope, 2001). Two non-lethal methods that are widely used to collect stomach contents from living fish are stomach tubes and pulsed gastric lavage. The former is a simple tube, usually glass or plastic, inserted through the esophagus of an anesthetized fish. Stomach contents are then removed by suction or compression of the sides of the stomach. Gastric lavage uses pulses of water, in connection with a tube, to flush contents from the stomach and is generally regarded as a more efficient technique. The use of antibodies, stable isotope ratios or nucleic acids (Rosel and Kocher, 2002) can be used to identify accurately even well-digested foodstuffs.

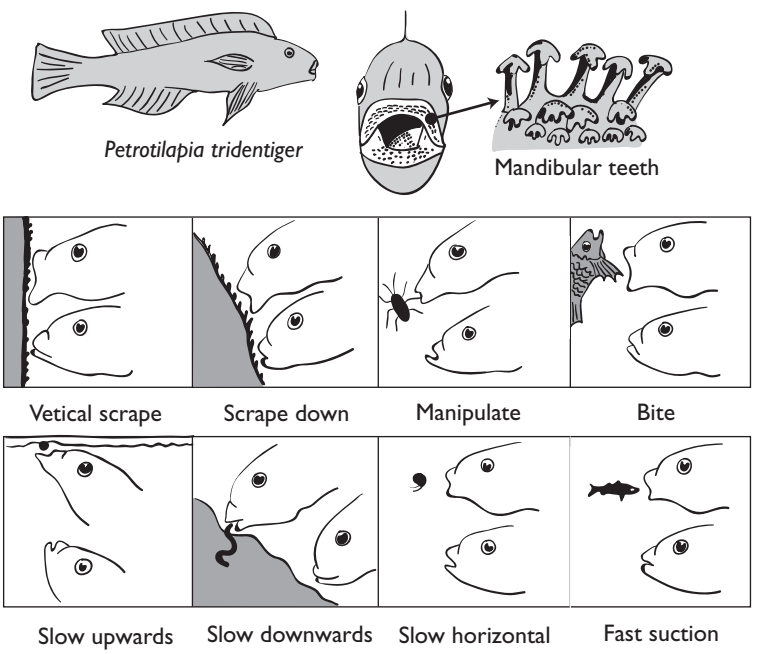

Figure 7.2 The trifid mandibular teeth and versatile feeding techniques of the Lake Malawi cichlid *Petrotilapia*. The three slow methods (bottom left) involve slow controlled suction. After Liem (1980).

Fish biologists today have come to rely on various high-tech devices and techniques to advance our understanding of feeding mechanisms. Underwater observation of feeding behavior may be recorded digitally or photographically. Data from EMG recordings, pressure transducers and fiber-optic endoscopes are combined with computer models to study and describe how food is handled and processed prior to its being swallowed (Ferry-Graham and Lauder, 2001). Digital particle image velocimetry (DPIV) utilizing lasers to illuminate reflective particles suspended in the water allow the visualization of water flow patterns inside of the fish's mouth (Higham *et al.*, 2006). High speed digital photography then shows the actual patterns of water movement that result from feeding activities.

7.3 Optimal Foraging Theory

In recent years, ecologists who are interested in how organisms exploit their environment have developed a theory of *optimal foraging*, which basically states that organisms will preferentially feed on food resources that provide the greatest long-term net nutrient return for the least energy investment or risk on the part of the consumer. Energy may be invested in finding food, capturing it, ingesting it, and finally digesting and absorbing its nutrition. Risk involves chiefly exposure to predation although some prey species possess defensive abilities that test their predators. Optimal foraging theory is subject to debate; however, actual observations on different feeding strategies tend to support it. What is not debatable is that fish must acquire enough energy from their food to survive and to reproduce.

7.4 Food Choices, Size, and Development

But optimal foraging strategies may not remain identical throughout an organism's lifespan and many species exhibit a *trophic ontogeny* or shift in food types and sizes, or feeding styles at different stages in their life cycle. These are necessitated by the fishes own increase in size, by morphological developments or by habitat shifts that accompany growth (Figure 7.3).

Adult fishes consume a great variety of foods and exhibit a great variety of feeding styles, although basically all feeding mechanisms may be categorized as either biting, ram feeding, or suction feeding based on how food gets into the mouth. Different types of food are then concentrated and processed for "swallowing" by several methods. Foods range in size from microscopic phytoplankton, bacteria, and detritus, through a progressively larger series of zooplanktons, and culminating in larger invertebrates and other vertebrates. Some fish also consume multicellular algae and vascular plants.

Although in general there is a positive correlation between the size of a fish's mouth and the size of the prey it consumes, there are some notable exceptions. Some of the largest fishes, for example whale sharks (*Rhincodon typus*) and basking sharks (*Cetorhinus maximus*), use their huge mouths to engulf and filter large quantities of small organisms. But what is "small" can be relative – a 20 meter whale shark may filter feed on juvenile tunas that are themselves 25–30 cm in length. At the other end of the size range of sharks, the diminutive 15–50 cm cookie-cutter sharks *Isistius brasiliensis* and *I. plutodus*

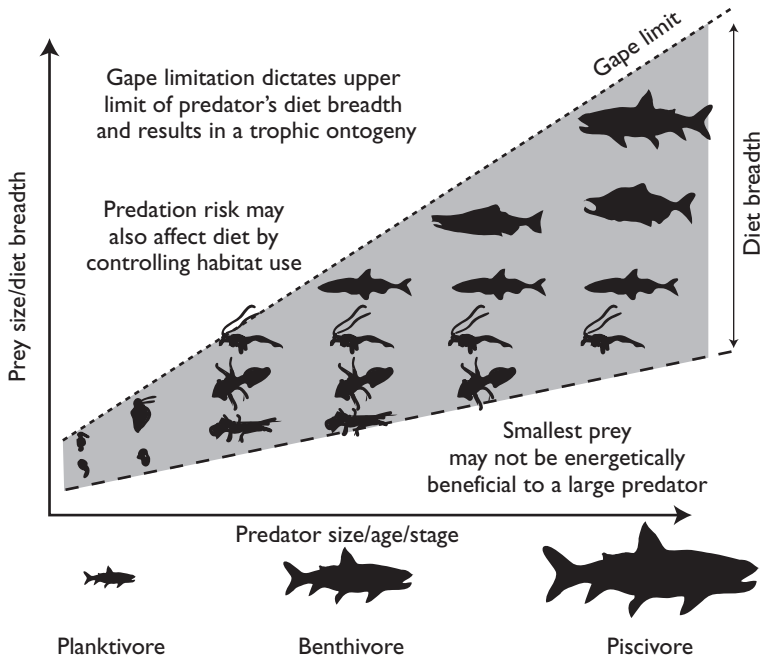

Figure 7.3 Feeding ontogeny of lake trout. Size of food as well as dietary breadth increases with growth. Adapted with permission from Brett Johnson. http://www.warnercnr.colostate.edu/class_info/fw300/flashcrd/ontogeny.gif

feed on whales and other large oceanic creatures, although they do so one small bite at a time (Figures 7.4A, B).

7.5 Food Capture

According to Karel Liem (1980) most of the enormous variety and range of foods eaten by fishes is obtained through only three basic feeding styles: ram feeding, suction feeding, and manipulation or biting. Virtually all species use one, and, because they are not mutually exclusive, most species use two of these styles.

Much of the evolutionary change that has occurred in lineages leading to both modern sharks and bony fishes has involved the development of mouths and jaws that are more efficient in food gathering. Primitive Actinopterygians (Paleoniscoids) as well as living polypterids provide examples of jaw structures that are adapted to engulfing their food whole. The relatively inflexible jaws of these older lineages were limited to more-or-less opening and closing and contrast sharply with the protrusible jaws of modern teleosts that actually reach out to engulf their prey (Lauder, 1980a, b). Jaw protrusion is believed to have evolved independently at least five times: in sharks, in chondrosteans, in ostariophysans, and again in higher teleosts (chiefly acanthopterygians, but also in cods (Gadidae) perhaps uniquely among the paracanthopterygians). A key factor in the evolution of protrusible jaws in the acanthopterygian perciform lineage has been the increased importance of a highly mobile

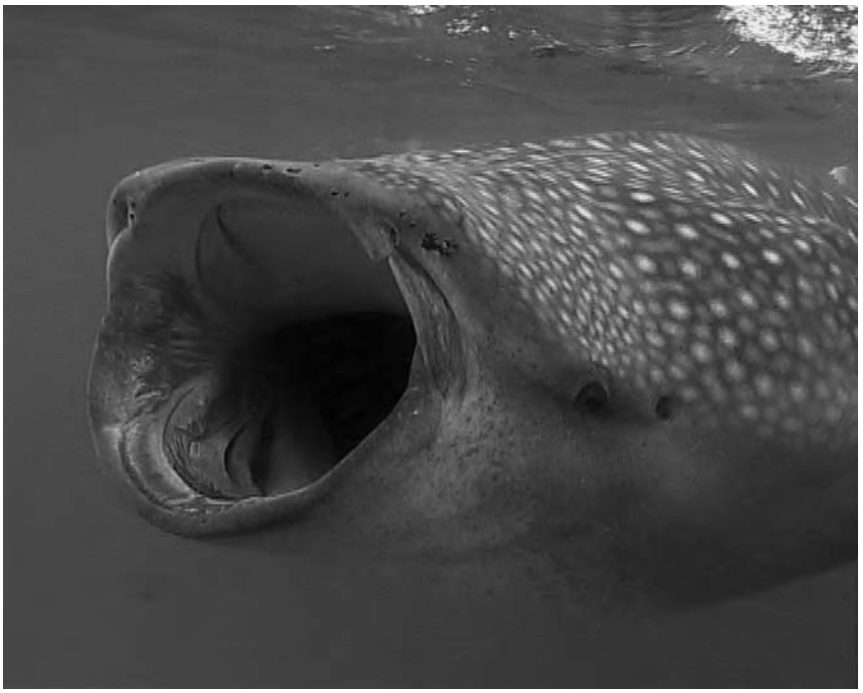

Figure 7.4A A whale shark opens its mouth, bringing in plankton-rich water. © Werner Mischler.

Figure 7.4B Cookiecutter shark dentition. © George Burgess.

premaxillary and commensurate restriction of the maxillary as the primary structure in the upper jaw (Ferry-Graham and Lauder, 2001).

These fishes open their mouths by utilizing the hypaxial/sternohyoideus muscle to lower the mandible, but can also use their epaxial muscles to hinge the neurocranium upwards relative to the vertebral axis and thus increase the size of their gape (Figure 7.5). In its simplest form, expansion of the buccal cavity is very limited in these cases and a small, but adequate, negative pressure is developed; however these fishes may enhance their feeding efficiency by ram feeding – using body velocity to overtake and capture prey.

Although ram and suction feeding may be viewed as extremes of a spectrum of feeding modes (Norton and Brainerd, 1993), many fish use a combination of forward motion of the body or jaw in addition to generating suction pressure to entrain prey. Barracuda (*Sphyraena barracuda*), pike (*Esox*), and gars (*Lepisosteus*) typically rely entirely on ram feeding, engulfing their prey by making a rapid lunge with their jaws wide open (Porter and Motta, 2004). The long-jawed butterflyfish (*Forcipiger longirostris*) rapidly extends its jaws while enlarging its gill chamber and reaching out, often into confined spaces where suction feeding alone would not be as effective. (Ferry-Graham *et al*., 2001).

Ram feeding occurs when a fish swims with its mouth open through a concentration of food. The mouth may be held open continuously (or for very long periods) or intermittently. Food may then be sieved from the water by gill rakers, collected in sticky mucus, or otherwise routed into the esophagus where it is then swallowed.

Ram feeding is sometimes referred to as "passive feeding," however, the fish must actively expend energy for swimming and a streamlined fish swimming with its mouth open loses much of its hydrodynamic efficiency thus increasing the cost of swimming and making ram feeding much less energy efficient than it might at first appear to be. This hydrodynamic loss may be partially compensated for by the injection of water from the gill openings into the boundary layer similar to what happens during respiration (see Chapter 5).

Studies on herring and other species have shown that a fish may use ram feeding at high concentrations of plankton, but switch to "picking" individual prey (suction feeding) at lower concentrations. At moderate concentrations, when prey would be equally available by either means, fish often favor picking over ram feeding, suggesting that suction feeding is energetically more efficient (Gibson and Ezzi, 1992).

In contrast to ram feeding, most fishes use suction to ingest their prey. The buccal and opercular chambers expand, drawing water, and any prey organisms in that water, into the fish's mouth.

Suction feeding is used to capture many different types of foods, for example: suspension feeding on dense aggregates of microorganisms or detritus, plankton picking, crevice feeding, bottom vacuuming, and by sit-and-wait predators. Suction feeding typically consists of four phases: (1) preparatory – a decrease in volume of the mouth by compression of the head and elevation of the hyoid; (2) expansive – which occurs progressively from front to rear, beginning with the opening of the mouth and subsequent enlargement of the gape, depression of the hyoid, abduction of the suspensorium, and abduction of the operculum (during which the opercular opening remains closed to prevent the escape of water); (3) compressive – closing of the jaws, adduction of the

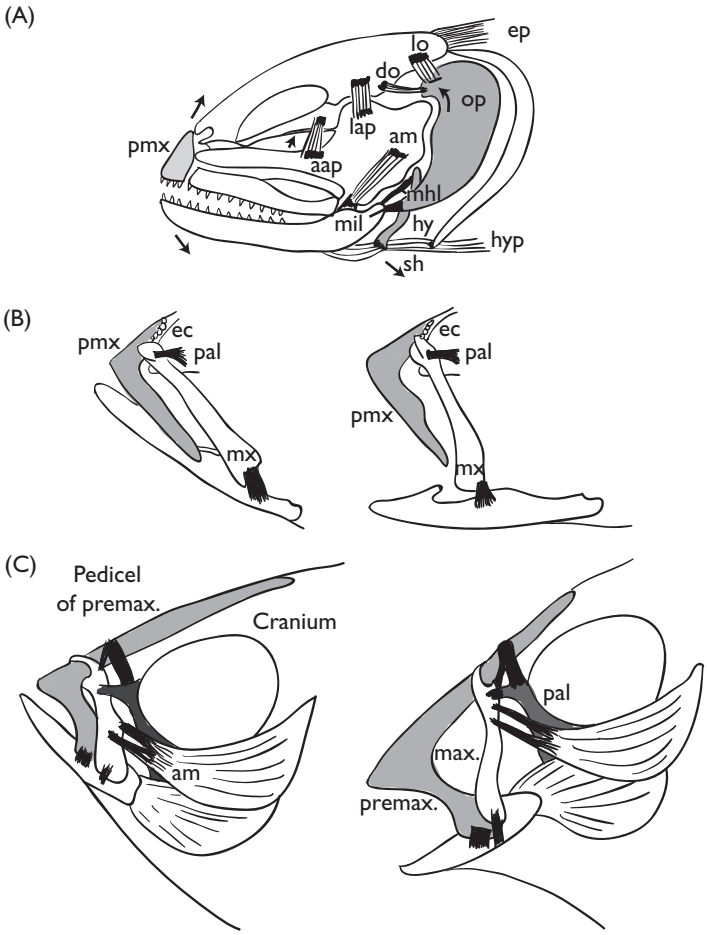

Figure 7.5 Protrusible and nonprotrusible jaws in teleosts. (A) The jaw muscles, ligaments and jaw movements during feeding in the Arctic char *(Salvelinus);* (B) open and closed jaws of unspecialized gadoid or percoid, showing how the pedicel of the premaxilla rocks on the rostral past of the cranium via the ethmoid cartilage as the lower jaw is depressed; (C) advanced protrusible jaw mechanism of the mojarra *(Gerres)*. The premaxillary pedicel slides under the palatine ligament to protrude the mouth, and as it protrudes the maxilla rotates, aap: adductor areus palatini; am: adductor mandibularis; do: dilator opercularis; ec: ethmoid cartilage; ep: epaxial muscles; hy: hyoid; hyp: hypaxial muscles; lap: levator arcus palatini; lo: levator operculi; mhl: mandibulohyoid ligament; mil: mandibulo-interopercular ligament; mx: maxilla; op: operculum; pal: palatine; pmx: premaxilla; sh: sterno-hyoideus. After Alexander (1970), Lauder and Liem (1980), and Schaeffer and Rosen (1961).

suspensorium and protraction of the hyoid, while opening the opercular flap; resistance of the gill arches delaying the outflow of water and permitting retention of food; and finally (4) recovery – a return to conditions preparatory for phase 1 (Horn, 1998).

Suction feeding occurs in a wide variety of fishes, and has been suggested as the primitive feeding style for all bony fishes (Hulsey *et al.*, 2005). The absence

of teeth associated with the earliest fossils from the Lower Silurian (420 mya) suggests to some that these were also filter feeders. Functional convergence in suction feeding between living sharks and bony fishes reflect similar hydrodynamic constraints resulting from evolutionary pressures to develop greater feeding efficiencies through protrusible jaws in both lineages. Relationships between jaw suspension and feeding in sharks are not, however, as clear as formerly believed (Wilga, 2002). Upper jaw protrusion in elasmobranchs (Figure 7.6) is related to morphology of the upper jaw-chondrocranium articulation rather than to the type of jaw suspension as had been previously suggested. Interestingly, the same jaw morphology and musculature that permits suction feeding can also be adapted to allow the fish to eject a stream of water that can be used to uncover buried prey or manipulate prey so it can be more easily consumed. Dasyatid and myliobatid stingrays forage by jetting water out of their ventrally oriented gill openings to blow large pits in the bottom (Wilga *et al.*, 2007), and these have been recognized in fossil sediments. The ability of archer fish (*Toxotes*) to "shoot down" insects with jets of water has already been mentioned and triggerfishes (Balistidae), which feed on echinoderms, use a jet of water to uncover buried sand dollars or overturn long-spined sea urchins, allowing them to attack the undefended ventral side.

Biting, as defined by morphologists and biomechanicists, involves the use of the jaws to grasp prey and includes feeding on only parts of a larger prey organism, taking bites of flesh, or of fins, scales, eyeballs, parasite picking, or benthic scraping found in many species of coral reef fishes and rock-dwelling

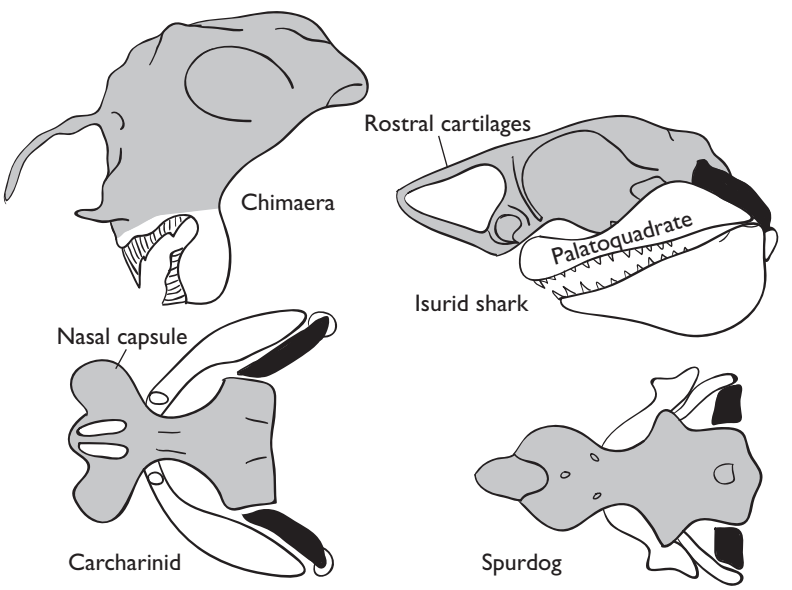

Figure 7.6 Cranium and jaws of elasmobranchiomorphs. Lateral views above, dorsal below. Myomandibula, black; neurocranium and capsules, shaded. Note bizarre shape of holocephalan skull with palatoquadrate fused to braincase and different angles of hyomandibula in the carcharinid and spur dog *(Acanthias)*. After Daniel (1922), Devillers (1958), Young (1981), and Moss (1977).

cichlids in which the jaws are applied to the substrate or directly to the prey. When defined this way, biting becomes the most specialized mode of feeding, and is believed to have evolved several times: in sharks (and perhaps earlier in placoderms), in cichlids, plectognath fishes (triggerfishes and their relatives), and in algal scrapers in diverse families including loaches, catfishes, blennies, and cichlids.

Regardless of the mode of feeding the feeding apparatus of most fish consists of four-bar-linkage systems constructed from the bones of the skull that form an expandable mouth cavity shaped like a truncated cone (Figure 7.7A–C). In contrast to simple lever systems, such as the lower jaw, most of which possess an input and an output link that rotate around a fulcrum provided by the jaw joint, four-bar linkages have a third link that transfers the transmission of motion and force as well as a fourth, stationary, link that anchors the assembly. Four-bar linkages are found not only in the mouth but also the pharyngeal and opercular apparati and these multiple linkages work together to permit the capture and processing of food. Expansion of the mouth cavity produces negative (suction) pressure that assists in the ingestion of food. Naturally, the actual feeding mechanism of fishes is more complex than this simple model and differences in the relative sizes and position of each bony element in the linkage system as well as associated muscles and tendons can produce a great variety of results, ranging from very rapid and forceful to very slow and precise feeding movements (Higham *et al.*, 2006; Figure 7.8). Most suction feeders are intermittent, however, lampreys and their ammocoete larvae possess anatomical structures that permit more-or-less continuous suction feeding currents.

7.6 Handling and Ingestion

Once food is captured it must (except in aerial and terrestrial feeding species) be separated from the water, transported to the rear of the mouth and oriented in such a way so that it can be swallowed or ingested. Fishes that feed on larger prey make use of the hydrodynamic properties of their oral chambers to insure that the food is moved towards the esophagus and does not escape through the mouth or gill openings. After food is taken in through the mouth, expansion of the mouth and gill chambers creates a movement of water that continues to transport food toward the rear of the mouth. Food transport may be assisted by teeth on the tongue and palate, or by the gill rakers. Although the same muscles and bones are used for food capture and handing, experimental results demonstrate that handling is a distinct process, although obviously one that is closely tied to food capture.

Versatility of the expanding cone model is restricted not only to prey capture. Pressure differences in different areas within the cone can be generated by modulating muscle actions. In this way captured prey can be moved or turned within the cone – it acts as a hydrodynamic tongue.

Suspension feeding occurs when water laden with microscopic organisms or detritus is drawn into a fish's mouth. These particles can then be removed by filtration or sieving, trapped in sticky mucus, or by other means that are not fully understood. Apparently, fishes have been utilizing this method for nearly as long as there have been fishes. Not only do the living cephalochordates feed this way, but studies done on the feeding apparatus of jawless heterostracans

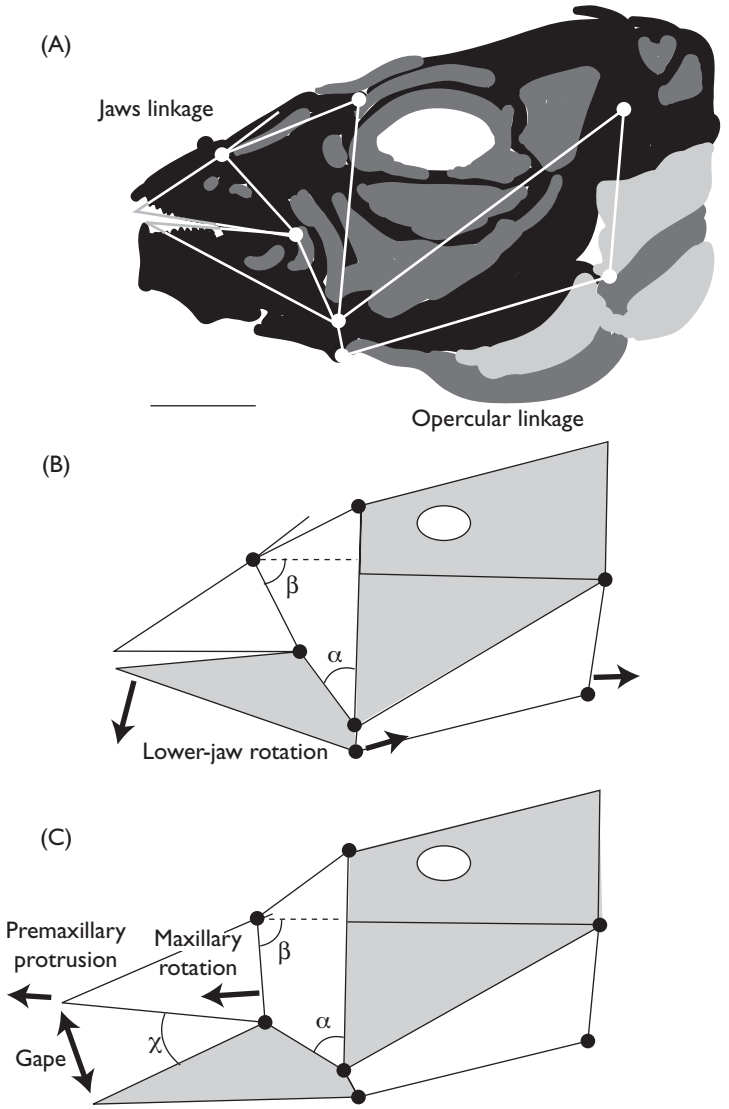

Figure 7.7 Linkage mechanism of maxillary rotation and premaxillary protrusion in percomorph fishes. (A) Skull morphology of *Oxycheilinus digrammuss*, a piscivorous labrid fish, with lines showing the geometry of the mandibular lever, the opercular 4-bar linkage, and the four-bar linkage that drives jaw motion; (B) the input motion that drives the linkage is ventral depression and rotation of the mandible (increase in angle α); (C) the output motions of the linkage are maxillary rotation (angle β), maxillary displacement ventrally, sliding and protrusion of the premaxilla, and increase in mouth gape and gape angle (angle χ). Westneat (2004).

indicate that they were primarily suspension feeders. But this is not to say that modern fish feed in an identical manner. Suspension feeding occurs in 11 orders of fishes, and is found in many of the world's economically important species.

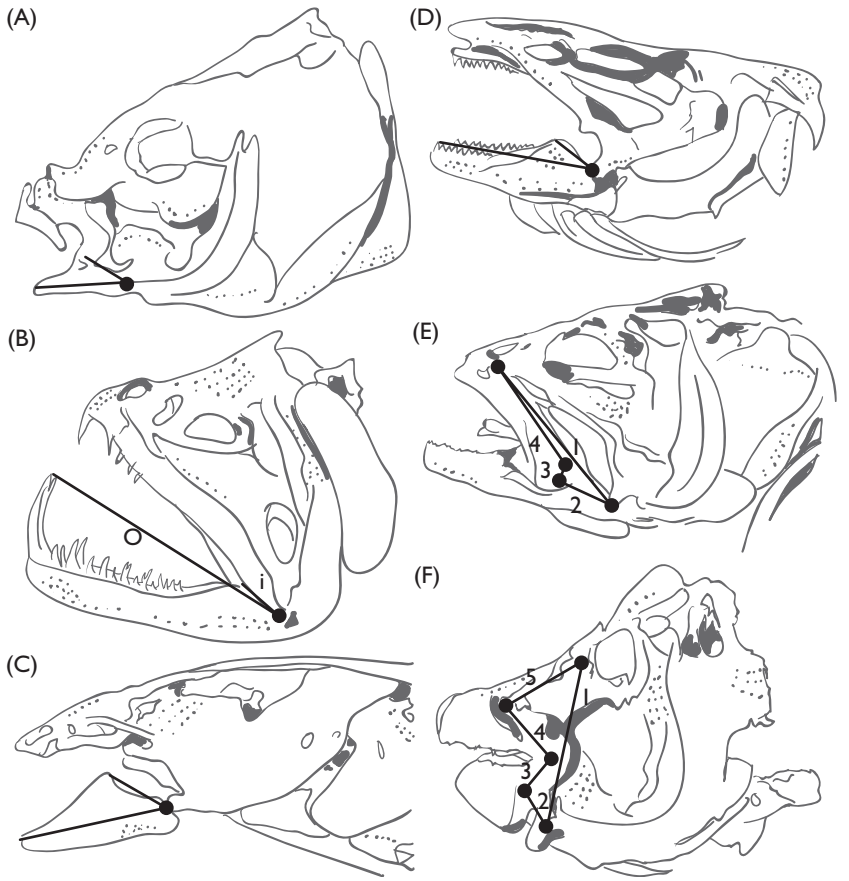

Figure 7.8 Skull diversity, mandibular lever variation, and linkage structure in actinopterygian fishes. Skull and jaw lever dimensions of the ostariophysan fishes (A) carp, *Cyprinus carpio*, (B) vampire characin, *Hydrolycus scomberoides*, with inlever (i) and outlever (o) labeled, and (C) catfish *Arius felis*. (D) Lever dimensions of the cod *Gadus morhua*. (E) Skull of the large-mouth bass, *Micropterus salmoides*, with diagram of four-bar linkage for maxillary rotation; 1. fixed link; 2. articular input link; 3. maxillomandibular ligament coupler link; 4. maxillary output link. (F) The parrotfish *Scarus guacamaia*, with a novel five-bar linkage for control of upper and lower jaws; 1. fixed link; 2. articular input link; 3. dentary coupler link; 4. maxillary output link; 5. palatine and palatomaxillary ligament coupler link.

Traditionally, filter and suspension feeding were thought to be associated with ram feeding, however, recent work shows that suction pump feeding plays an important role. Along with the whale shark and the megamouth shark (*Megachasma pelagios*), the basking shark is one of three species of large, filter-feeding sharks (Figure 7.9). However, it, together with the manta and devil rays (*Mobula*), is the only elasmobranch that relies solely on the passive flow of water forced through the pharynx by swimming. In contrast, the whale shark and megamouth assist the process by suction or actively pumping water

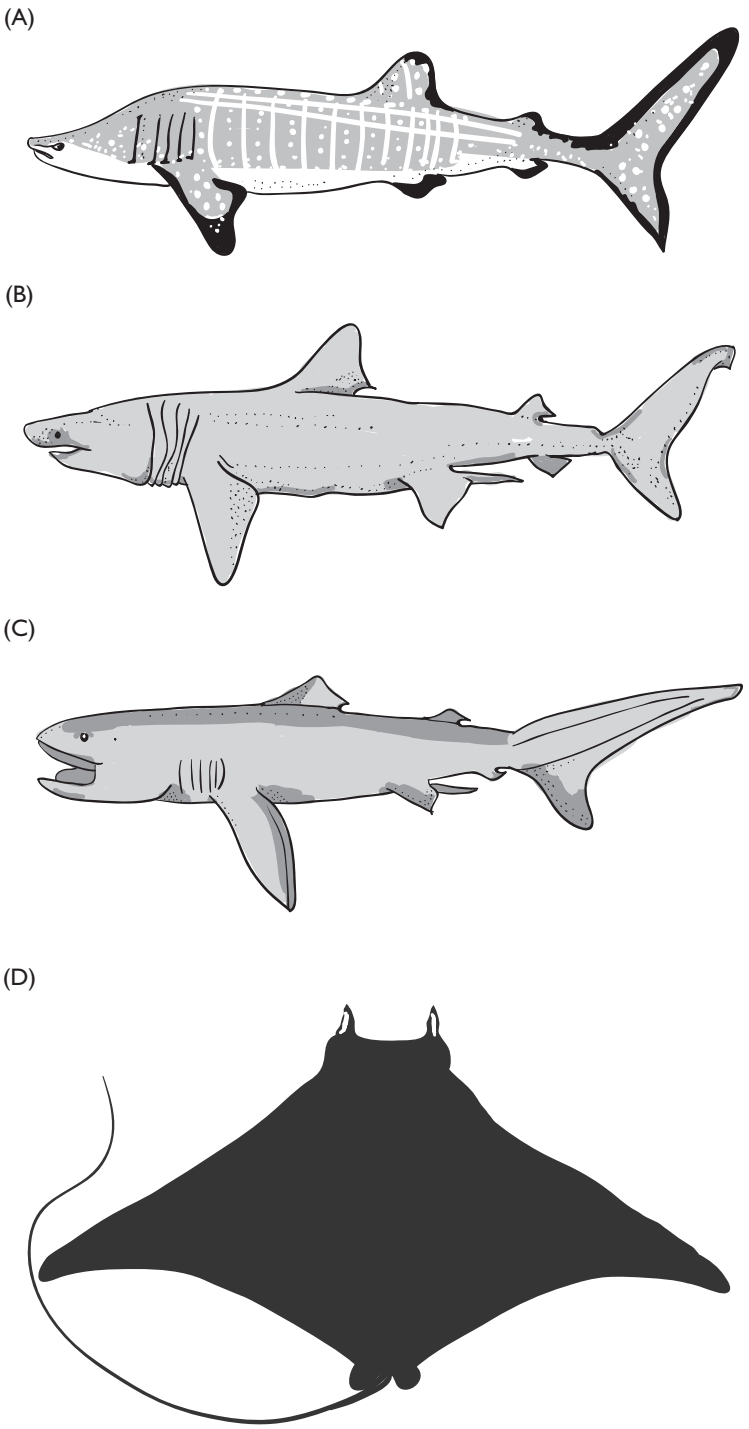

Figure 7.9 Filter-feeding elasmobranchs: whale shark, basking shark, megamouth shark, and manta ray. A, B, and C from Compagno (2001) and D is from De Bruin *et al*. (1994).

into their pharynxes. Not only whale sharks but various bony fishes also draw water into their mouths at higher velocities than in passive filter-feeders, enabling them to capture larger more active prey as well as larger aggregations of zooplankton.

Varied methods are also employed to separate food from the water. As it turns out, fish do not swallow much water with their food, so somehow food and water are separated. In many fishes the muscular esophagus or oropharyngeal sphincter, which separates the esophagus from the pharynx, serves to prevent the passage of too much water, in some cases probably wringing unnecessary water from the food as it being swallowed. In recent years several studies have demonstrated that the classic view in which food is sieved from the water by the gill rakers is inadequate. In the classic model, the gill rakers serve as a mechanical sieve, trapping food as the water flows between them (Rubenstein and Koehl, 1977; Silvester, 1983). Particles that are too large to pass through the pores defined by the rakers, or by microbranchial processes, small projections that extend out from the rakers, are trapped on lateral gill rakers, and are then passed forward to the medial rakers of the preceding arch before being transported posteriorly to the esophagus. Channel size may be varied by bony tips of medial gill rakers on the preceding arch, which are moved into the inter-raker channels; but not all fish have this ability. Alternate sieving models suggest that the upper surface of each branchial arch between the rakers forms a functional sieve, not the rakers themselves (Van Den Berg *et al*,. 1994). For example the numbers of gill rakers increase in young herring up to 50 mm in length, however, above 50 mm length the increase dramatically slows. The length of the gill rakers and the distance between adjacent rakers increase throughout life making a constant sieve size based on inter-raker distance impossible, however, the area provided along the length of the rakers increases proportionally to the fish's size (and food requirements).

But food may also be effectively separated from the water by methods that do not involve mechanical sieving. The gill arches cause a swirling vortex to form at the roof of the mouth in some species. Food particles are trapped in the vortex, stick to mucus on the roof of the mouth and are then swallowed. Plankton filterers and detritivores such as the thread-fin shad (*Dorosoma*) and the anchovy (*Ctengraulis*), both clupeoids, the osteoglossid (*Heterotis*), the milkfish (*Chanos*), and mullets (*Mugil spp.*) possess paired epibranchial organs, muscular diverticula containing mucus cells at the back of the pharynx which probably squeeze concentrated food particles into a bolus before swallowing.

Aerosol (or hydrosol) filtration, separates particles using adhesive properties of the filter elements, rather than pore size. The mucus covered surfaces of the arches themselves may serve as the sites of filtration with inter-raker spaces serving only for the removal of water from the oral chamber.

Alternatively, the barrier formed by the rakers may act as a crossflow filter. Crossflow filtration is widely used in industrial applications such as winemaking, brewing, and water purification. It is a valuable method for filtering large volumes without clogging filters. Instead of passing a mixture of water and food particles through the filter, the mixture is passed across the surface of the filter. In a process that is not well understood, the liquid is drawn through the filter, while solids accumulate on the "downstream" end of the filter where mucus entrapment may also help in capture and retention of food particles (Figure 7.10).

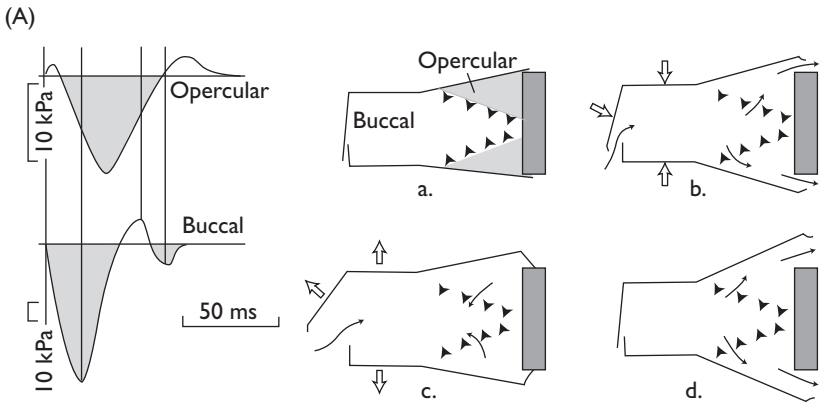

Figure 7.10A Suction feeding. Left: buccal and opercular pressure records from pumpkinseed (*Lepomis*) – note large negative pressures giving high inflow velocity. Right: model showing successive stages in feeding, incorporating backflow from opercular chamber (lightly stippled in A). After Lauder (1980c).

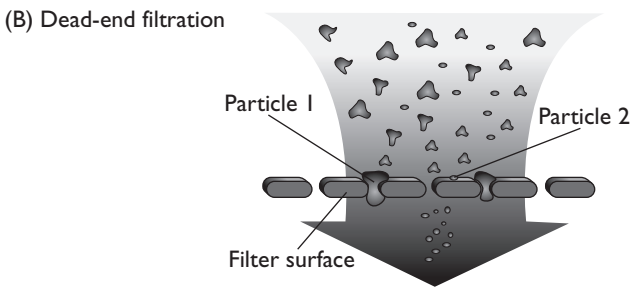

Figure 7.10B In dead-end filtration, fluid flow is perpendicular to the filter surface and the filter rapidly becomes clogged with particles. Particles may be retained by sieving when they are larger than the filter's pore size (particle 1), or by hydrosol filtration when they are smaller than the pore size (particle 2); in this case, the small particles stick to the elements of the filter. Brainerd (2001).

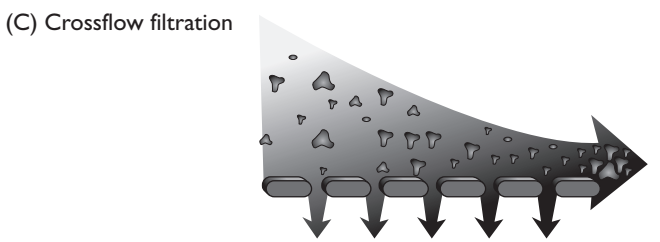

Figure 7.10C In crossflow filtration, fluid flows parallel to the filter surface and particles become more concentrated as filtrate leaves through the filter's pores. Brainerd (2001).

Both aerosol and crossflow filtration could possibly reduce the energetic costs of feeding since there is reduced hydrodynamic resistance and no clogging to overcome (Sanderson *et al.*, 2001).

Thus, mesh sieving is only one of numerous mechanisms described by engineers by which solids may be separated (filtered) from a suspending fluid. It appears that fish may use most, if not all, of these, with more than one mechanism occurring sometimes in a single species or even an individual, depending on the size and concentration of the food.

Carp sieve out larger food particles (insects, etc.) while using crossflow filtration, not sieving, to concentrate smaller (detrital and microbial) food particles. At the same time, similarly sized inorganic particles are expelled through their opercular openings or by spitting through a process known as oral winnowing. Oral winnowing is the separation of food from non-edible materials, and occurs when the flow of water reverses within the mouth cavity. During winnowing, only a little of the water escapes through the mouth. Most, together with the indigestible materials, is forced out of the opercular openings or the mouth during a subsequent contractile effort.

7.7 Anatomy and Physiology of the Digestive Systems

The capture, processing and reduction of prey into nutrients is the function of the digestive system which can be thought of as a series of interconnected tubes through which food passes, each with specialized anatomical and physiological features, and associated secondary organs that provide the means of mechanical and chemical digestion, absorption of nutrients, and the final elimination of undigested materials. Beginning anteriorly, these organs are the mouth and oropharynx with its teeth, gillrakers and other structures for the initial capture and ingestion of food, the esophagus, the stomach, the intestine with its associated digestive organs: the liver, pancreas, and gall bladder, and last the rectum, or in some fish a spiral valve. The operation of the digestive system is under direct and complex control systems of hormones related to food ingestion and movement through the gut. The production of these hormones makes the intestine the largest endocrine organ in the body (Chapter 9). In addition to digestive functions, the intestine, especially the posterior portions, plays an important role in excretion and immune functions, as discussed in the appropriate chapters.

Teeth

Living Agnathan fishes possess conical, rasping tooth-like structures made of keratin, the same structural protein found in human hair and nails, on their tongue, rather than enamel covered, bony teeth typical of other vertebrates (Figure 7.11). The lamprey also has similar teeth around its mouth. They also have no identifiable esophagus or stomach. The intestine is straight with little regional differentiation, although parasitic/predatory lampreys have a single fold (typhlosole) that runs in a spiral along the length of the intestine increasing the absorbent surface. In non-parasitic lampreys such as the North American and British brook lampreys (*Lampetra appendix* and *Lampetra planeri* respectively), some North American species of *Ichthyomyzon*, and the southern hemispheric *Mordacia praecox*, the larval stage persists for several

years as a filter feeder and following metamorphosis the adult digestive system is non-functional and no food is consumed from when they metamorphose until they spawn and die one-half year later. An intermediate condition is found in the non-parasitic Caspian Sea lamprey, *Caspiomyzon wagneri*. Ammocoete larvae live in bottom deposits and feed on diatoms and detritus, however, adults feed on algae and higher plants as well as scavenging dead fish and are known to attach themselves to trout, presumably for transport.

Jawed fishes possess many types of teeth both in their jaws and elsewhere in their mouth cavities. Jaw teeth located on the premaxillary, maxillary, and

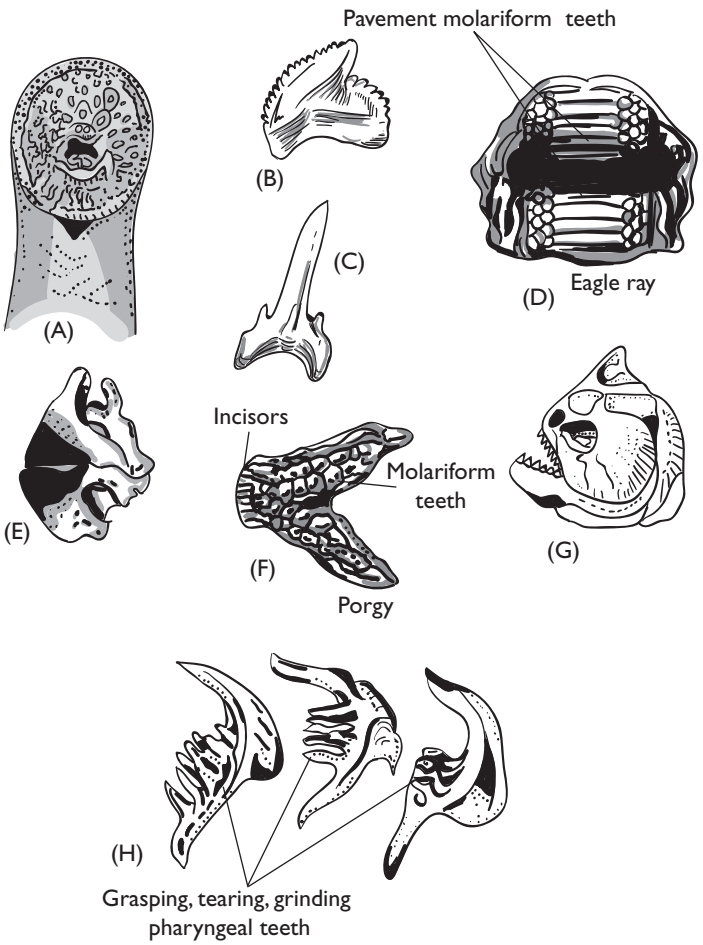

Figure 7.11 Varieties and shapes of teeth in fishes. (A) Tooth-like, rasping mouth of lamprey; (B) broad shearing tooth of tiger shark (*Galeocerdo cuveri*); (C) pointed, spearing tooth of sand tiger (*Odontaspis taurus*), (D) pavement molarform crushing teeth of eagle ray (*Myliobatis*); (E) beak-like structure of fused teeth in parrotfish (*Pseudoscarus*); (F) cutting incisors and grinding molars of porgy (Sparidae); (G) cutting teeth of piranha (*Serrasalmus*), (H) grasping, tearing and grinding pharyngeal teeth from minnows (Cyprinidae). Adapted from Norman (1931) and Lagler *et al*. (1977).

mandible (dentary) are used for biting, grasping, shearing, rasping, or scraping. Depressible teeth allow food to move into the mouth but not outwards. Many fish possess only small pointed teeth suitable for immobilizing prey while relying on suction mechanisms to finish the job of prey ingestion. Barracuda and similar predatory species possess large, shearing teeth that cut their prey nearly in two, allowing it to fold for easier ingestion. The bladelike teeth of predatory sharks are justifiably celebrated for their ability to remove "chunks" of flesh from large victims (Williams, 2001).

In addition, or sometimes instead of jaw teeth, many fish possess teeth on their tongue or the roof of the mouth (palatine and vomerine teeth), which also help hold food and direct it to the esophagus. One entire major group of fish derives its name from the presence of teeth on their tongues; the Osteoglossomorpha, a name which translates as "bony tongues."

Pharyngeal teeth located on, above, and below the pharyngeal arches are important for shearing or crushing prey. They often occur in fish that otherwise lack teeth, such as carps, minnows, and suckers; however, many families such as parrot fish, cichlids, and drums possess pharyngeal teeth in addition to well developed jaw teeth. As illustrated by drums that feed on hard-shelled mollusks, the form of pharyngeal teeth may change with growth, cardiform teeth found in small fish which feed on softer-bodied prey are replaced by villiform teeth, which in turn are replaced by heavy molariform teeth as the size class of drum and the hardness of their favored prey increases.

The digestive tract

The digestive tract of fishes is divided into four regions: the foregut (esophagus and stomach, if present), mid-gut, hindgut, and rectum. The foregut begins at the posterior boundary of the gill cavity or pharynx and includes the esophagus, the stomach, when present, and the pylorus.

Typically, the esophagus is a short muscular tube connecting the oropharynx with the stomach. Often the esophagus can expand to accommodate almost anything a fish can get in its mouth. In marine and euryhaline fishes such as tilapia, eel, and flounder, the esophagus also plays an important role in maintaining water balance, serving as a site for the absorption of water imbibed by the fish in order to offset osmotic loses to a hypertonic environment (see Chapter 6). Fishes of the order Stromateidae often possess pharyngeal sacs that may or may not be equipped with tooth-like projections. Many of these species feed on soft-bodied coelenterates.

A stomach may be present or absent. In its simplest form, the stomach is an elastic sac that receives and stores food, and begins chemical digestion; however, in mullet, *Corregonus*, *Sardinella*, or *Mormyrus* – most of which are microphagous detritivores or herbivores – part of the stomach may be modified into a gizzard-like structure (Figure 7.12).

Fishes which lack stomachs include Holocephalans, which typically feed on mollusks as well as some fish; lungfish, which are predatory on fish, mollusks, and arthropods, barnacle eating blennies, and a variety of herbivorous fishes.

In most fishes the esophagus enters the stomach anteriorly and the intestine exits posteriorly (Figure 7.13A). Typically, there is no anterior (esophageal or cardiac) sphincter, however, most fish possess a well developed pyloric sphincter that regulates the passage of partially digested food from the

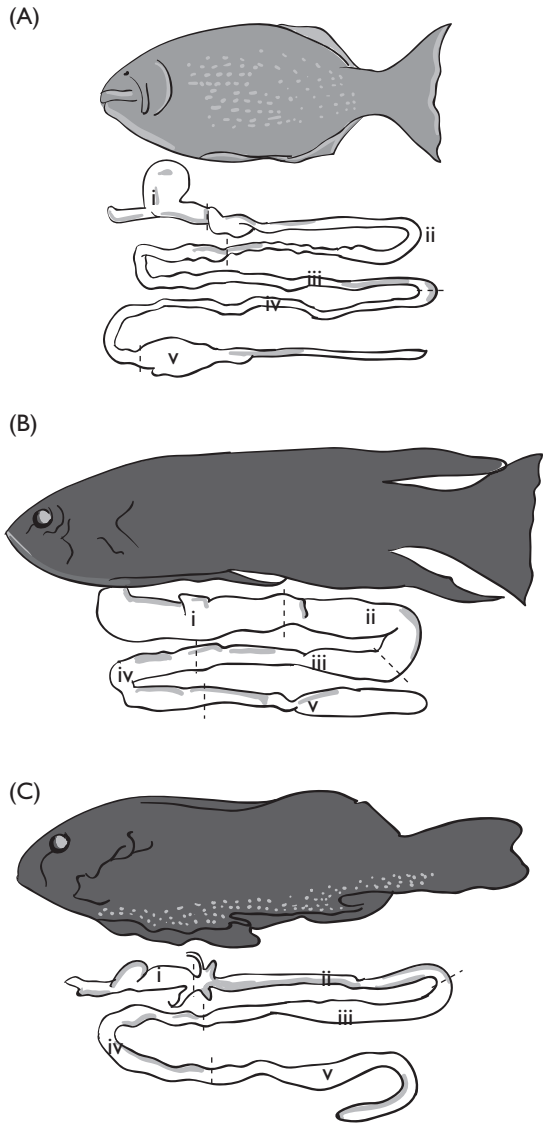

Figure 7.12 Intestines of three herbivorous fishes. Roman numerals i–v designate segments in which differences in biochemical activities were analyzed. Drawings based on photographs of *K. sydneyanus* (A), *O. pullus* (B), and *A. arctidens* (C) Mountford *et al.* (2002).

stomach. An interesting variation on this basic design is found in the Lake Magadi tilapia (*Alcolapia grahami*) that dwell in extremely alkaline waters (Bergman *et al.*, 2003). In this species the intestine connects directly to the esophagus and the stomach at a three-way junction formed rather like an upside down letter "T" (Figure 7.13C). When the stomach contains food, the pyloric sphincter will close, permitting alkaline water to pass directly into the

intestine, and thus preserving the acidic environment of the stomach. A somewhat similar situation is found in other tilapine cichlids in which the pyloric sphincter is located anteriorly in the stomach, but not in direct opposition to the esophagus (Figure 7.13B).

The stomach is a site of protein digestion initiated by the enzyme pepsin. Hydrochloric acid (HCl) secreted by gastric glands provides proper pH for pepsin, and also serves as a chemical barrier to bacteria and parasites. It may also assist with the breakdown of hard, shelly materials. The stomachs of many fishes also exhibit chitinase activity, although whether this enzyme is secreted in the stomach or esophagus or both is not clear.

The remainder of the gut is differentiated into regions, but what the physiological role is of each is not clear. Aside from the obvious roles in digestion and absorption of nutrients, posterior sections also play roles in salt and water balance and immunity.

The mid-gut includes the intestines posterior to the pylorus, and often merges without anatomical distinction into the hindgut, although, in some fish, the beginning of the hindgut is marked by an increase in gut diameter. The mid-gut often includes a variable number (from zero to 1000) of pyloric caecae near the junction of the stomach and intestine. Pyloric caecae occur in fishes of almost every feeding variety and their function is not clear. It has been suggested that they serve to increase surface area or as sites for the absorption of certain nutrients (fats and waxes; Buddington and Diamond, 1986). They have been shown to contain digestive enzymes including pepsin which has a pH optimum of about 1.5–4, which is certainly not typical of intestinal pHs

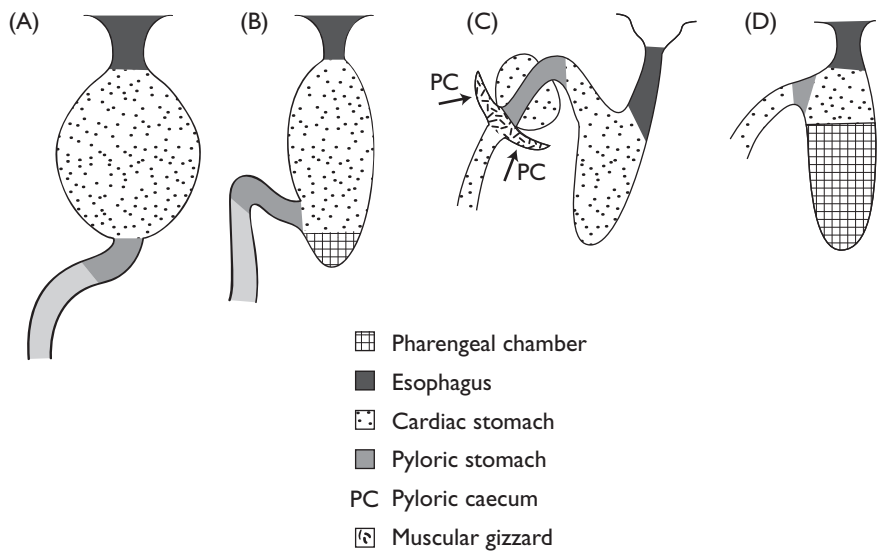

Figure 7.13 A variety of stomach types found in fishes: contrasting the "straight" stomach of *Esox* (A) with "typical" J-shape in *Anguilla* (B), the heavy walled "gizzard" in *Mugil* (C) and "T" shape in *Alcolapia grahami* (D). Adapted from from Harder (1975).

(Horn, 1989). Pyloric caecae are always absent in fishes that lack stomachs. The mid-gut is typically the longest portion of the gut, ranging from less than one body length to over 20 body lengths and may be coiled into complicated loops (often characteristic for each species) when longer than the visceral cavity. The length and complexity of the mid-gut often provides a clue to the feeding habits of the species (de Groot, 1971), however this relationship is not infallible.

The remainder of the digestive tract may be differentiated into a hindgut and a rectum, and at the posterior end the hindgut exits the body cavity via the anus or cloaca; a chamber formed from infolded body wall, receiving both the anal and urogenital openings which occur in sharks, rays, and Sarcopterygians, but never in teleost fish or holocephalans. A pre-rectal ileorectal valve may be present, as in many teleosts, and some fishes also possess a single large hindgut caecum which may be used as a final site for digestion and absorption (*Polypterus*), fermentation of herbivorous materials or even as a respiratory chamber (Gee and Graham, 1978).

Digestive enzymes

Like other animals, fish possess an array of digestive enymes by which large macromolecular nutrients are broken down into smaller molecules that can be assimilated. Most fish possess seven main digestive enzymes – trypsin, maltase, amylase, two +aminopepsidases (carboxypepsidase a, carboxypepsidase b), lipase, and alkaline phosphatase. Almost all the major enzymes are present in all fish regardless of their food habits, however, the relative concentration and activity varies according to food preference. Pepsin is localized in the stomach where it functions at optimum pHs between 1 and 4, while the others are found in the intestine at more alkaline pHs. The optimum pH for each enzyme varies with different regions and between different species. In general, the optimum pH for trypsin lies between 6.8–7.8, for carbohydrases 5–7, and for lipases the most alkaline > 7.8. There are two sources of enzymes for the mid-gut – the pancreas and the secretory cells in the gut wall. Since the pancreatic tissue is often diffuse and closely adhering to the liver, portal veins, and gall bladder, it is often difficult to determine the exact origin of many digestive enzymes. Many fish appear to produce amylase and other carbohydrases, but others rely on the activities of gut microflora to supply these enzymes.

Chitinase has already been mentioned as occurring in the stomach, but in some fish it is found only in the intestine where it is secreted by the pancreas.

Absorption efficiencies of carnivorous and herbivorous teleosts differ significantly. In carnivorous species the energy absorption efficiency is around 80% (up to 97% for adult sea lampreys feeding on blood), and for herbivores much less, around 40–50%. The only elasmobranchs examined have been lemon sharks (*Negaprion brevirostris*), which have been found to have absorption efficiencies up to 83%. It is interesting that lemon sharks and carnivorous teleosts should have similar energy absorption efficiency values, for the shark digestive strategy is very different to that of most teleosts. Thus, compared to teleosts, these sharks have a low rate of food intake (1% of body weight per day in the sandbar shark, *Galeaspis*), extended retention time, greatly increased surface area – with the spiral valve at the hinder end of the gut – and grow slowly.

Other organs

The pancreas is an important source of digestive enzymes. It may be a discrete organ or a diffuse mass of tissue (Hilliard and Potter, 1988), often interwoven among pyloric caeca. Islet cells, the sources of insulin and glucagons, may be found within the pancreatic mass as is typical of higher vertebrates, or separately, often in association with liver tissues. Endocrine pancreas islet cells are often consolidated into large tissue masses, known as Brockmann bodies. Newly absorbed nutrients are transported to the liver by the hepatic portal vein. The liver, which may be the largest organ besides swimming muscles in a fish's body, is not directly involved with digestion, but assimilates nutrients, produces bile, and detoxifies toxins from both endogenous (metabolic) and exogenous sources. A final organ associated with digestion is the gall bladder which secretes bile, produced by liver, that aids in emulsification and increases intestinal pH. The gall bladder also excretes absorbed toxins and metabolic wastes back into the gut for elimination.

7.8 Food Types, Characteristic Adaptations, and Feeding Guilds

Suites of characteristics, including anatomical, physiological, and behavioral adaptations, come together to permit fish to best utilize an amazing variety of foods (Wainwright *et al*. 2000). Fishes sharing such dietary specializations are often regarded as belonging to the same Feeding Guild.

Carnivorous fishes

The vast majority of living fishes are predatory. Of some 1100 species of extant elasmobranchs, only 13 (1.2%) – the basking shark (*Cetorhinus maximus*), megamouth shark (*Megachasma pelagios*), whale shark (*Rhincodon typus*), manta ray (*Manta birostris*), and about nine species of devil rays (genus *Mobula*) – have forsaken the actively predacious habits of their kin and adapted to a more placid "grazing" lifestyle.

Predatory (piscivorous) fishes typically have large, terminal or subterminal mouths with well developed grasping and biting teeth. Many also have teeth on the tongue and roof of mouth. Among predatory fishes adapted to feeding on hard, shelly prey some jaw or pharyngeal teeth are usually heavy and molariform. Gill rakers, if present, are typically short or blunt. They have a well defined stomach, and typically possess pyloric caecae. The intestine is relatively short. They may be strong swimmers or sit-and-wait ambushers. And, if the latter, they may possess camouflage, lures and the like.

Some predatory fishes feed on the sea's abundant gelatinous animals such as ctenophores, jellyfishes, and salps. Correlated with a gelatinous diet are enlarged digestive tracts, exceptionally large stomachs, and extremely long intestines. Fish feeding on gelatinous animals also may have pharyngeal or esophageal modifications, presumably to prevent regurgitation (Fänge and Grove, 1979). *Mola mola* has three rows of recurved pharyngeal teeth. In the stromateoid fishes, there are esophageal sacs with denticulate papillae. *Genicanthus personatus*, the masked angelfish, includes hydromedusae and siphonophores in its diet and has finger-like esophageal papillae that point posteriorly. The esophagus of chum salmon is strongly muscular with a well defined sphincter.

Plankton filterers

Another major feeding guild consists of plankton filterers. A successful plankton filterer needs to be able to separate plankton from the water. Typically this occurs through mechanical filtration by means of gill rakers, but, as noted in "Food choices, food and development", aerosol filtering is employed as well. Some filter-feeders, especially those in which phytoplankton is an important source of food, have exceedingly long guts. The gut in the menhaden is ten times the length of that in the herring and has 400 pyloric caecae compared with 20 in the herring, reflecting the greater difficulty of digesting plant food. Filter-feeding appears during ontogeny; it is not found in young larvae, which are usually microzooplankton particle-feeders. In herring and menhaden, in the paddle fish *Polyodon* and in certain cichlid species filtering appears as the gill rakers develop. Most of the clupeoids must reach a length of 80–100 mm before starting to filter.

Large zooplankton filter-feeders

There are a few large fishes that are able to filter sufficient water to be able to filter-feed on zooplankton when this is abundant. In freshwater, the paddle fish (*Polyodon*) of the Mississippi Basin filters cladocerans, cruising around with its lower jaw dropped almost to 90° (Figure 2.18 on p. 52). In the oceans, the huge whale sharks (*Rhincodon*), basking sharks (*Cetorhinus*), and manta and devil rays (*Mobula*) all filter-feed. In whale sharks and manta rays, the filter is spongy tissue formed from modified denticles, while in basking sharks, there are long gill rakers (again modified denticles; Figure 1.19 on p. 23) on each gill arch. Whale sharks sometimes filter in the vertical position, pushing their heads slowly out of the water, allowing the pharynx to drain, and then subsiding slowly again below the surface. Basking sharks feed on copepods and live in temperate waters, where zooplankton varies widely in abundance during the year. While early calculations based on assumed zooplankton calorific value and abundance on the one hand, and estimates of the energy expenditure of the shark when swimming with wide open mouth while filtering on the other, suggested that these enormous fish are actually balanced on the knife edge of using more energy capturing their food than they can obtain from it. Several of the assumptions behind these calculations were recently shown by David Sims (1999, 2000; Sims *et al.*, 2003) to be faulty, resulting in an overestimation (by about three times) of the energy basking sharks require to swim. Also, contrary to earlier reports, basking sharks (although not all of them) do not shed their gill rakers and hibernate in the winter, but instead they feed on deeper-dwelling populations of mesopelagic zooplankton that do not experience such dramatic seasonal variations in abundance. However, at least some sharks do shed their gill rakers; one stranded at Polperro in Cornwall in November had the rakers covered with epithelium and shorter than those of feeding adults.

Plankton pickers or particle feeders

Particle-feeding or picking zooplankton is seen also in some rather unexpected fishes. The deep-sea tripod fishes (see Figure 2.9 on p. 44) pick copepods from the benthopelagic plankton, as they sit perched on their fins facing into the current. Curiously, the spur dog (*Squalus*) may also feed in this way on

the planktonic ctenophore *Pleurobrachia*, which it sometimes takes in such amounts as to fill its gut, and it has recently been suggested (from their parasite communities) that eels of all sizes in rivers and lakes in England feed regularly on planktonic copepods.

Bottom feeders, detritivores

Many detritivores are bottom vacuumers/suckers that typically possess small, undershot, inferior mouths equipped with suctorial lips and barbels, sensory pits, etc. positioned to help detect buried prey. Other detritivores feed on phytoplankton and mud particles, triturated by the same kind of gizzard as in mullets (Mugilidae). Although mullets typically browse on the algal film over mud (leaving a characteristic series of depressions where they have gulped in surface mud), they can also feed on planktonic algae at the surface–air interface using the gill rakers as a sieve (Figure 7.14). Detritivores may have either a one- or two-part stomach. Those with two-part stomachs have short intestines and those with a one-part stomach, a long intestine.

Herbivorous fishes

Herbivorous (phytophagous) fishes generally have small, often inferior, mouths equipped with rasping or nipping teeth. Pharyngeal teeth may be present. Stomachs are generally absent, but if present are thin walled and elastic. Because plant material is usually difficult to digest they may use cellulase and other enzymes produced by gut microflora (Jobling, 1995), hind gut fermentation by bacteria and protozoans of carbohydrates to short-chain fatty acids that can be directly absorbed with intervention of bile salts, or mechanical processing (trituration) by a muscular stomach/gizzard or mastication by pharyngeal teeth.

Overall, herbivorous fishes are very much in the minority, and are least common in the sea and in temperate freshwaters. Only some 5% of all fish families (18 marine and at least 20 freshwater) contain herbivores, although herbivorous individuals may be among the most numerous in any fish community (Horn, 1989). Perhaps this is because although more energy has to be expended by herbivores than by carnivores in obtaining their food, herbivo-

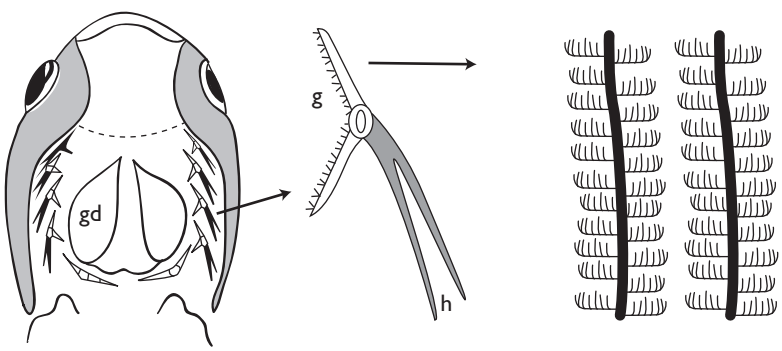

Figure 7.14 The filter-feeding apparatus of the mullet (*Liza*), a facultative filter feeder. Left: schematic horizontal section of head showing position of gill rakers on branchial arches. Right: the filtering gill rakers: g: gill rakers; gd: gizzard; h: gill hemibranchs. After Guinea and Fernandez (1992).

rous food resources tend to be quite abundant. Certainly, compared with carnivores, herbivores have to spend a good deal more of their lives feeding. Some phytoplankton feeders filter continuously, while a parrot fish spent 8 h day $^{-1}$, and *Haplochromis* and *Tilapia* 14 h day^{-1}, this in comparison with 1–3 h day^{-1} for salmonids. Whatever the diet, all fish have a high protein requirement (around 50% of dry weight of the diet). How do herbivores satisfy this requirement? As detritivores and herbivores they ingest large amounts of attached protein-rich microorganisms, even if they do not eat protein-rich algae.

Most marine herbivores are found around the coral reefs of the tropics, where surgeon fishes (Acanthuridae) crop algae growing over corals and on sandy patches near the reef, while parrot fishes (Scaridae), rabbit fishes (Siganidae), and damsel fishes (Pomacentridae) browse algae scraped off coral surfaces. Some temperate shore fishes, such as blennies and gobies, feed largely on seaweeds, and the much prized temperate freshwater ayu (*Plecoglossus*) of Japanese rivers, feeds solely on moss scraped from its territory of mossy stones. Ayu are an expensive delicacy since they are caught in a curious and somewhat inefficient way; fishermen place a small ayu on their line, below which is a series of unbaited hooks. Attracted by this decoy, another ayu is foul-hooked as it comes out to defend its feeding territory.

Herbivory is, however, much more common in tropical freshwaters, where seasonal flooding inundates forests and plains. Here fishes such as the grass carp (*Ctenopharyngodon*) can feed on grasses, and decaying vegetation, while fruits, flowers, and seeds that fall into the water are seized by fishes such as the cyprinid *Puntius* or fruit and nut eating characins, including the tambaqui (*Colossoma macropomum*) which can attain a weight of 30 kg, and species of piranhas (*Serrasalmus*). As in phytoplankton feeders, filter-feeders, and mammalian herbivores, herbivorous fish have longer guts with greater surface area for absorption than carnivores or omnivores. As a rough rule, the ratio of gut length to body length is greater than 3 in herbivorous fishes, from 1 to 3 in omnivores and less than 1 in carnivores.

Unusual food types

Fish have also evolved adaptations that permit them to feed on the fins, scales, mucus, blood (lampreys, candiru (*Vandellia cirrhosa*), male angler fish), even the eyes (*Haplochromis compressiceps*) of other organisms. Such species typically possess highly specialized, often bizarre morphological and behavioral adaptations. Cleaner fish that feed on ectoparasites also provide interesting examples of symbiotic relationships between the small cleaner and the usually much larger host species as described in Chapter 13.

Envoi

Food and feeding presents some of the most interesting applications of current ecological theory and modern technology in the study of the lives of fishes. How fishes acquire their food, what they eat, and how that food is utilized are all fundamental questions facing investigators. Traditional studies, often based on stomach contents, are now enhanced by computer modeling, electromyographic recording, and digital photographic techniques that allow for real-time, *in-vivo* data collection on feeding habits.

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8 Reproduction, and Life Histories

8.1 Types of Life History

In contrast to other vertebrate groups, reproduction in fishes exhibits great diversity and many original features. Reproductive strategies are as diverse as the adaptations to numerous aquatic environments that are found in fishes. This diversity may concern sexuality, spawning, and parental behavior, sensitivity to environmental factors, and specific features of gametogenesis (Jalabert, 2005). Knowledge of fish reproduction and life history is important. Fishes represent models for reproduction in higher vertebrates (at least in some species), successful fisheries and aquaculture depend on understanding, even controlling reproduction (Imsland and Jónsdottir, 2003). The prospects of breeding fish that exhibit desired characteristics for food, recreational fisheries or the aquarium trade requires an understanding of reproductive physiology, breeding behavior, and genetics (Purdom, 1993).

Fishes vary in where they reproduce, some, such as the annual killifishes of the tropics, being confined to a single small pool their entire lifetimes, others undertaking lengthy migrations between feeding and breeding sites. Some fishes lay eggs, others produce living young, and even their manner of determining which sex an individual belongs to shows greater variation than is found in any other group of vertebrates. Numerous reviews of fish reproductive physiology and development exist, perhaps the best still being W. S. Hoar's (1969) paper. More recent reviews (Carrier *et al.*, 2004; Yaron and Sivan, 2006) include increased coverage of endocrines and other molecular aspects of reproduction. Other worthwhile resources include Nikolsky (1963), Potts and Wooton (1984), Blaxter (1969, 1988) and Hamlett (2005).

Among the most remarkable life histories are those of the marine lampreys, Pacific salmon and several species of freshwater eels that spend most of their lifetimes in either fresh or seawater, but migrate to the other habitat for spawning. The physiological (and in some cases anatomical) changes that are necessary to permit these fish to survive in their alternative habitats are considerable, and it is little wonder that among these species death generally occurs after spawning. In keeping with their well established penchant for categorizing things, biologists have developed terminology to describe these habits. As noted earlier in Chapter 2, fishes that spend most of their lives in freshwater but spawn in the ocean are deemed to be catadromous, while those

with the opposite life history strategy are anadromous. The term diandromy may be applied to either type.

Pacific salmon afford us with the textbook example of an anadromous fish, although other species including Atlantic salmon, several other salmonids, lampreys (*Petromyzon* and *Geotria*), sturgeons (*Acipenser*), shads (*Alosa*), and striped bass (*Morone*) also exhibit similar migratory and reproductive patterns. Salmon are hatched in freshwater streams, often at considerable distance from the sea, spend their first year (more or less) of life working their way downstream and then live for several years at sea, the exact time and extent of their travels (see Figure 8.1) varying among species and even populations

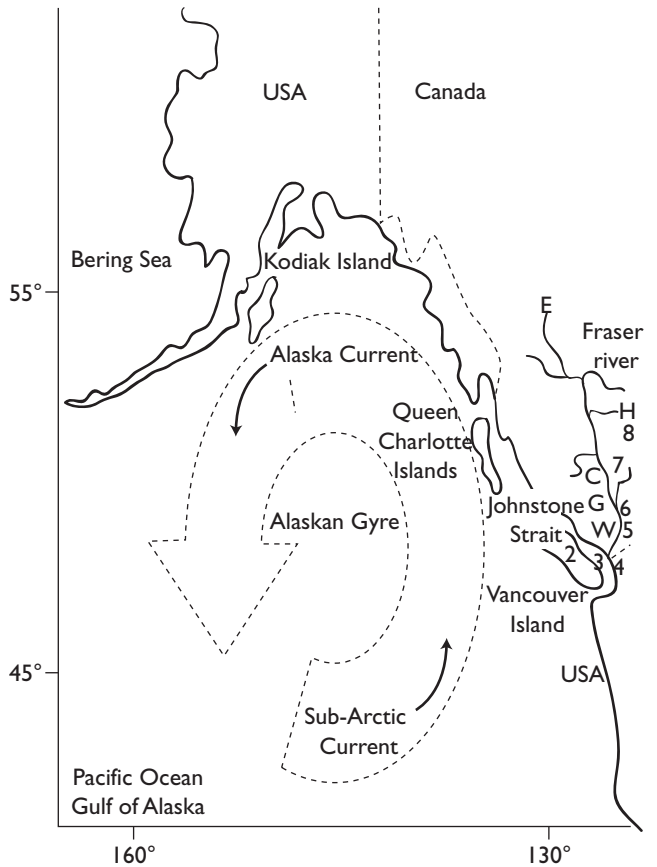

Figure 8.1 Map of northern Pacific Ocean and northwestern coast of North America showing locales, landmarks, and major current patterns associated with salmon migration phases. *Oncorhynchus* species differ in extent and time of main migration. Coho remain in coastal waters near their area of origin (although some individuals are more wide-ranging), Chinook feed in both coastal and offshore waters – divided into two "populations" (although there may be more): "coastal type" and "ocean type." The latter migrate into sea water during their first year of life, while coastal or stream type remain in freshwater a full year or more, but when they do go to sea they rapidly move offshore – off the continental shelf and across to the Asiatic coast, whereas the ocean types remain close to the North American shore, over the shelf or even in estuaries.

(Eikaas *et al.*, 2006), before undertaking the arduous migration back to the same stream in which they were born.

Anguillid eels demonstrate the opposite pattern, utilizing freshwater (or estuarine) habitats for growth and feeding, and returning to the sea to spawn. In the North Atlantic, eels from both North America and Europe spawn in the Sargasso (van Ginneken and Maes, 2006), while, in the Pacific, anguillid eels utilize sea mounts as spawning centers (Tsukamoto, 2006; Figure 8.2). Among the so-called "southern trouts" of the family Galaxiidae, some species are anadromous, others catadromous, while others are confined to freshwaters.

In most fishes the male and female reproductive functions occur in separate individuals, some producing sperm, others producing eggs. Fertilization is external and the large number of eggs produced by each female (on an annual basis the "fecundity") are left to develop, hatch, and grow without parental care. The newly hatched young are a few millimeters long, usually in the form

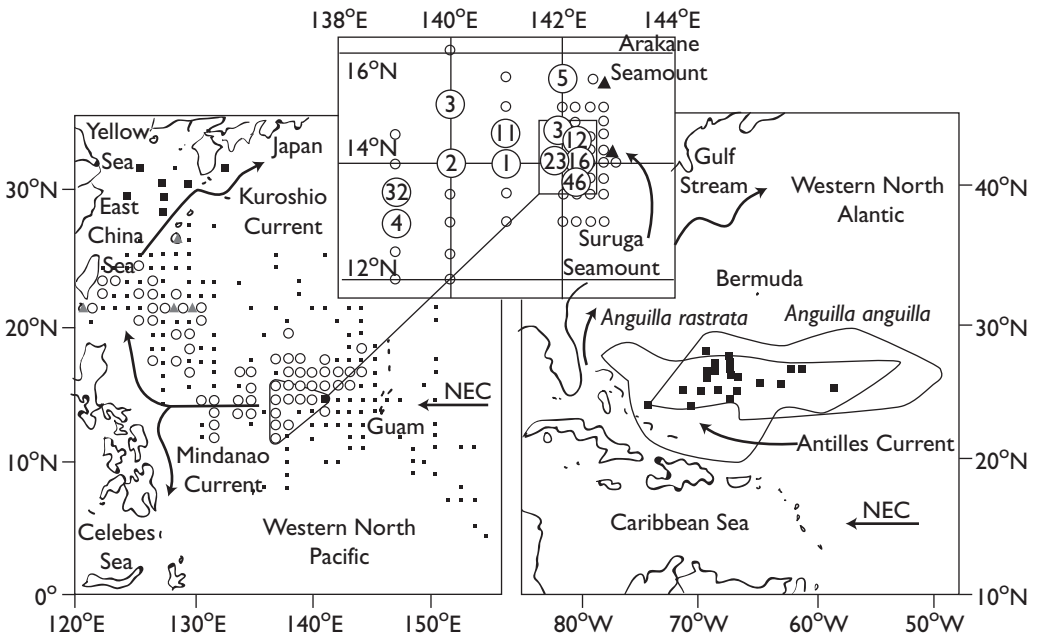

Figure 8.2 Pacific and Atlantic eel spawning sites. Left: collections of Japanese eel, *Anguilla japonica*, in the western North Pacific Ocean between 1961 and 2002 (n = 2418), circles indicate sites where leptocephali larger than 7 mm were collected. Metamorphosing leptocephali (shaded triangles) and oceanic glass eels (squares) were also found. Black dots: sites where no larvae were collected. Right: eel collections in the North Atlantic 1913–1985. Shaded areas: sites where leptocephali ($\leqslant$ 10) of both European or American eels were found. Solid squares mark sites where preleptocephali were collected from both oceans. NEC = North Equatorial Current.

Inset, distribution and number of Japanese eel preleptocephali ($\leqslant$ 7 mm) shown by shaded circles and leptocephali > 7 mm, by open circles) collected during the time of the new moon June 2005. Black triangles indicate location of seamounts, black circles stations where no Japanese leptocephali were found. Adapted from Tsukamoto (2006 p. 929).

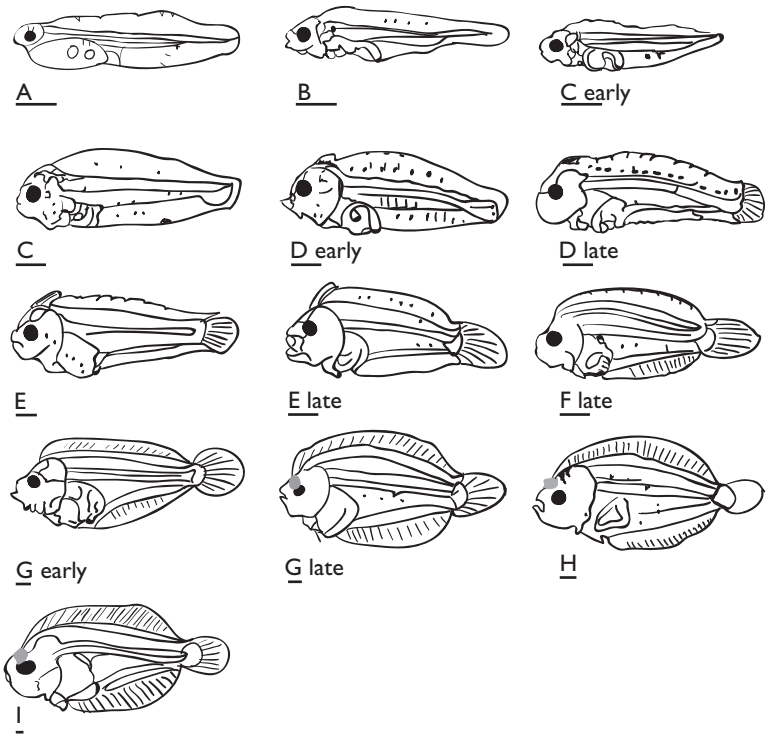

Figure 8.3 Hatched larval stages of *Paralichthys dentatus*. Scale bars = 100 μm. Camera lucida drawings from fixed specimens; pigment cell morphology, and distribution are not representative of live larvae. Although finrays begin to develop by late stage C/23, they are not readily visible until stage F/26. In stages F/26–I/29 the position of the migrating right eye is shown in gray. From Martinez and Bolker (2003).

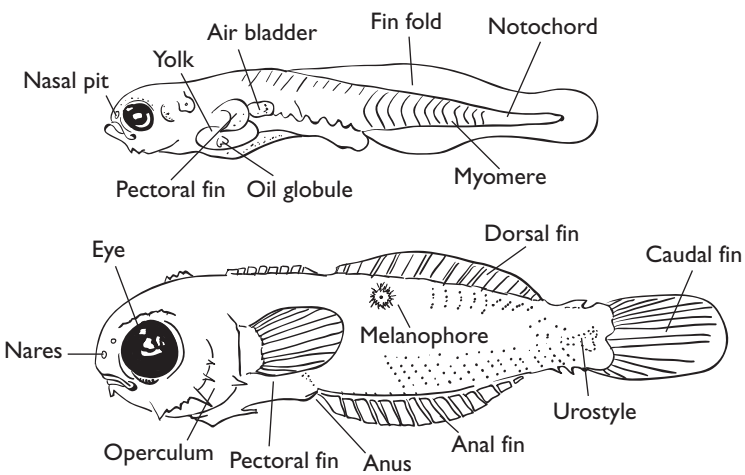

Figure 8.4 Yolk-sac larva and late stage larva of a blenny. Redrawn from Fahay (1983).

of larvae quite unlike the adult, with a yolk sac and relatively undeveloped body form (Figures 8.3 and 8.4). Once the yolk is resorbed, the larvae must find food on its own. After a period of growth the larvae metamorphose into the juvenile, immature adult, form.

This typical reproductive or life-history strategy is open to many modifications. Some species are hermaphrodite or change sex during their lifetime; others have internal fertilization, usually leading to live-bearing; others guard their eggs or young for varying periods of time. Balon (1985) describes "guilds" of fishes grouped into a classification of reproductive styles such as non-guarding, guarding, and bearing which emphasize the very wide range of strategies evolved by fishes for the care of their eggs.

There is also a very significant relationship between fecundity and egg size. Since the size of the ovary is limited by the size of the female, high-fecundity females must necessarily have small eggs and vice versa. A further strategy is determined by the spawning season. In the tropics, spawning may be year-round but in more temperate regions spawning is usually seasonal, most often in the spring. Some species have one-off spawning, the eggs all being produced in a single batch; others are batch spawners, producing several batches of eggs over a more prolonged spawning season.

Because fish are poikilotherms, the rates of development and growth are very dependent on temperature (Figure 8.5). Generally speaking, tropical fish have rapid growth, small final size and a short generation time, although there are obvious exceptions suchs as whale sharks, marlin, barracuda, and jacks. Fish in the deep sea or at high latitudes often grow slowly to a considerable size and can live to a greater age. Determination of age depends on counting rings on the scales or otoliths (p. 249) and it is often far from certain whether each ring corresponds to a year of age or not. A lifespan of 10–25 years is common for many of the commercial species with which we are familiar, but some species (e.g. the redfish *Sebastes*) live for 50 years or so, and tagging studies in the northwest Atlantic have shown that the sandbar shark, for example, may live for 40 to 50 years not reaching sexual maturity until it is 30 years old.

Age determination from scales or otolith annuli has been supplemented for some species by radiometric analysis of otoliths. This involves determining the ratio of the naturally occurring radionuclide ^{226}Ra and its decay product ^{210}Pb in the otolith. The ratio of the two (which have different half-lives) depends on their decay rates since the time that ^{226}Ra was incorporated into the otolith. Remarkably enough, such determinations have shown that the orange roughy (*Hoplostethus atlanticus*, first discovered off the Azores and later also found around sea mounts in the Atlantic and Indo-Pacific) from south-east Australian waters matures at around 32 years, when it is 32 cm long, and is very slow growing, fish 38–40 cm long turning out to be no less than 77–149 years old (Fenton *et al.*, 1991).

The quantification of nucleic acids, especially the ratio of RNA to DNA, is a recent method of assessing larval condition, nutritional status, and growth. RNA levels are indicative of protein synthesis, while DNA content provides a measure of the total cell number/volume. This ratio has been shown to respond to changes in a fish's feeding and growth status after only 3 days (Rooker and Holt, 1996).

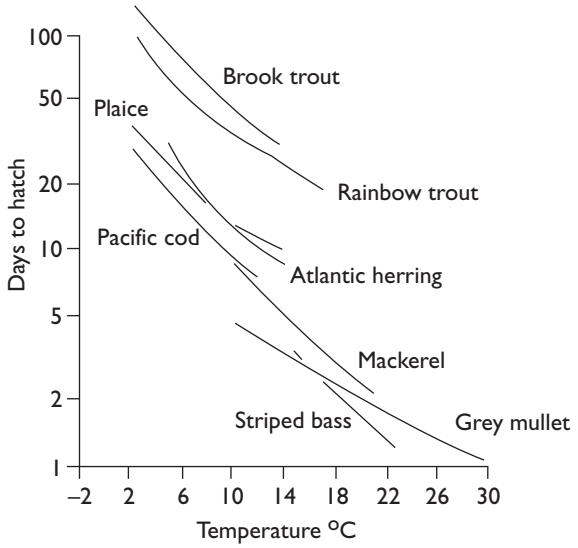

Figure 8.5 Time from fertilization to hatching in various species depending on temperature. Redrawn from Blaxter (1988).

8.2 Fecundity and Egg Size

It is not at all clear why some species have evolved a strategy of producing many small eggs, and others the opposite strategy of fewer, larger ones. The relationships between these characters and the implication for the species can be summarized (Blaxter, 1988) as follows:

- Fecundity and egg size are inversely related when comparisons are made between species (Figure 8.6). In batch spawners, egg size tends to decrease as the spawning season progresses.
- Fecundity increases with age and size of the female within a species (Figure 8.6).
- Large eggs take longer to develop than small eggs, when interspecific comparisons are made.
- Large eggs produce larger larvae at hatching with a longer period of feeding on yolk reserves.
- Fecundity tends to be high in marine fish that release their eggs into open water; it is lower in freshwater species and those species that provide parental care (Figure 8.7).

Winemiller and Rose (1992) note that the life history strategies of fishes fall within a trilateral continuum (Figure 8.8), the endpoints of which comprise three basic strategies: opportunistic spawners such as anchovies, silversides, and most cyprinodontiformes, with high reproductive effort, early maturation, and small body size, low per batch fecundity but multiple spawning bouts per season, little parental investment, and short generation times; periodic spawners, such as salmon, most clupeids and other marine species with moderate reproductive effort, delayed maturity, and large body size contributing to high batch fecundity, little parental investment, and long generation times; and equilibrium spawners, such as cichlids, centrarchids, syngnathids,

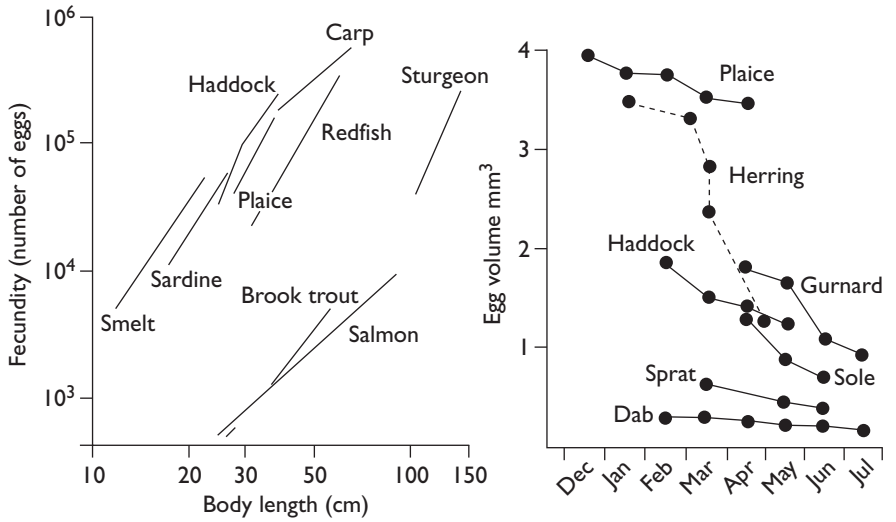

Figure 8.6 Left: the fecundity of various species depending on their length. Redrawn from Blaxter (1969). Right: the size (volume) of the eggs of various batch spawners as the spawning season progresses. Data from Bagenal (1971).

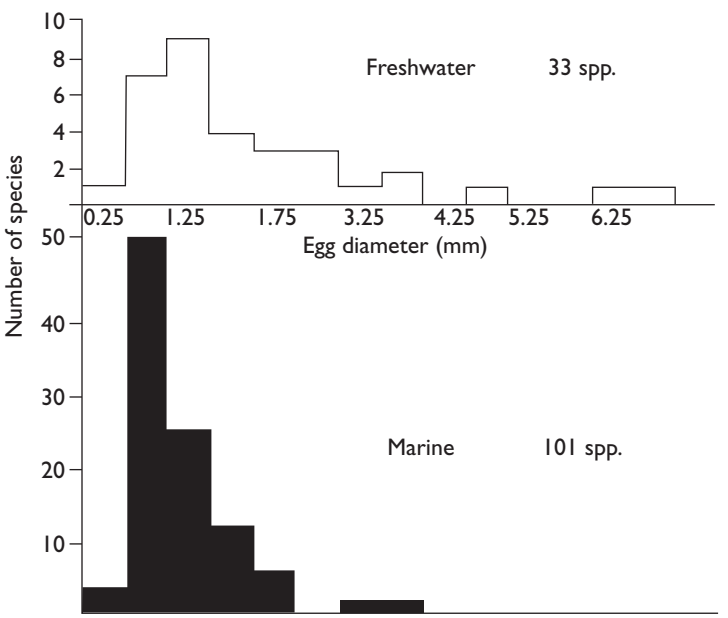

Figure 8.7 Frequency distribution of egg diameter of European marine and freshwater fishes. After Wooton (1979).

nest-building coral reef fishes, and many catfishes with low reproductive effort, variable body size and age at maturity producing low batch fecundity but with high parental investment, and moderate to long generation times. While the three apices of the triangular model correspond to extremes, there

are many intermediate examples representing trade-offs between the different factors that determine overall reproductive success in many environments. The second and third categories correspond respectively more-or-less to the "r" and "K" selective strategies applicable to many phyla that have been discussed by MacArthur and Wilson (1967) and other theoretical ecologists. A K-strategy is appropriate in stable crowded environments where a low fecundity (large egg) and long developmental period may be favored. In less stable, uncrowded environments, where there are chances for maximal population growth, the r-strategy is optimal with high fecundity (small egg) and a short developmental period to exploit any opportunities for expansion.

8.3 Maturation

Vertebrates of both sexes possess a germinal epithelium consisting of germ and somatic cells. The gonads are mesodermal in origin and develop in close association with the nephric system. In elasmobranchs a lateral cortex gives rise to the ovary, and a more medial area becomes the testis. One of these usually

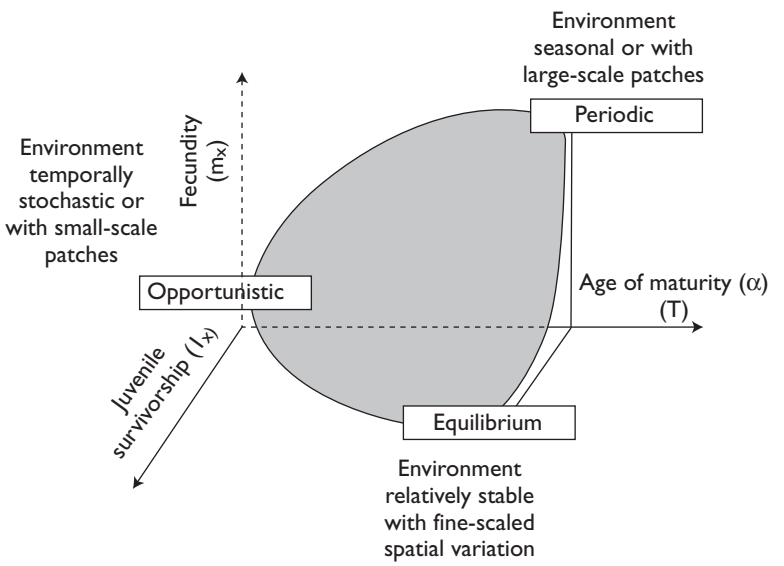

Figure 8.8 Model for an adaptive surface of fish life-history strategies based on fundamental demographic trade-offs and selection in response to different kinds of environmental variation. The opportunistic strategy (small T, small m_x, small l_x) maximizes colonizing capacity in environments that change frequently or stochastically on relatively small temporal and spatial scales. The periodic strategy (large T, large m_x, small l_x) is favored in environments having large-scale cyclic or spatial variation. The equilibrium strategy (large T, large l_x, small m_x) is favored in environments with low variation in habitat quality, and strong direct and indirect biotic interactions. Curvilinear edges of the surface portray diminishing returns in the theoretical upper limits of bivariate relationships between adult body size and clutch size, adult body size and parental investment/offspring (a correlate of juvenile l) and clutch size and juvenile survivorship. Adapted from Winemiller and Rose (1992, Figure 6, p. 2212).

atrophies early in development when the sex is determined. In cyclostomes and teleosts the gonads derive from a single region equivalent to the cortex. While most fishes have paired gonads, fusion of two primordia in the cortex of lampreys leads to a single gonad, whereas in the hagfishes one gonad fails to develop, and there is only a single ovary in some sharks (Lagler *et al.*, 1962).

The gametes first appear as primordial germ cells, the maturation of which is regulated by hormones (see Chapter 9 and Figure 8.9). The ovary is usually hollow, although the wall may become folded as its internal surface area increases.

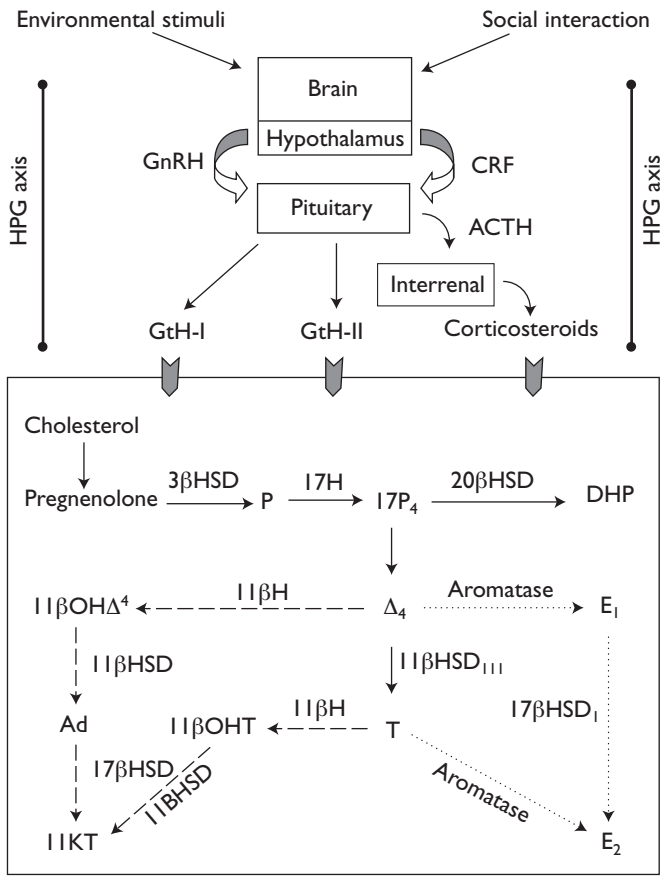

Figure 8.9 A simplified representation of the endocrine pathways relevant to the regulation of sex-change in sequentially hermaphroditic fish. Steroidogenic pathways predominating in male and female fish are denoted by dashed and dotted lines (respectively). Feedback relationships are not illustrated. Abbreviations: HPG, hypothalamic-pituitary-gonadal; GnRH, gonadotropin releasing hormone; GtH, gonadotropic hormone; HPI, hypothalamic-pituitary-interrenal; CRF, corticotropin releasing factor; ACTH, adrenocorticotropic hormone; P, progesterone; $17P_4$, 17-hydroxyprogesterone; DHP, 17,20β-dihydroxyprogesterone; Δ_4, androstenedione; Ad, adrenosterone; T, testosterone; 11KT, 11-ketotestosterone; 11βOHT, 11β-hydroxytestosterone; $11\beta OH\Delta_4$, 11β-hydroxyandrostenedione; E1, estrone; E2, estradiol-17β; H, hydroxylase; HSD, hydroxysteroid dehydrogenase. From Frisch (2004, p. 484).

The oogonia become surrounded by a single layer of follicular cells in cyclostomes and teleosts but a multilayer in elasmobranchs. In the primary oocyte stage the developing eggs are supplied with yolk by the follicular cells during vitellogenesis. The yolk attains a granular texture in most fishes; however in atherinomorph fishes it retains a liquid composition. As will be noted below, this is not the only reproductive peculiarity of the Atherinomorpha. At the completion of maturation the oocyte becomes free of the follicle during ovulation and water is taken up, causing the egg to swell. In batch spawners, populations of different-sized oocytes can be seen, each corresponding to a future batch of eggs for release. In some species the mature eggs are released into the body cavity and pass into the funnel-shaped opening of the oviduct and so to the exterior; in other species the ovarian lumen and oviduct are continuous.

Elasmobranch and bony fish testes are unique among vertebrates in that spermatocyte development occurs in cysts (spermatocysts) that contain spermatocytes of a single genetic lineage (Miura and Miura, 2003). During development, spermatocytes migrate from their site of origin and empty eventually into an efferent duct. Despite this similarity, the teleost and elasmobranch testes are differently organized.

In most sharks the testes are paired cylindrical organs with a ridge of germinal tissue running along each lateral surface. Spermatocyst development proceeds across (diametrically) each testis leading toward the medially located efferent ducts. In contrast to this pattern, the testes of lamniform sharks are cylindrical organs with several germinal zones surrounded by seminiferous follicles. Spermatocyte development occurs radially, with the mature spermatozoa being released in efferent ducts that surround each group of follicles (Jones and Jones, 1982). Skates and rays appear to have a different structure termed "compound" that shows characteristics of both other types (Pratt, 1988).

As described by Parenti and Grier (2004), bony fishes show even greater variation of testis morphology. *Latimeria*, non-teleost Actinopteryigii, and basal teleosts are characterized by testes with anastomosing tubules (although it must be noted that these are fundamentally different from the tubular testes of amniotic vertebrates) whereas derived taxa (Neoteleosts) have a lobular testes, which in turn can be divided into a so-called "perciform" testes (because it was first observed among perciform fishes, but in fact is also found in all major groups except Atherinomorphs) in which spermatocytes are distributed along the entire length of the lobules and an "atherinomorph" testis (which is restricted to and found universally among atherinomorph taxa) in which spermatocytes are restricted to the distal ends of the lobules.

The spermatogonia pass through a spermatocyte and spermatid stage before becoming spermatozoa. During spawning these pass directly to the exterior via the seminiferous tubules and vas deferens. En route the spermatozoa are diluted with secretions of seminal fluid. Some elasmobranchs, for instance the basking shark, and live-bearing teleosts produce spermatophores or packets of spermatozoa.

8.4 Intersexes, and Unisexual Species

The sexuality of teleost fishes is extraordinarily complex with genetics, environment, and other endogenous and exogenous factors all playing a role in

some species (Baroiller *et al.*, 1999). Genetic determination may include factors located on autosomes or sex chromosomes, with either heterogametic males (XY or XO) or females (WZ; Devlin and Nagahama, 2002). Many species are gonochoristic: individuals are either males or females throughout their reproductive life, although in what is termed "juvenile hermaphroditism," the gonads of some fishes develop first as ovaries before differentiating into testes, even when these individuals never become functional females (Frisch, 2004). But in other species the same individual may posses both functional male and female gonads during its lifetime. Although hermaphroditism is almost unknown in the ostariophysans, the dominant freshwater group, it is common in marine actinopterygians, especially in families represented in coral reef fauna such as labrids (wrasses), sparids (sea breams), serranids (sea basses), scarids (parrot fishes), pomacentrids (damsel fishes), and polynemids (threadfins). In the deep sea, intersexuality is found in a number of groups, for example *Gonostoma* and *Cyclothone* in the bathypelagic stomiatoids and in the benthic chloropthalmid tripod fish *Bathypterois*.

Hermaphroditism may be synchronous where both parts of the ovitestis mature together as in *Bathypterois*, or successive with the individual starting as a male and becoming a female, as in *Gonostoma gracile* and *Cyclothone microdon* or vice versa. One of the potential advantages of being synchronously hermaphrodite, viz. to allow self-fertilization in a sparsely distributed population, does not seem to occur in the tripod fish but, perhaps, the meeting of two individuals allows the fertilization of two batches of eggs as has been observed in several species of shallow-water fishes such as the hamlets (*Hypoplectrus*). In the successive protandrous (male first) hermaphroditism of the stomiatoids the males are obviously smaller than the females (Figure 8.10). In such fishes size is much more at a premium for the females in that they can accommodate a large number of eggs. The small male, having fertilized the eggs of its older and larger conspecifics, can then be saved to become a female itself. Successive hermaphroditism in which smaller males transform into

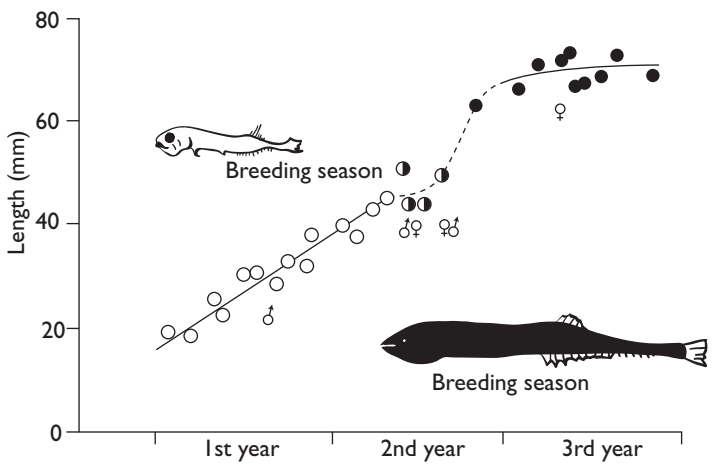

Figure 8.10 Male to female sex change during life of *Gonostoma gracile*. After Kawaguchi and Marumo (1967).

females is found in many coral reef fishes such as groupers and many species of sparids. In parrot fishes and wrasses the hermaphrodites are protogynous (where the individuals change from plain-colored females to brightly colored males). In this instance the larger males may be less susceptible to predation when searching for females or better at defending their territories that typically contain more than one large female. If something should happen to the male, the largest female will undergo transformation and become the new territorial male. Interestingly enough, most species of wrasses and parrot fishes also possess gonochoristic males which spawn en-masse with schools of smaller females (Robertson *et al.*, 1982). In other reef species, such as anemone fishes, the small size of the territory (a single anemone) again makes the reproductive potential of a large female the more important. Viewers of the movie "Finding Nemo" should take consolation in the fact that Nemo's widowed father Marlin would eventually have become Marlene, a replacement mother for Nemo.

In addition to fishes, the individuals of which may belong to both sexes, some populations (which arguably may be regarded as species) may consist solely of females. The African cyprinodontoid *Rivulus marmoratus* is a self-fertilizing hermaphrodite, in which each successive generation contains only genes supplied by the mother. Males of this species do exist and gonochoristic reproduction is possible and permits genetic recombination. The live-bearing Amazon molly, *Poecilia formosa*, found in northern Mexico and south Texas exists only as females. These mate with males of closely related species; however, the male's sperm is only required to stimulate development of the diploid eggs produced by the Amazon molly, a phenomenon known as gynogenesis (Figure 8.11A). Gynogenetic species or populations are also known from other genera including *Menidia*, *Carassius, Phoxinus, Cobitus, Rutilus*, and *Fundulus*. Additional gynogenetic strains have been produced artificially, chiefly for aquaculture purposes, in other species. A similar reproductive phenomenon, known as hybridogenesis (Figure 8.11B), is found on the Pacific coast of Mexico where all female populations of another poeciliid live-bearer, *Poeciliopsis*, are found. Females of these populations exist as triploid hybrid strains (which unlike *P. formosa* have not attained the distinction of a formal name, but instead are known by combinations of the presumed parental lineages, e.g. *P. monacha-2 lucida*) and also mate with males of related species, but in these cases the male genome is incorporated and may or may not be expressed in the subsequent generation. Regardless, the male chromosomes are segregated and discarded at the time of meiosis, so that the eggs represent a clonal lineage.

In theory, such asexually reproducing populations have at least only a short-term advantage over their sexually reproducing neighbors as all individuals, rather than only ~50%, are reproducing females. Sexual reproduction, on the other hand, is supposed to confer an instantaneous as well as long-term advantage, in that offspring will show increased resistance to parasites and diseases (W. D. Hamilton's take on van Valen's Red Queen hypothesis) and so asexual strains should be short-lived as there is no way to eliminate deleterious mutations once they occur. Molecular evidence has indicated that *Poecilia formosa* as well as the lineages of *Poeciliopsis* have existed for 100 000 years, or well beyond the 10^5 generations that are predicted as theoretical maximums for them (Schlupp, 2005).

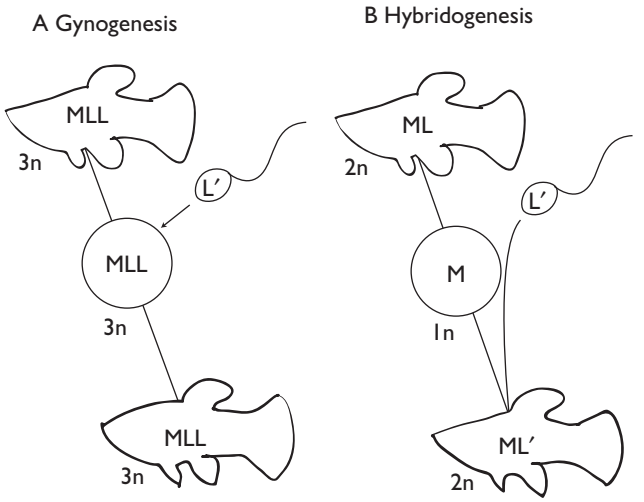

Figure 8.11 Parthenogenesis in Mexican livebearers. In gynogenesis (A) triploid females, designated MLL for *Poeciliopsis monacha-lucida-lucida*, produces triploid eggs that are activated, but not fertilized by sperm from male *P. lucida* (L′) producing a daughter genetically identical to the mother. In hybridogenesis (B) a diploid female (ML) produces haploid eggs that contain only the maternal genome (M). Sperm from male *P. lucida* (L′) combine to produce diploid females (ML′) but again, the paternal genome (L′) is discarded during oogenesis so all future eggs contain only the M genome. After Vrijehoek (1984) and Allendorf and Ferguson (1990).

8.5 Fertilization to Hatching (Incubation)

The released egg is protected by a fairly tough chorion or egg case. Within this the cytoplasm and yolk are contained by a vitelline membrane. Often one or more oil globules are present. Fertilization occurs by a spermatozoon passing through a funnel-shaped micropyle leading to a fusion of the pronuclei of the sperm and egg. This leads to activation when the oocyte, arrested before fertilization, then resumes development. The vitelline membrane separates from the chorion creating a perivitelline space, and the micropyle is plugged, preventing further spermatozoa entering. Some elasmobranchs have polyspermy in which a number of spermatozoa may enter the micropyle but only one fuses with the nucleus of the egg, the rest being resorbed and perhaps used as an additional nutrient. In salmonids, water activation takes place as the egg absorbs water regardless of whether fertilization takes place or not. In river water, the vitelline membrane becomes opaque and its permeability changes. If spermatozoa are not present, the fertilizability of the egg is soon lost. After fertilization the chorion hardens, protecting the egg. This is especially valuable in waves or surf or if the eggs are buried in gravel. The chorion remains permeable to water and small molecules but the site of osmoregulation (p. 177) is the vitelline membrane. Fish eggs are usually round although the hagfish (*Myxine*), anchovy (*Engraulis*) and bitterling (*Rhodeus*) have ovoid eggs, and in some gobies they are pear-shaped (Figure 8.12). Most species are telolecithal with yolk

concentrated at the vegetative pole and the cytoplasm at the animal pole giving a "polarity" to the egg. The egg goes through a process of cleavage and morphogenesis as the cells divide, which form layers, and then organs. In lampreys cleavage is holoblastic, the entire egg dividing to form smaller cells or micromeres at the animal pole and macromeres at the vegetative pole. In hagfish, elasmobranchs and teleosts development is meroblastic (Figure 8.13). Here, cleavage at the animal pole leads to a blastoderm or cap of cells. The blastoderm overgrows the yolk (epiboly) eventually enclosing it to form a gastrula, a hollow sphere of cells containing yolk with a small opening into the perivitelline space – the blastopore. The embryonic axis is laid down in relation to the dorsal lip of the blastopore and the neurula stage forms with the future head, spinal cord, and body musculature soon visible. After a time, the tail region grows away from the neurula and coils around inside the perivitelline space. The optic cups (the future eyes) and heart are the first organs to be identified easily. The heart starts to beat well before hatching and in some species the eyes become pigmented and functional, and the embyonic fish often wriggles and rotates within the chorion. The prelude to hatching is a softening of the chorion as a result of enzymes secreted by glands on the head. Some of the chorionic material is probably utilized as a nutrient by the embryo before hatching. The embryo then breaks free from the chorion to become a larva.

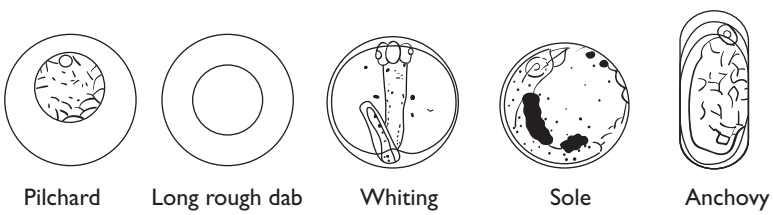

Figure 8.12 Eggs of various species. Redrawn from Russell (1976).

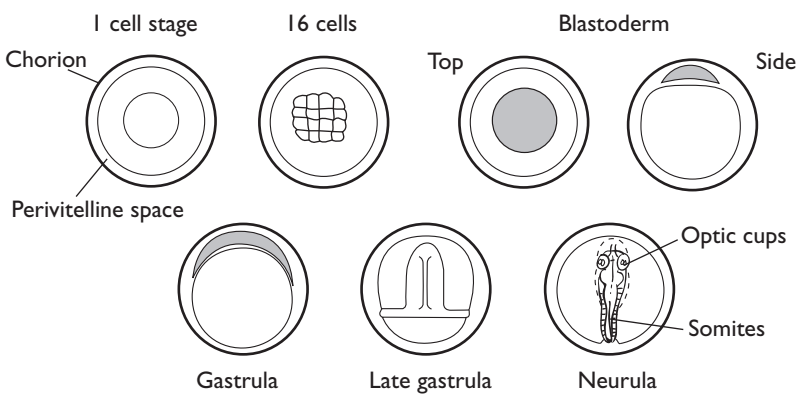

Figure 8.13 Stages in development of the meroblastic fish egg of *Fundulus heteroclitus*. Redrawn from Lagler *et al.* (1962).

8.6 Parental Care

Ovoviviparity

Retention of the eggs within the body of the female for subsequent development is necessarily preceded by internal fertilization but, even where fertilization is external, some species then take the eggs into their bodies to protect them. The male marine catfish (*Ariopsis felis*), for example, takes up to 50 eggs (20 mm in diameter) into its mouth and broods them until hatching, and even shelters the young for 2 weeks afterwards. Similarly, the male in seahorses and pipefish (Syngnathiformes) takes the fertilized eggs into special pouches or marsupia, whence they emerge as they hatch. Curiously enough, in the related *Solenostomus* the female lays eggs, which are fertilized, and she then takes them into a marsupium formed by the pelvic fins.

In ovoviviparous fishes the eggs develop in the "uterus" – the oviduct of elasmobranchs and the ovary of teleosts. During the "gestation" period – note that many of the terms are borrowed from mammalian reproduction – the eggs hatch within the mother and are eventually born alive as larvae or juveniles. The gestation period can last from a day or two in small tropical teleosts to 1 or 2 years in sharks. During gestation the eggs are protected from predation (although if the mother is eaten all the young are lost!) and live in physiologically regulated surroundings. At birth the young are larger than their oviparous counterparts and have better locomotor powers to avoid predators and to feed on a wider range of food organisms. Presumably such advantages compensate for a lower reproductive rate.

In ovoviviparous species there are two reproductive strategies: the eggs may merely develop on their endogenous yolk reserves so that the young are born at a lower weight than the original egg as in *Sebastes*; or, more commonly, the developing young take in nutrients from the uterus, either by mouth from uterine secretions, as in the electric ray *Torpedo*, or via the thread-like extensions of the uterine wall (trophonemata) which pass into the esophagus of the young through its spiracles, as in some stingrays and the butterfly ray, *Pteroplatea* (Figure 8.14). In these cases, the new born pup may be 50 times the weight of the unfertilized egg. In lamnoid sharks, the embryos develop a precocious dentition after their yolk reserves are used, and generally only a single young shark in each uterus survives pregnancy, reaching a size over 1.0 m. This huge size at birth is because the survivors have fed within the uterus on the unfertilized eggs or on their smaller siblings.

Viviparity

In viviparous species, an intimate connection is established between the maternal and embryonic circulation akin to that in the mammalian placenta. In the small cyprinodont *Heterandria formosa*, for example, the embryos are retained in the ovarian follicles, where a maternal capillary network is in intimate contact with the external surface of the embryo, and nourishes it. In the four-eyed fish *Anableps*, the placenta is derived from a large expansion of the pericardial wall, while in the small cyprinodont *Jenynsia*, the placenta links the maternal circulation with the branchial capillaries of the embryo (Meyer and Lydeard 1993). But it is in hammerhead and carcharhinid sharks that placental structure is best known (see p. 234).

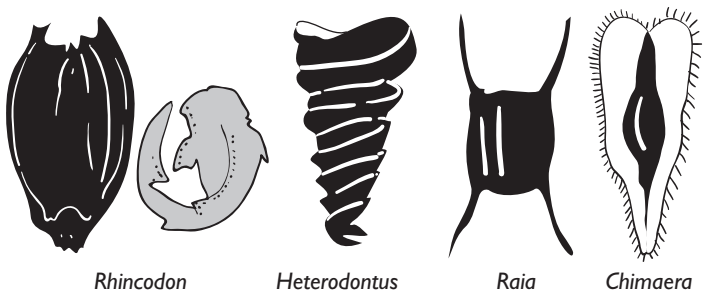

Figure 8.14 Egg cases of ovoviparous (*Rhincodon*, with late term embryo to right) and oviparous elasmobranchiomorphs (not to same scale). After Lineaweaver and Backus (1970), Dean (1906, p. 32), and Daniel (1922).

Nest building and brooding

Some littoral species of bullheads (Cottidae), blennies (Blenniidae), and gobies (Gobiidae) protect their eggs which are simply attached to the substratum, but many species from a range of taxonomic groups build nests in which the eggs are guarded, and sometimes ventilated, by one or both parents. These include the catfish *Ictalurus*, the stickleback *Gasterosteus* (p. 416), centrarchid sunfish, cichlids, the bowfin (*Amia*), and the lungfishes *Protopterus* and *Lepidosiren* (but not *Neoceratodus*). The Siamese fighting fish, *Betta splendens*, makes a nest of bubbles that may help to aerate the eggs as well as protect them. Fertilization is almost always external in species with paternal care and internal with maternal care, an exception being the sculpin, *Artedius harringtoni*, in which the male guards the eggs when they are laid some time after internal fertilization. Other species have brood pouches for carrying the eggs as in seahorses (*Hippocampus*), and pipefish (*Syngnathus*). The marine ariid catfish, the apogonid cardinal fish (*Apogon semilineatus*), and some tilapias are mouth brooders. *Tachysurus barbus* (see Figure 8.20, p. 240) incubates its eggs intestinally and *Platystachus cotylephorus* over its abdomen. The bitterling (*Rhodeus amarus*) lays its eggs within the gills of a freshwater mussel, an interesting turnabout as larval mussels, known as glochidia, are parasitic on fishes. These modes of parental care have not been adopted by elasmobranchs as a reproductive strategy since internal fertilization is universal. Elasmobranchs are much more commonly ovoviviparous than teleosts. All types of parental care are associated with small numbers of larger young, often with a fairly long reproductive period, and are therefore examples of K-selection.

8.7 Agnatha

The eggs or sperm from the single gonad of lampreys and hagfishes are shed into the body cavity and thence into the water through abdominal pores. In the large anadromous sea lamprey (*Petromyzon marinus*) the fecundity ranges from 24 000 to 236 000 but is only 400–9000 in smaller non-parasitic species. The eggs are laid in the stream bed and hatch in about 2 weeks as small pro-ammocoete larvae. They soon change into active ammocoete stages that burrow into silt banks and filter-feed. After a period of up to 5 years, longer in

parasitic species, they metamorphose into adults at a length of 12 cm or more. Some species descend to the sea to hitch rides on, and rasp blood from, other fishes, but the non-parasitic brook lamprey (*Lampetra planeri*) remains in freshwater.

The mortality of the ammocoete stages is relatively low judging by the decrease in population size of different year classes. A protracted ammocoete stage and delay of metamorphosis is probably favorable to the parasitic species where an adult existence is hazardous if host fishes of suitable size are scarce. The ammocoete is unique among fish larvae in having such a long sheltered existence and microphagous mode of feeding. Because of their size at metamorphosis and slow maturation rate, lampreys would be categorized as periodic spawners (Winemiller and Rose, 1992) but have many the characteristics of K-selected organisms. However, their high fecundity and unlimited food supply, at least in the pre-adult stage of parasitic species, suggest a typical r-selected species; once again demonstrating the difficulty of neatly compartmentalizing theories.

Hagfish are probably functionally dioecious, although their gonads pass through an hermaphroditic phase. They fit better as K-selected organisms and tend to be deep-sea forms where the environment is rather stable but food-limited. They lay linked batches of five or six large ellipsoidal eggs, 14–25 mm long, on the sea bed, or perhaps within their burrows. After 2 months or more, these hatch as juveniles. Curiously, although the eggs of *Bdellostoma* and the Australian *Geotria* are well known, the eggs of the European *Myxine* have very rarely been found. Holmgren (cited in Powell *et al.*, 2005) found only 131 during a 20-year search and all but three were undeveloped. The impecunious and optimistic reader will be disappointed to learn that the prize offered in 1865 by the Copenhagen Academy of Sciences for a description of the embryology of *Myxine* remained unclaimed, but has now been withdrawn!

8.8 Elasmobranchiomorpha and *Latimeria*

Reproduction

In elasmobranchs and holocephalans (chimaeras) fertilization is internal, the inner elements of the pelvic fins, the claspers, being rolled up to form a tube with overlapping edges. The claspers transfer sperm to the oviduct of the female. After fertilization the eggs may be laid on the sea bed or retained within the uterus.

Oviparity is believed to be the primitive condition for all elasmobranchiomorph fishes, (Dulvy and Reynolds, 1997) although today it is confined to only 40% of living species: the chimaeras, some families of skates (Rajoidea), and several (four perhaps eight depending on classification) families of sharks – horn or Port Jackson sharks (Heterodontidae), bamboo sharks (Hemiscyllidae), one or two species of the carpet sharks (Orectolobidae), and all but one species of scyliorhinid dogfishes including the collared and frilled catsharks (Parascylliidae and Proscylliidae) if these families are distinct. Egg laying appears to be most common among small species (< 1 m) which perhaps do not have enough internal volume to accommodate and sustain developing embryos. Live bearing (either ovoviviparity, carrying fertilized eggs to term inside the female, or true viviparity) has evolved independently nine or ten times, with maternal input

into nutrition of the embryo occurring in four or five of these cases. Egg laying seems also to have reappeared in two lineages – the skates of the family Rajidae, which comprise the most speciose family of living elasmobranchs, and the monotypic stegostomatid zebra shark, *Stegostoma fasciata*.

For many years, based on the occurrence of a briefcase-sized egg case collected in a shrimp net in the Gulf of Mexico, it was thought that the whale shark was also oviparous. Recently, however, a female whale shark containing over 300 near-term embryos was taken, providing convincing evidence that whale sharks, like all other large shark species, bear live young (Joung *et al.*, 1996).

Elasmobranch eggs within their horny cases range in size from over a centimetre in dogfishes to 25-cm long in the chimaeroid *Callorhynchus*. The egg capsule is horny and may be equipped with tendrils to attach it to weed or stone. By elasmobranch criteria, fecundity is quite high. Some species of skates (*Raja*) lay 100 eggs or so. The eggs incubate for several months or even longer. Recorded incubation times are 2.5–3 months in a carpet shark (*Chitoscyllium griseus*), 4.5–8 months in *Raja* species, 6–8 months in *Scyliorhinus*, and 9–12 months in the Port Jackson shark (*Heterodontus*) and the chimaera *Hydrolagus colliei*.

Although there are no documented examples of nest guarding or other forms of parental care among oviparous sharks, the mother sharks may take care to deposit their eggs in relatively safe locations. Port Jackson sharks (*Heterodontus portusjacksoni*) produce corkscrew-shaped egg cases (some egg cases are "right-handed", while others are "left-handed") which initially are soft and pliable so that the mother can push them into a rocky crevice. After a few hours in seawater the egg case hardens, the spiral flange making it necessary to literally unscrew the egg case to remove it from its hiding place.

Ovoviviparity and viviparity

Most sharks and rays with parental care are ovoviviparous (aplacental), the embryos depending on their yolk reserves, eating other eggs in the uterus (oophagy) as in the mackerel shark (*Lamna nasus*), or even consuming other embryos (adelphophagy) as in the sandtiger shark (*Carcharias taurus*), shortfin mako (*Isurus oxyrinchus*), and a few other species. *Lamna* and the thresher shark (*Alopias vulpes*) have a gestation period of about 2 years and the young (five individuals or less) are born at a length of about 60 cm. The ovoviviparous rays depend on uterine milk rich in protein and lipid, and produced by processes very similar to those seen in the production of mammalian breast milk. Such rays lack placentae, but thread-like trophonemata extend from the uterine wall into the mouth and gill chambers producing the milk and acting as respiratory membranes, as in the stingray (*Dasyatis violacea*), the eagle ray (*Myliobatis bovina*), and the butterfly ray (*Pteroplatea* = *Gymnura*; Figure 8.15). The viviparous (placental) elasmobranchs all belong to the Carcharhinidae, the gray sharks (the smooth dogfish (*Mustelus*) is a member of this family) or the closely related hammerhead sharks (Sphyrnidae; Figure 8.15). In *Sphyrna* a placental cord forms, bearing fine processes which aid in the absorption of the maternal secretions. In some species, such as *Mustelus canis*, the embryo depends upon its yolk for the first 3 months, and only then does a placenta develop, to nourish the embryo for the remaining 7 or 8 months of pregnancy (Figure 8.16; Wourms *et al.*, 1988; Hamlett *et al.*, 2005).

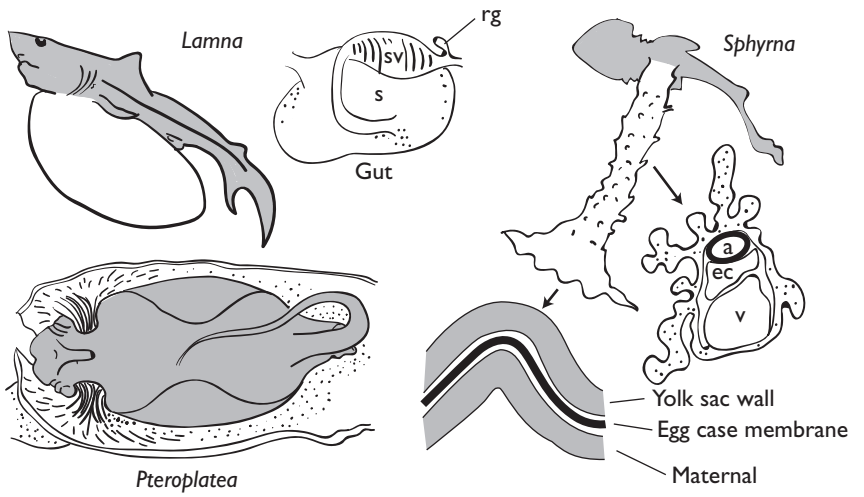

Figure 8.15 Elasmobranch embryonic nutrition. Upper left: egg-eating embryo of porbeagle (*Lamna*) showing greatly enlarged stomach (seen in detail on right). Lower left: embryo of butterfly ray (*Pteroplatea* = *Gymnura*) showing trophonemata entering spiracles. Right: placenta of the hammerhead (*Sphyrna*). The embryo is attached to the placenta by a cord bearing absorptive processes, seen in section to the right. a, artery; v, vein; ec, extra-embryonic coelom; rg, rectal gland; s, stomach; sv, spiral valve. After Wood-Mason and Alcock (1890), Schlernitzauer and Gilbert (1966), and Shann (1923).

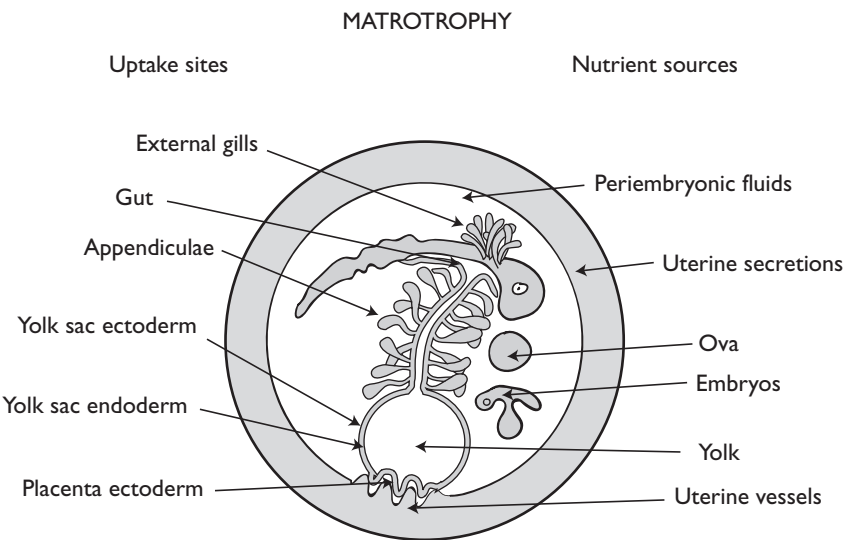

Figure 8.16 Schematic diagram of nutrient sources and uptake sites in chondrichthyan gestation. From Hamlett *et al.* (2005, Figure 13.1, p. 398).

Such sharks often come close inshore to give birth in shallow water nursery areas (often in bays), where adults that might eat the young do not normally occur. For example, at least eight species of carcharinids and hammerheads use Cleveland Bay in North Queensland as a nursery area. While in these nurseries, the adult females do not feed (Simpfendorfer and Millward, 1993).

Oviparous sharks and rays tend to be bottom dwellers of inshore waters and of relatively small size, with young usually less than 30 cm long at birth. The larger sharks and rays are viviparous and produce young mostly 30–70 cm in length. The largest shark, the whale shark, develops from a 60 cm, 1 kg embryo, of which an 11 meter female contained over 300. *Lamna nasus* produces young about 70 cm long and weighing about 10 kg, whereas the blue shark (*Prionace glauca*) has a maximum litter size of 54, the pups being 31–47 cm long and with a mean weight of 0.14 kg. As one might expect, there is a relative trade-off between numbers and size in species that have evolved parental care.

The genus *Mustelus* is of particular interest in the way that different species have adopted different reproductive strategies. While *Mustelus laevis* and *M. canis* are viviparous, although the placenta only develops after a period of ovoviviparity in the latter species, *M. vulgaris* and *M. antarcticus* are ovoviviparous.

Latimeria

Although a bony fish, the coelacanth *Latimeria* more closely resembles elasmobranchiomorphs in many of its physiological characteristics, including reproduction, and so is best considered here. *Latimeria* incubates very large eggs (like tennis balls) in the oviduct for an estimated 13 months. The embryos, 20–30 in number in a 2-meter-long female, have large yolk sacs which can also be seen in some fossil coelacanths (Figure 8.17). The adults live to an age of at least 30 years, possibly twice that long.

8.9 Teleosts

Teleost life histories are diverse. Although parental care is widespread, viviparity is only well represented in two major groups: the ophidioids and cyprinodontoids. The majority of teleosts are oviparous and much more fecund than

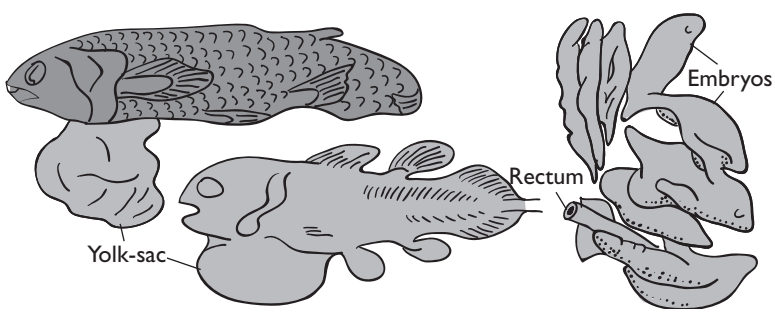

Figure 8.17 Coelacanth embryos. Above: *Latimeria* embryo from oviduct, shown schematically on right. Below: *Rhabdoderma* (Upper Carboniferous) – note yolk sacs in fossil and recent embryos. After Smith *et al.* (1975, pp. 190, 1105) and Schultz (1977, p. 277).

elasmobranchs. The incubation period is short and the newly hatched larvae have a relatively short period with high mortality before they metamorphose; they are therefore r-selected species. There are always exceptions to such generalizations, the 3-year drift of the leptocephalus larvae of European eels (*Anguilla anguilla*) across the Atlantic being an example.

One characteristic that distinguishes bony fishes (but agnathans as well) from cartilaginous fishes is the presence of a larval stage: incompletely developed, often with its own unique specializations, organism that must undergo anatomical and physiological reorganization, known as metamorphosis, before achieving their basic adult form. Larval forms are prevalent in every animal phylum, although there are many clades in which the larval period has been reduced, or even lost, providing opportunities for speculation by evolutionary biologists as to the relative advantages and disadvantages of metamorphosis in an organism's life cycle (Penchenik, 1999). Advantages may include the capacity for producing a much larger number of young (when size and number of young are inversely correlated), reducing competition within a species by allowing the young fish to inhabit a totally different habitat from that of adults, reducing the risk of predation (although there are many predators upon planktonic organisms), or allowing for dispersal over very great distances. For example, many coral reef fishes have pelagic larvae that are capable of dispersal by currents across vast stretches of open ocean in which the adults would find no suitable habitats. Such advantages may be purely speculative and must be balanced against a possible disadvantage of being hatched at a relatively small size and in an incompletely developed state. Additionally, larvae may possess features not found at all in the adults and thus requiring a significant energy expenditure associated with the anatomical rearrangement of metamorphosis into the juvenile. Like all biological processes, metamorphosis is ultimately controlled by genes, often working through the endocrine system. Manipulation of the timing of genetic induction can speed up metamorphosis and endocrine disruptors can postpone or even prevent it. The reduction or loss of a larval stage in many lineages has led to the suggestion that there may be selective pressures against it, but the retention of the larval period and subsequent metamorphosis in so many diverse lineages suggests that the cost of eliminating it may be evolutionarily too great.

There are no particular distinguishing characteristics of freshwater compared with marine eggs and larvae, although they have different osmotic problems. Freshwater eggs are more likely to be larger (1.0–2.0 mm in diameter, see Figure 8.7) and to be attached to weed or stones, and some are laid in nests or brooded in various ways. Although a few species, such as gouramis and grass carp, produce buoyant eggs, non-floating kinds may be best suited to freshwater, to prevent excessive drift in rivers and streams. Marine eggs are usually smaller (about 1.0 mm in diameter) and almost always buoyant and liberated into the pelagic zone. The herring and capelin (*Mallotus villosus*) are exceptions, laying sticky demersal eggs that adhere to weed or gravel on the sea bed or in the intertidal zone. The mummichog (*Fundulus heteroclitus*), Atlantic silverside (*Menidia menidia*), and Californian grunion (*Leuresthes tenuis*) lay their eggs on various substrata in the intertidal zone where they are theoretically less susceptible to predation, although the eggs must have greater

resistance to desiccation than the average fish egg (Horn *et al.*, 1999). Spawning is usually linked with tidal lunar cycles such that eggs are deposited during a very high or spring tide and hatch on a subsequent spring tide.

Marine eggs can easily be arranged to be buoyant since the solute concentrations within the yolk can be maintained osmotically below that of the ambient seawater, and hence less dense. There is usually a massive uptake of water at ovulation; the fertilized eggs of plaice, for example, are over 90% water. The specific gravity of the whole egg is then kept below that of seawater, although the yolk material itself is of higher specific gravity. A globule of low specific gravity oil may also help flotation. In freshwater, solute concentrations within the egg are of course higher than in the surrounding water, so buoyant eggs require large oil globules.

The larvae that hatch from teleost eggs are rather various in form, as seen in Figure 8.18, and often quite unlike the adult, for example leptocephali (Figure 8.19; some over 1 m long!) having curious forms with bizarre shapes. Unfortunately, there is insufficient space to cover the full range of teleost reproductive habits; all that can be done is to outline a few case histories, and to recommend the reader to seek further in the byways of teleost reproductive adaptations. References such as Leis and Carson-Ewart (2004), Richards (2004), or Smith and Heemstra (1986) provide information on and illustrations of larval fishes of the world's oceans.

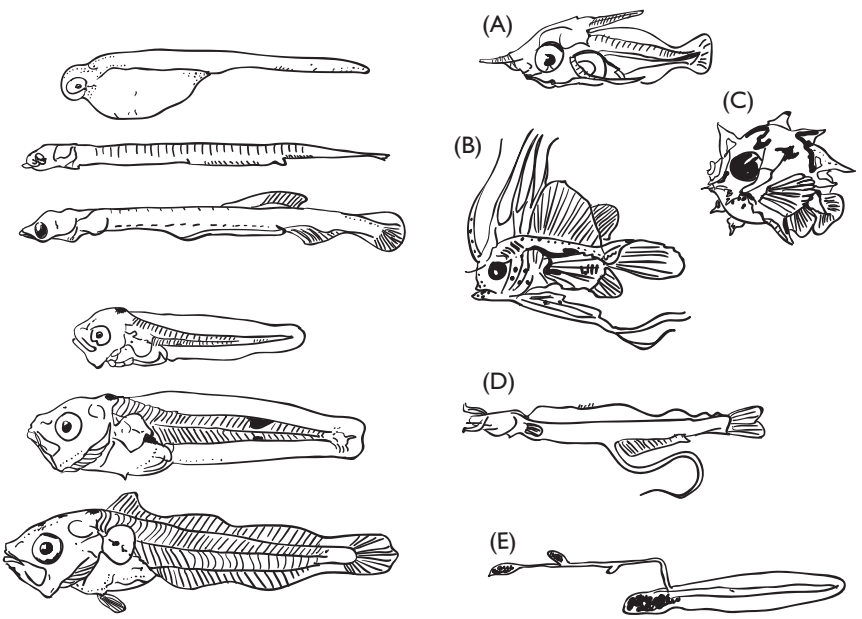

Figure 8.18 Teleost larvae. Left above: three stages in larval development of the northern anchovy (*Engraulis mordax*). Left below: three stages in development of the hake (*Merluccius productus*). Right: more unusual larvae (A) *Holocentnts vexillarius;* (B) *Lophius piscatorius;* (C) *Ranzania laevis;* (D) *Myctophus aurolaternatum;* (E) *Campus acus*. From Blaxter (1988).

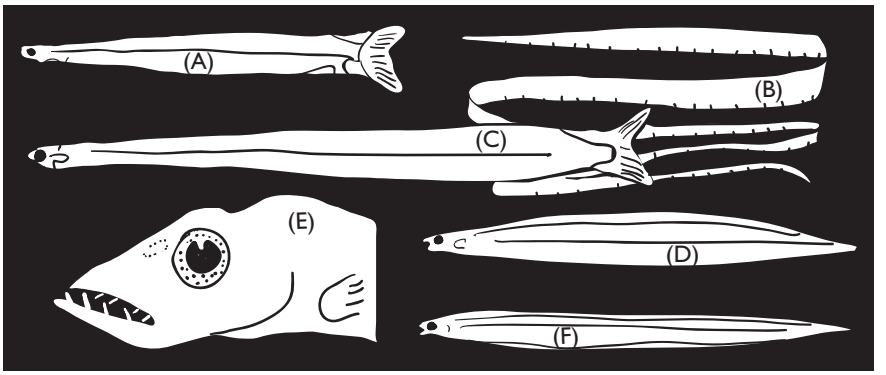

Figure 8.19 Leptocephalus larvae (A) *Elops*; (B) notacanth (perhaps *Aldrovandia*. Figure 2.8 – this larva can certainly exceed 1 meter); (C) *Albula*; (D) *Platuronides* (Serrovomeridae); (E) head of *Nemichthys* (Snipe eel); (F) *Gnathophis* (Congridae). Not to same scale. After Alexander (1961), Beebe and Crane (1937), Castle (1963, 1969), Smith (1970), and Hildebrand (1963).

Freshwater species

Freshwater environments include the stable and mainly non-seasonal great tropical lakes, the great lakes of more temperate regions where conditions are more seasonal with ice-cover in the winter, the highly seasonal flood plains of rivers such as the Amazon and Nile, and the many rivers of temperate regions where there is a strong seasonality of temperature and water flow. Life-history adaptations are found to all these conditions.

In the African great lakes, such as Lake George and Lake Victoria, ripe individuals of most kinds of cichlid fishes exist at any time of the year, although there may be seasonal peaks in the number of spawning individuals (Keenleyside, 1991). The dominant cichlid fauna forms "flocks" of over 300 species in Lake Malawi, over 200 in Lake Victoria, and 120 in Lake Tanganyika. For the most part these cichlids produce small egg batches that are guarded on the substratum or are mouth-brooded, usually by the female. The substratum-guarders are usually monogamous and the parents form pair bonds. The mouth-brooders are polygamous and the parents separate soon after mating. In flood-plain rivers, the entire life of some fish communities may be geared to the rains, flooding not only greatly increases the available habitat, it also releases nutrients that evoke blooms of phytoplankton and an increase in microzooplanktonic food organisms. Many of the larger fishes, especially the ostariophysans (carp, roach, etc.) spawn just before or during the flood, while others spawn in grass swamps at the edge of the advancing flood. For many tropical fish, the high-water period is also the main feeding and growing season, when they build up fat stores to carry them through the dry season.

In some genera of the oviparous killifishes (Cyprinodontidae) there are "annual" species with a life history adapted to exploit the temporary pools that appear each year in the tropical and sub-tropical regions of South America and Africa. At the beginning of the rainy season, killifish eggs buried in the mud hatch quickly, and the young grow rapidly on the abundant (but temporary) food supply, and are ready to spawn in 6 to 8 weeks. They produce

drought-resistant eggs with thick chorions which can go through an insect-like suspension of growth and development in a diapause phase, although there are also "escape" eggs that avoid diapause.

In the viviparous killifishes (Poeciliidae), fertilization is internal. The male is smaller than the female (unlike the oviparous species), and some of the anal finrays are elongated to form an intromittent organ, the gonopodium (Figure 8.20). In the mosquitofish, *Gambusia affinis* or *G. holbrooki*, so called for their great liking for the larvae and pupae of *Anopheles* among other things, the male is about 2.0 cm and the female 3.5–4.5 cm long. Broods of 43–205 young are produced every 21–28 days. The mosquitofish occurs naturally in south-eastern parts of the United States but has been introduced to many other parts of the world to help in the control of malaria, where an unintended effect has been its negative impact due to its consumption of eggs and fry of small native fishes. In Australia, introduced mosquitofish are referred to as "Dambusia."

If frequent enough, the repeated broods of viviparous killifishes may make them almost as fecund as their oviparous relatives. In some poeciliid species, sperm is stored in the ovarian wall and the embryos gestate within intact egg follicles in a process called "superfoetation." By a successive ripening and fertilization of the egg batches, up to nine broods, each in its own state of development, can be gestated in the ovary, for example in *Poeciliopsis retropinna, P. elongates*, and *Heterandria formosa*. Viviparity in killifishes and other small species is largely confined to tropical and sub-tropical environments, where there is less risk of marked seasonal fluctuations. In North America, the killifishes most at home in more temperate conditions, belong to the oviparous genera *Fundulus* and *Cyprinodon*.

Finally, salmonids deserve special mention because of their varied life histories (McDowall, 2002; Quinn and Myers, 2005). The Atlantic salmon (*Salmo salar*; p. 424), the Pacific salmon (*Oncorhynchus*) and their relatives – the trouts such as the rainbow trout (until recently called *Salmo gairdneri*, but now *Oncorhynchus mykiss*), charr (*Salvelinus*), grayling (*Thymallus*), and whitefish

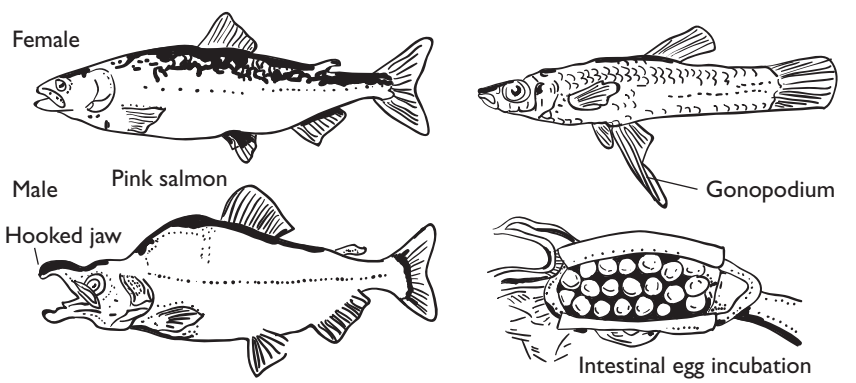

Figure 8.20 Left: female and male pink or humpback salmon (*Oncorhynchus gorbuscha*) showing hooked jaw and hump of the male at spawning. Right, top: male intromittent organ (gonopodium) of *Gambusia* (mosquito fish). Right, bottom: intestinal egg incubation in *Tachysurus barbus*. Redrawn from Nikolsky (1963).

(*Coregonus*) are all of considerable economic importance. Salmonids have such a great and lengthy association with humans that this has led to the development of a distinctive vocabulary to describe the reproduction and development of salmon. Initially applied to *Salmo salar*, terms are now used for other species of salmonids. Female salmon deposit their eggs in gravel nests known as *redds*. The eggs hatch into fry or larvae known as *alevins*, which later metamorphose into *parr*. In many salmonid species the parr gradually work their way downstream, undergoing a second, even greater transformation into *smolts*. After varying periods at sea, maturing salmon, now known as *grilse*, return to their native steams to spawn, *jacks* and *jills* being precocious males and females that return at younger than usual ages, or perhaps remaining in freshwater having never undertaken the journey to the sea. In many species the adults die after spawning, but sometimes a few adults, known as *kelts*, survive to spawn again. Clearly these are words used by English speaking cultures (most having their origins in Middle English), but the vocabularies of other northern European, Asiatic, and North American peoples are just as rich.

Salmonids comprise both landlocked as well as anadromous species and are characterized by their large size for freshwater fish, the adults usually growing to at least 1–2 kg and in some salmon 25 kg or more. Large size in these latter species is associated with the life-history characteristic of not surviving spawning, referred to as semelparity (Crespi and Teo, 2002). Semelparous species, consisting chiefly of the Pacific species of *Oncorhynchus*, put energy into growth (larger fish having the capacity to produce more young), longer marine residencies, and longer migrations while at sea, longer upstream migrations to spawning sites, and in defense of nesting sites after spawning. In contrast to the semelparous species, iteroparous species, including Atlantic salmon and various trouts and charr, are generally smaller and often as not survive spawning. But even among these iteroparous species, larger individuals are less likely to survive than are their smaller relatives. Because of their size and the excellent quality of their flesh they are much sought after for food and sport. Salmon, rainbow trout, and charr, in particular, are farmed in cages and raceways in both the sea and freshwater (p. 451) and many efforts have been made to transplant and establish them in new habitats, for example in the southern hemisphere (McDowall, 1988). The salmonids are oviparous, producing a few thousand rather large eggs, 5–7 mm in diameter. The male develops secondary sexual characters at spawning, the hooked jaw of the Atlantic and pink salmon being especially conspicuous (Figure 8.20). Salmonid eggs are laid in the shallow gravel beds of fast-running streams and take several weeks to hatch. The young of the Atlantic salmon hatch as fairly well-developed alevins about 20 mm long, with red-pigmented blood and a large yolk sac. They pass through a parr stage, and migrate to the sea as smolts at a length of 10–15 cm, usually after 1 or 2 years in freshwater. The typical development of a salmonid, the rainbow trout, is shown in Figure 8.21.

Marine species

Distribution

The spawning grounds of commercially important marine species are well known, especially in temperate latitudes, from the distribution of the fishing

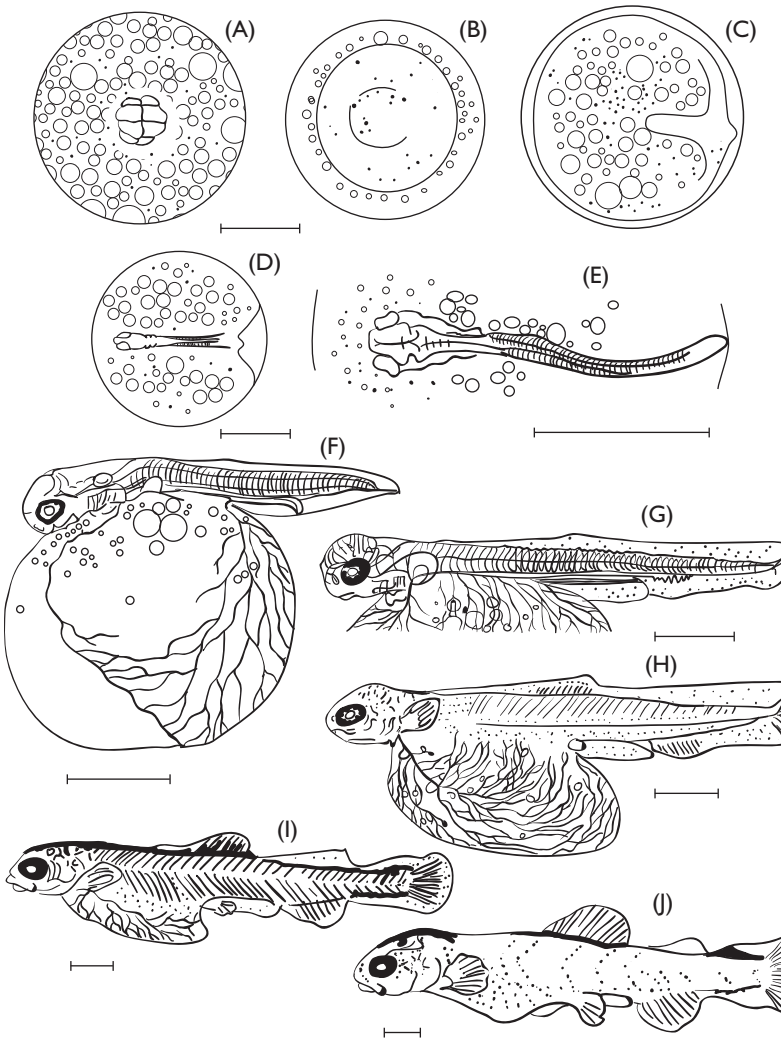

Figure 8.21 Development of the rainbow trout *Oncorhynchus mykiss* (A) 8-Blastomeres; (B) early embryo apparent, one-third epiboly; (C) 0–5 somites, one-half epiboly; (D) otic placodes, three-fourths epiboly; (E) caudal bud with 10–20 somites, total somites 51–58, heart beating; (F) posterior cardinal veins formed, choroid of eye pigmented; (G) near hatching, pelvic fins develop; (H) hatched alevin, first anal and dorsal fin rays. (I, J) Later alevin stages as yolk is resorbed. Scale bars 2 mm long. Redrawn from Vernier (1969).

fleets and sampling of their catch. Data from zooplankton research cruises on the distribution of eggs and larvae can also be back-plotted to identify spawning grounds and can be used to measure mortality rates and assess paths of dispersal. Much less is known about the spawning behavior of the adult fish. At one time it was thought that spawning was a rather hit-or-miss process in which large numbers of females and males milled around together in midwater. There are doubtless many exceptions to such a scheme. We now know

that the haddock, for example, goes through a quite elaborate courtship involving visual displays by the male (Figure 8.22) and vocalization. The male of the coral reef angelfish (*Pygoplites diacanthus*) produces a vortex ring of the eggs and sperm by flexing its tail, presumably improving the success of spawning. In the herring (a demersal spawner) males and females interact, often on a one-to-one basis, and the female will only release its eggs onto a suitable substratum. Some species such as the seahorse are monogamous, staying with the same mate for an entire breeding season, possibly several.

About three-quarters (9000 out of 12 000) teleost species produce buoyant eggs so that the most common habitat for the eggs and larvae is the pelagic zone. Here the young stages comprise that part of the zooplankton called the ichthyoplankton. They have little control of their own destiny; apart from the older larvae making limited diel vertical migrations, dispersal is mainly under the control of the currents. As they grow towards and through metamorphosis and become independent, there is a tendency for the juveniles to collect on inshore nursery grounds such as estuaries or tropical mangrove swamps or, in the case of flatfish, to settle on the sea bed (Figure 8.23).

As almost all fish are poikilotherms, the rate of development depends on temperature (Figure 8.5). In tropical regions, not only is development fast but the generation time is short and spawning almost year-round. The eggs of coral fishes require only a day or so to hatch, and larval life lasts from a few days to several months and is often dependent upon their arrival at a suitable reef habitat. This extended period of larval existence allows for widespread dispersal of many reef fishes across oceanic expanses. Many families of coral fishes such as the sea basses (Serranidae), snappers (Lutjanidae), red mullet (Mullidae), and butterfly fishes (Chaetodontidae) lay pelagic eggs but some gobies, blennies, and damsel fishes lay non-buoyant eggs, which may

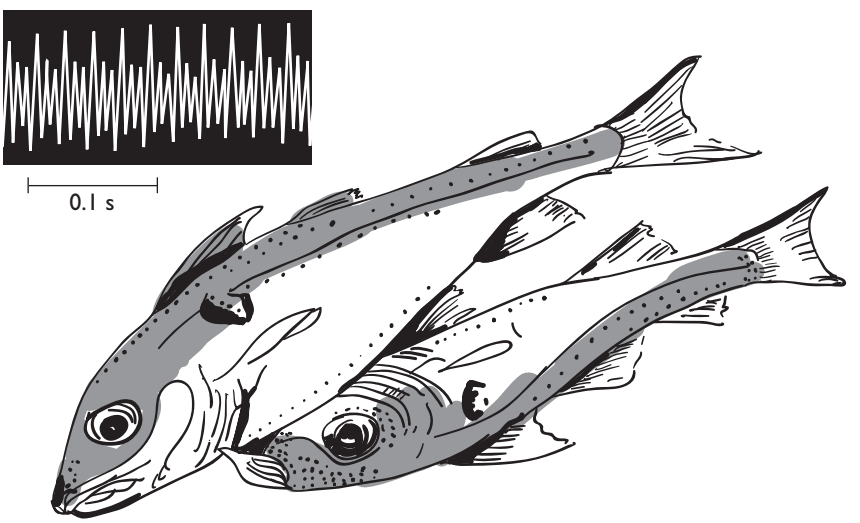

Figure 8.22 Haddock (*Melanogrammus aeglefinus*) spawning behavior; the male is below. Inset: audiogram of humming sound made by the male as it leads the female. Redrawn from Hawkins *et al.* (1967, p. 923).

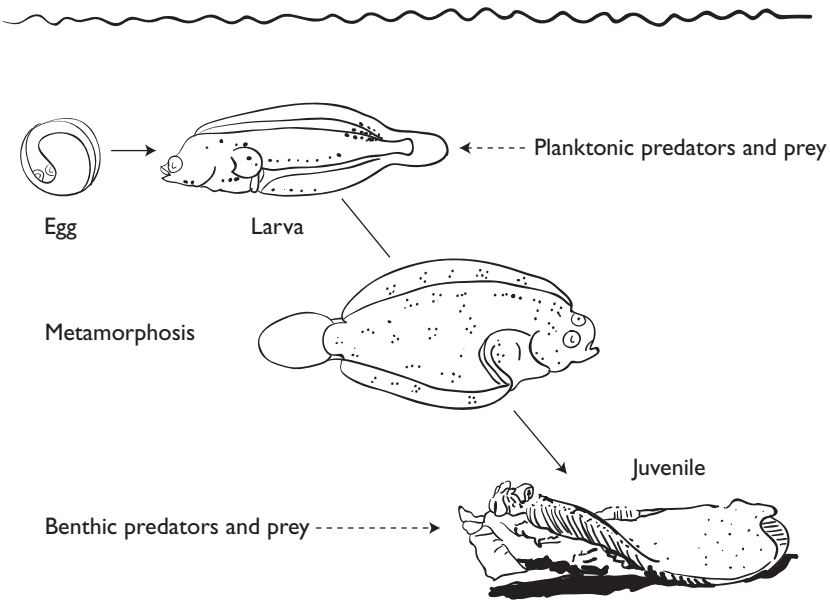

Figure 8.23 Early life history of plaice (*Pluronectes platessa*) a typical flatfish which spends its larval life in the plankton and settles on to the sea bed at metamorphosis. Drawing by Dr. R.N. Gibson.

be scattered over the bottom or guarded by one or both parents. Total fecundities may range from tens of thousands to millions of eggs. Mortality is high and only small numbers return to the reef as juveniles, and presumably even fewer disperse to other areas. An unusual characteristic is found in eggs and larvae of the sharpnose puffer fish (*Canthigaster valentini*) which are unpalatable to reef fish predators.

In the open ocean of the tropics and sub-tropics, epipelagic teleosts such as flying fishes (Exocoetidae), sauries (Scomberesocidae), sailfishes and marlins (Istiophoridae), and tunas and ocean sunfishes (Molidae) produce numerous buoyant eggs as do the species in the mesopelagic and bathypelagic zones. Species at both these levels are presumed to spawn where they live, and the eggs float upward toward the surface. The young stages then join the ichthyoplankton for a time before moving down again to the adult habitat.

Both in the open ocean and in coastal waters, species diversity drops markedly between sub-tropical and temperate latitudes. Of the 111 species of British fishes, 68 or 61% lay floating eggs. The remainder produce grounded eggs (sand eels, blennies, and clingfishes), are nest-building (wrasse, stickleback), viviparous (*Sebastes*) or have brood pouches (pipefishes). Such adaptations no doubt reduce mortality in inshore areas where the eggs could be stranded or damaged by wave action.

At high latitudes the inshore waters are near freezing and often covered with ice. Many species lay large eggs on the sea floor. The Antarctic notothenioids (ice fish) have eggs 2–5 mm in diameter and fecundities of 2500–12 000. Growth is slow and maturity is not reached for several years. Fish in these waters must exploit the short growth season with a short-lived larva or become

less dependent on seasonality by producing large demersal eggs. These hatch into bottom-feeding larvae with substantial yolk reserves that allow them to grow quite large by endogenous nutrition, increasing the range of prey available. Polar fishes are typically K-selected with their large eggs, low fecundity, and delayed maturity.

8.10 Larval Ecology

The high commercial value of food fishes which are mainly marine (p. 440), has resulted in a great deal of research over the last hundred years aimed at clarifying the factors that control the recruitment of the young stages to the fishable stock. A full understanding of the ecological mechanisms underlying recruitment would help in the scientific management of stocks and enable the prediction of yields to the fishing fleets.

Life is tough when you are small. Even the small size of most newly hatched fishes seems to conspire against them as it produces in a low Reynolds number (see page 81) in which the viscosity of the water is the dominant factor in their locomotion. This results in a short swim-and-rest pattern of searching the surrounding water for food which also uses the minimum amount of energy. Mortality rates of both eggs and larvae are of the order of 5–30% of the population per day. At one time it was thought that this mortality was mainly caused by starvation, the larvae being unable to find adequate microzooplanktonic food, with a particular "critical period" first postulated by Hjort (1914) at the end of the yolk-sac stage. More recently, it has become fashionable to consider that predation is of equal or greater importance as a mechanism of mortality (Bailey and Houde, 1989). Careful experiments have shown that the mortality rates of eggs are similar to those of larvae (the eggs cannot die of starvation) and that there is little evidence of a critical period at the completion of yolk resorption. Furthermore, experiments on rearing larvae of species like cod in shore-based tanks show very high survival rates in the absence of predators. But of course fish in these tanks have virtually unlimited food supplies.

Estimation of natural mortality is difficult. Of late, fishery biologists have been investigating marine food chains in which fish larvae are involved and measuring predation rates under experimental conditions. It is thought that the transparency of eggs and younger larvae, and the disruptive camouflage of their melanophores reduces their conspicuousness to predators, at least under some conditions of illumination and background. It is important for survival that growth be rapid to reduce the spectrum of predators; generally speaking, larvae have to be less than half the size of their predators to be vulnerable. As larvae grow they become more conspicuous but their development of sensory and swimming abilities allows them to better avoid predators as well as find food. Changes of behavior such as schooling or settling on the sea bed are also likely to reduce predation pressure.

That is not to say that feeding (p. 247) and starvation do not play a significant role in these high mortalities. Many species possess distinctive larval adaptations to help find food and reduce energy requirements. Larvae of species such as anchovy, herring, plaice, sole, turbot, and cod sight their prey at a distance of only a few millimeters (usually less than one body length), and

bend their bodies into an S-shape before darting forward to seize it. The ability to catch the prey depends on its size compared with the larva's jaw gape, and on the experience of the larva, catching efficiency increasing with age as well as larval size (Fuiman *et al.*, 2006). The volume of water searched for food obviously depends on the sighting distance, the field of view of the larva and its swimming speed (Figure 8.24). Species have evolved some remarkable larval adaptations to save energy and increase the effectiveness of their searching for food. Spines and filamentous processes increase buoyancy. Some mesopelagic fish larvae, such as *Idiacanthus*, have extraordinary stalked eyes supported by cartilaginous rods that probably increase the visual field by 80 times, but even a much smaller peduncle, as in *Myctophum*, provides a significant improvement in the field (Figure 8.25). Remarkably enough, stalked eyes have evolved entirely independently not only in the hammerhead sharks (*Sphyrna*), but also in the Australian platystomatid flies such as *Achias*!

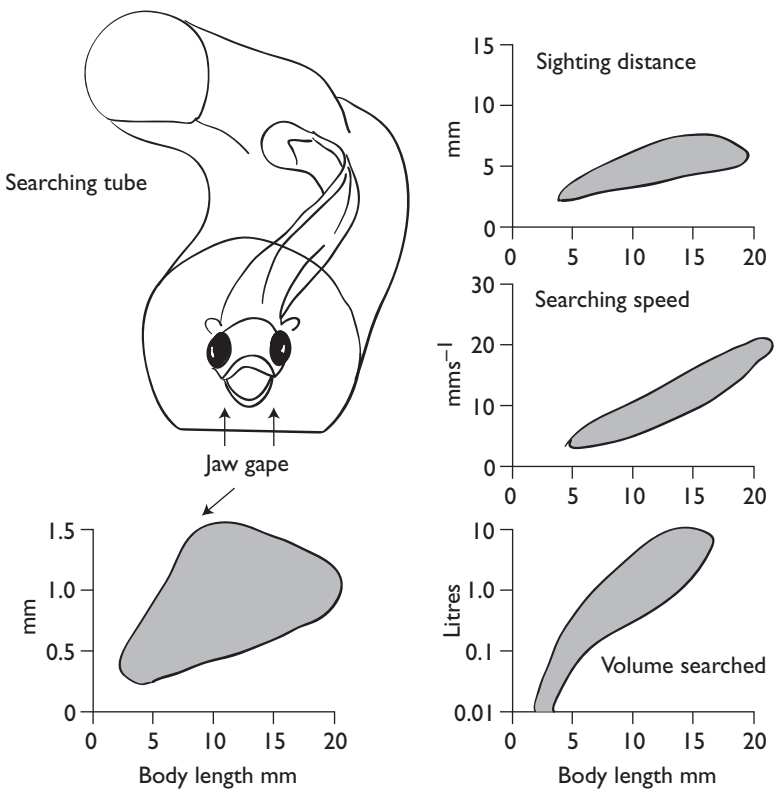

Figure 8.24 Food acquisition changes in developing larvae. Top left: the feeding "tube" searched by a fish larva. Bottom left: graph showing how the gape of the jaw changes as larvae grow. The shaded area in this and the other graphs is an envelope containing a number of regression lines showing the relationship between jaw gape and body length in several species. Right: three graphs, the relationship between prey sighting distance, searching speed and volume of water searched as the larvae of several species grow. Redrawn from data in Rosenthal and Hempel (1970), Hunter (1981), and Blaxter (1985).

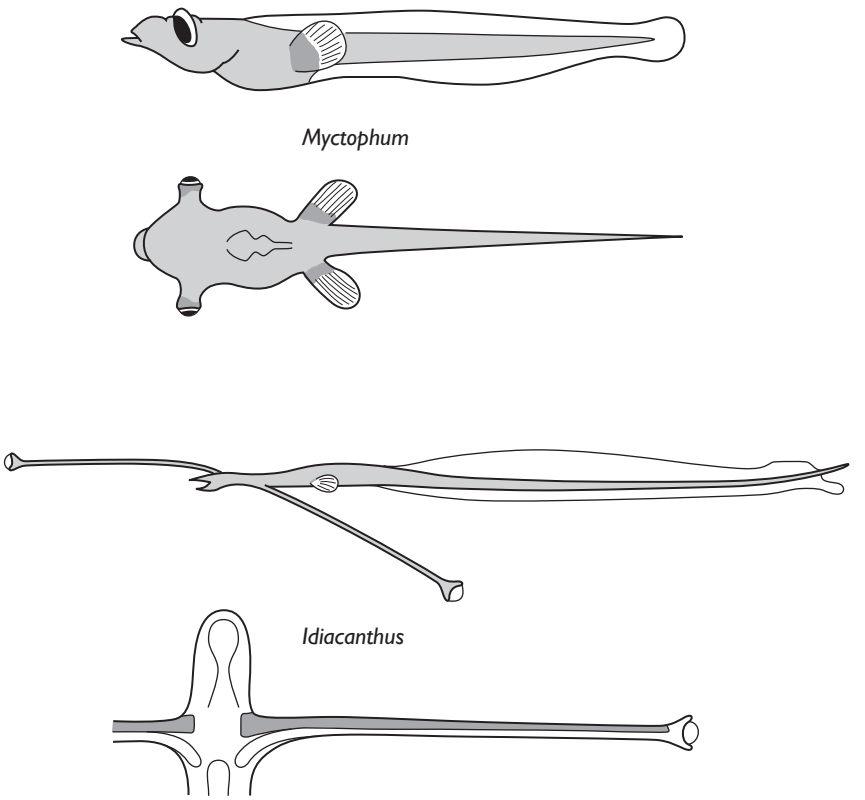

Figure 8.25 Mesopelagic fish larvae with extended eyes which increase search area. After Weihs and Moser (1981, p. 31).

Larvae of species such as herring and plaice can survive for about a week without food when they are small, and plaice can withstand starvation for as long as 3 weeks as they approach metamorphosis. Starving larvae reach a point where they are still alive but are too weak to feed if food becomes available (the so-called point-of-no-return (PNR) or "ecological death"). Considerable efforts have been made over the past decade to categorize the nutritional status or condition of sea-caught larvae, to ascertain their chances of survival. To this end, condition factors (weight divided by the cube of the length), body dimensions, organ histology, fat content, and DNA:RNA ratios have been used in a wide range of species including anchovy, cod, herring, sardine, and many species of flatfish.

One of the most interesting findings is that microzooplankton density in the sea is rarely adequate to sustain survival and growth unless the food is distributed patchily, for example at hydrographic "fronts" or discontinuities (p. 211). This has led to the concept of "Lasker years," named after a distinguished marine biologist from the US. It was found in the California current that good brood survival was associated with calm weather when such fronts (and patches) could be built up and maintained, so providing circumstantial evidence for the advantage of patchiness.

The importance of a match between larvae and their microzooplanktonic food has led to the match–mismatch hypothesis of larval survival expounded by Cushing (1990). The main production of larvae should be geared to the production of their food. Since the youngest larvae have very small mouths and most fishes' prey capturing ability is gape limited, it is essential that their food, such as copepod eggs and nauplii, is available when the larvae are very small. Batch spawning may help in matching larvae to their food but, of course, the numbers of larvae will then be less than with a one-off spawning. As well as a match with the food, a mismatch with the predators is also advantageous for survival. Thus larvae have to be considered both as predators and prey and this leads to the concept of cohort competition. The best way to survive is to grow fast, to enable larger food items to be eaten and larger predators to be avoided. If the potential prey can "outgrow" the potential predator it has a greater chance of survival at the predator's expense. Evolution must put a high premium on such fast growth to yield a good brood and high recruitment.

8.11 Growth

Fishery biologists are interested in growth because (obviously) it is a major component of the fishery equations that are used to calculate the yields at different levels of exploitation. It is not only the numbers of fish that are important (resulting from recruitment and number of fish surviving from year to year) but also the addition to the biomass of stock caused by the growth of individual fish.

Growth is enhanced by a good food supply and high temperature. In high latitudes it is thus seasonal and overwintering fish may grow slowly or not at all. Growth can depend on intra- or interspecific competition and may thus be density-dependent. A considerable breakthrough in refining the fishery equations was made in the 1940s by applying the von Bertalanffy growth equation (p. 249) to the growth of commercially important teleosts as part of the study of fish population dynamics.

A key requirement in estimating growth is to be able to age individual fish. Fortunately, in many species, various hard tissues maintain a history of the individual's growth. In higher latitudes, with seasonality the overwintering growth checks are reflected in the growth patterns of a number of tissues, especially the scales and otoliths, giving rings equivalent to the age in years (Figure 8.26). In larvae it is possible to remove the otoliths and, after suitable treatment, observe *daily* growth rings. This fairly recent finding has enabled larval growth and mortality rates to be measured, giving much greater insight into the ecological pressures on populations of larvae. In low latitudes the lack of seasonality makes these techniques less applicable, or even useless. Periods of rainfall, even cloudy days produce recognizable variations in otolith structure and growth.

Elasmobranchs lack bony tissues, however, in species such as the spiny dogfish (*Squalus acanthias*) and the skate (*Raja clavata*), transverse sections of the spines or vertebral centra, respectively, show rings that correspond to changing seasonal patterns of growth. These annual rings are used to age the fish. In tropical or subtropical waters, where there is less seasonality, the annual rings may be less evident, or absent (Wintner, 2000).

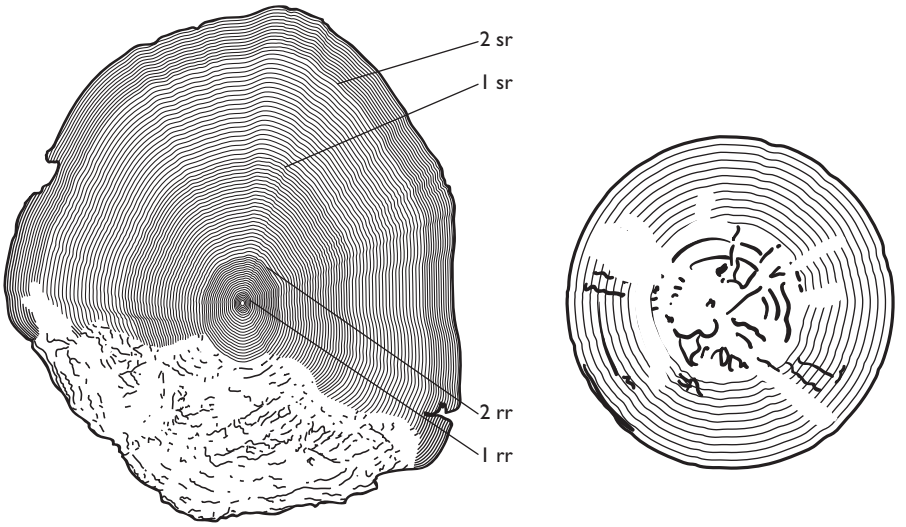

Figure 8.26 Left: scale of a 4-year-old salmon (2 years in the river, rr = a river ring, 2 years at sea; sr = sea ring). Redrawn from Jones (1959). Right: otolith of herring larva with 12 daily rings. Redrawn from photograph by Dr. A. Geffen.

Holden (1974) studied growth of spiny dogfish using a modification of the von Bertalanffy growth equation on the assumption that the growth curves of the embryos can be extrapolated to give those of free-living individuals (Figure 8.27). The derived equation is:

$$\frac{l_{t+T}}{L_{\infty}} = 1 - \exp(-KT) \qquad (8.1)$$

where l_{t+T} is the length at birth, L_{∞} (L-infinity) is the maximum theoretical length, T is the length of gestation and K is a constant. In European waters, $t+T$ is 27.5 cm, L_{∞} is 108 cm and the gestation period 2 years. Thus $27.5/108 = 1 - \exp(-2K)$ or $K = 0.15$. This derived growth constant may be compared with the value 0.11 from actual growth data of free-living individuals. In general, values of K seem to be 0.1–0.2 for sharks and 0.2–0.3 for rays but an exception is found in the seven species of smooth hounds (*Mustelus*) where K ranges from 0.22–0.53 for males, and 0.21–0.36 for females. In this genus, maturity is reached up to 4 years after birth, a much faster rate of growth than the spiny dogfish which takes 10 years to mature.

Elasmobranchs generally have low rates of reproduction, slow growth, and a long time to maturation compared with teleosts. The period for 50% to mature (the generation time) is probably 7–13 years in sharks and 5–6 years in rays. These K-selected species are very susceptible to overfishing and many of the fisheries that depend upon them are not sustainable as the result of a low rate of recruitment.

Envoi

The life histories of fishes present a varied and complex picture making them a fertile ground for studies of reproductive physiology, embryology, and

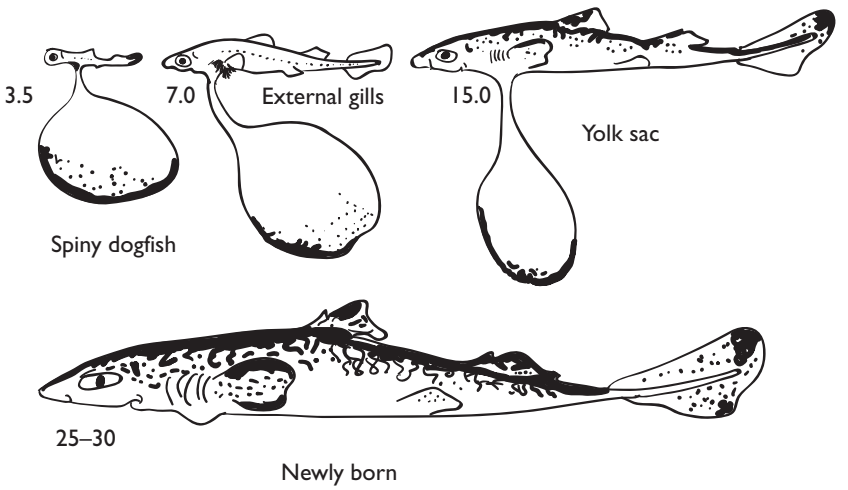

Figure 8.27 Stages in the uterine development of the young of the ovoviparous dogfish *Squalus acanthias;* young removed from the uterus. Lengths in centimeters. Redrawn from Hishaw and Albert (1947, p. 187).

evolutionary ecology. In addition, knowledge of the patterns and control of reproduction is essential knowledge for those interested in culturing fishes or managing wild populations.

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9 Endocrine Systems

From the comparative point of view fish endocrine organs, and the hormones they secrete are much more interesting to zoologists than are those of terrestrial vertebrates (Bern, 1985, 1990). It will not come as too much of a surprise to guess that various groups of fishes show different stages in the evolution of the endocrine systems found in other vertebrates. Possibly it may be less evident that these have led to new ideas about the possible ligand and receptor changes leading to those of terrestrial vertebrates, and they have supported genome duplication theories in vertebrate ancestry, first suggested by Ohno (1970) and by studies on Hox genes (see p. 11). Thus, for instance, Conlon and Larhammar (2005) discuss the evolution of the neuropeptide Y and tachykinin neuroendocrine families in relation to genome duplications.

Studies on fishes have significantly added to our knowledge of endocrine systems in mammals, including ourselves. It was in teleosts that Scharrer, more than 60 years ago, first demonstrated the link between brain neurons sending hormonal material down their axons, and the region of the pituitary where they are stored and later released. Indeed, as Lethimonier *et al.* (2004) remarked, in connection with the different forms of the gonadotrophin-releasing hormone (GnRH) "… fish in general, and teleosts in particular, have often played a leading part in changing established concepts." Rather surprisingly perhaps, a number of hormones first found in fish such as the urotensins, stanniocalcin, and glucagon-like peptide-1 (GLP-1) were later found to be important in mammals (Conlon, 2000a).

The advent of rapid molecular methods for isolating and sequencing peptide hormones, and continuing comparative functional studies in vertebrates have led to a vast increase in the understanding of these different hormone families, which can only be touched on here. Almost every issue of the journals *General and Comparative Endocrinology* and *Molecular Endocrinology* show the continuing importance of work on fish endocrine systems. In the space of this short chapter, we can only give some idea of the interest and importance of fish endocrinology, and look briefly at some hormone families: the reader should seek further for himself in this rapidly growing subject.

9.1 Why Fish Endocrinology is Important

Quite apart from the interest fish endocrinology has for the evolution of the vertebrate endocrine system, it is of very considerable practical importance. First, fish endocrine systems are very sensitive to environmental stress and to pollutants of different kinds, readily suffering endocrine disruption (see Kime, 2000; Arcand-Hoy and Benson (2000)), hence fish act as very useful monitors of environmental quality. Many such endocrine disrupters affect the long-term survival of fish populations even if they do not cause immediate mortality.

Second, understanding fish endocrine systems is obviously important in applied fishery sciences, where endocrine manipulations are used to control reproduction (together with genetic manipulation). In salmonids, for example, these approaches have led to unisex populations (females in salmonids which grow more quickly than males, and in tilapias to the larger males). Some idea of the complexity of salmonid endocrinology can be gained from Bern and Nishioka (1993). Again, in stocking game fish such as black crappies (*Pomoxis nigromaculatus*) monosex males produced by repeated androgen administration to the small fry, avoid the problems of stunted populations in small lakes. As fish farming is becoming more and more significant as a quality protein source, the hormonal control of farmed fish has benefits as well as some dangerous environmental aspects such as the accidental release of genetically modified fish (see Chapter 13). Last, fish make suitable and tough experimental models for the actions of hormones in higher animals.

9.2 Hormones and Receptors

Hormones (from the present participle of the Greek horman δρμαω. set in motion) are classically defined as chemical messengers secreted by special endocrine cells or organs into the blood, whence they reached specific distant target organs to exert their effects. This definition (which was fine for the first hormones characterized, secretin and gastrin, at the beginning of the last century) has had to be broadened more recently. This is because the hormonal messengers have been found to be delivered not only into the blood to act on distant organs in the classical way (*endocrine*), but also to neighboring cells by diffusion (*paracrine*), for example at neuronal synapses and in the gut, and even by *autocrine* secretion, where a cell produces a hormone that binds to receptors on its own surface.

However far their messages go, vertebrates only use four kinds of compounds as hormones:

1. peptides, such as vasotocin, insulin, and very many others;
2. steroids, such as testosterone;
3. lipid derivatives, such as prostaglandins;
4. amine derivatives, such as thyroxine and dopamine.

We cannot delve into the whole array of fish hormones, but the most familiar (apart from the steroid reproductive hormones and thyroxin) are probably the varied families of peptide hormones, such as the melanocortin family expressed by the proopiomelanocortin gene (POMC), recently reviewed by Melz *et al.* (2006).

Figure 9.1 shows how post-translational processing of POMC produces several different pituitary hormones, such as: adrenocorticotrophic hormone (ACTH) in the rostral adenohypophysis, and corticotrophin-like intermediate peptide (CLIP), γ-LPH, melanotropins (MSHs) and β-endorphin in the intermediate lobe. These POMC-derived hormones regulate a variety of physiological functions, which are associated with stress responses and environmental adaptation (Raffin-Sanson *et al.*, 2003). So ACTH stimulates the adrenal cortex to produce and secrete adrenocortical hormones (e.g. corticosteroids, glucocorticoids), MSH causes dispersion of pigment granules in melanocytes, producing a rapid change in skin coloration. β-Endorphin binds to the opioid receptors in the brain and produces analgesic effects (at least in mammals).

We are all too accustomed to sit back and accept simplifications and generalizations in texts, and to suppose that most vertebrates are fundamentally like mammals. It comes as rather a surprise to find that in fish such as the channel cat fish (*Ictalurus*), many other tissues than the pituitary express POMC (Karsi *et al.*, 2004), although, except in the kidney, expression is low (Figure 9.2). The corresponding receptors are also expressed at many sites, and in this case are G-proteins. None the less, if we bear in mind the "revised" definition of hormones, we should not be too surprised.

After reaching their target cells, hormones have to be able to activate systems within these cells, and to do so many peptide hormones bind to G proteins which span the cell membrane.

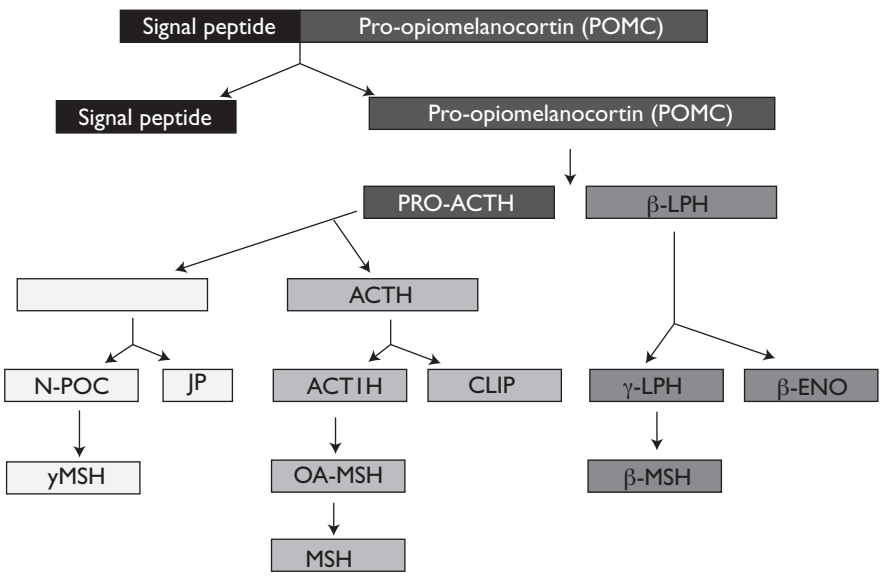

Figure 9.1 The proopiomelocortin (POMC) processing pathway in the hypothalamus. The POMC gene encodes a 32 kilodalton (kDa) precursor which is sorted to the regulatory secretory pathway by a signal peptide. The precursor protein has three main regions: the ACTH sequence cleaved to finally generate α-MSH; the C-terminal cleaved to yield γ-LPH, β-endorphin and β-MSH, and the N-terminal that contains the β-MSH sequence. After Melz *et al.* (2006).

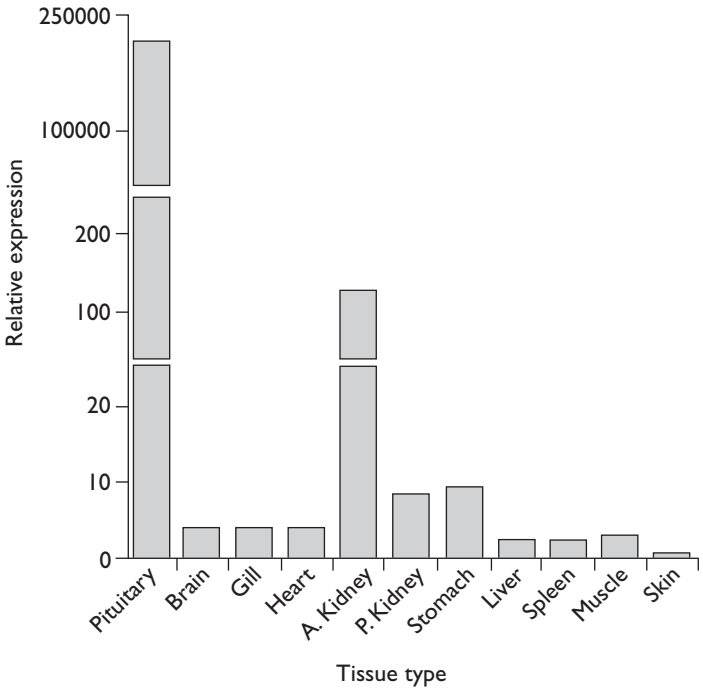

Figure 9.2 Molecular cloning of proopiomelanocortin cDNA and multi-tissue mRNA expression in channel catfish. From Karsi *et al*. (2004).

Binding to these receptors (G proteins because they are guanine nucleotide binding proteins), is the first step in the process of the hormone changing intracellular biochemical reactions. The G-proteins do this by switching guanosine diphosphate (GDP) for guanosine triphosphate (GTP) to signal the beginning or cessation of switching on or off second messenger intracellular enzyme cascades. G-protein receptors are regulated by a ubiquitous family of cytoplasmic proteins, the arrestins, found in every metazoan animal cell. There are three different arrestin types in fishes, interacting with hundreds of G-protein receptor subtypes (see Gurevich and Gurevich, 2006). There is a number of other second messengers, such as cyclic AMP, Ca^{2+} and NO, but we shall leave them and begin with fish hormones.

9.3 The Endocrine Organs of Fishes

The main endocrine organs of a teleost fish are shown schematically in Figure 9.3. By far the largest of these organs is the gastrointestinal tract. The list of hormones given in Table 9.1 is certainly incomplete, for it is being added to all the time. Naturally enough, when a new hormone is found in mammals, search is made for it in fish to discern the relationships and origins of the new hormone (and perhaps partly to try and add a rapid publication to one's CV!), and so the mammalian (rodent and human naturally) hormones leptin and ghrelin, involved in control of food intake, have been found fairly recently in teleosts.

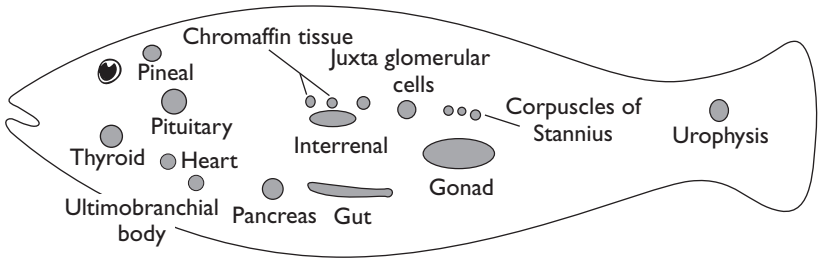

Figure 9.3 The endocrine organs of a teleost fish.

To determine the presence and functions of the hormones secreted in fish has sometimes been rather difficult, for despite a variety of approaches (e.g. immunocytochemistry and radioimmunoassay; bio-assays by injection of extracts from the organs into fish and other vertebrates, such as frogs and mice; injection of higher vertebrate hormones into fishes themselves; and the effects of surgical removal or chemical block of the organs), clear results have not always been obtained.

Thus injection of extracts from the teleost caudal neurosecretory organ (urophysis) into rats produces vasoactive effects, while in the fish itself, the extracts have osmoregulatory effects. Even if a well-characterized mammalian hormone, such as gastrin, is injected into a fish, it is hardly surprising that similar effects to those produced in mammals may not be obtained, because there may have been changes both in receptor structure, and in the hormone structure itself between fish and mammals.

However, uncertainty does not always appear, and an excellent example of a very clear effect was Fontaine's (1964) work on the hormone produced by the corpuscles of Stannius (p. 272).

Table 9.1 shows what is known of the presence and functions of hormones in fish. Many hormones are named from their function in mammals, so that their piscine homologs are often inappropriately named. For example, prolactin (PRL) was named for its stimulatory action on mammalian mammary glands. We might reasonably expect that without the same target organs, PRL would have a different function in fish. Maybe not so very different, for in at least some elasmobranchs while there may not be breasts, there are intrauterine secretions known as uterine milk nourishing the embryos (Chapter 8). But in teleosts (and, indeed, it later became evident, in higher vertebrates) PRL has a remarkably wide spectrum of action, also being involved in osmoregulation (particularly in regulating chloride cells in freshwater teleosts, see Manzon, 2002), reproduction, growth, lipid metabolism and steroid synergism. As neurons containing PRL-like material are revealed immunocytochemically in various brain regions in fishes, we can also add neurotransmitter action to this remarkably varied list. Indeed, some peptides such as angiotensin may be delivered to their target receptors in several ways: as hormones, neurotransmitters, or by local paracrine cell secretions.

Origins

Were fish endocrine organs and their hormones inherited from protochordates? Since in living Agnatha most of the typical gnathostome endocrine

Table 9.1 Endocrine organs in teleosts, their hormones and the effects produced

Site and hormones produced	Target organ	Effects
Urophysis Urotensins I–III	Kidney, gills	Changes salt water balance
Pituitary Prolactin (PRL)	Several	Multiple, including osmotic balance
Adrenocorticotrophic hormone (ACTH)	Interrenal	Stimulation of cortisol; links with immune system
Somatostatin (SS)	Liver, brain	Inhibition of growth hormone (GH) release, metabolic effects
Growth hormone (GH)	Several	Stimulation growth
Thyroid stimulating hormone (TSH)	Thyroid	Stimulates thyroxin, controls growth, metabolism, migratory changes
Follicle stimulating hormone (FSH)	Ovaries	Stimulates vitellogenesis
Luteinizing hormone (LH)	Gonads	Stimulates ovulation and spermiation
Melanophore stimulating hormone (MSH)	Skin and others	Darkens skin only in some species
Arginine vasotocin (AVT), isotocin, mesotocin	Blood, many tissues	Increase blood pressure, constricts gill vessels, systemic vasodilation, many other effects
Thyroid Thyroxine	Many	Various, but not calorigenic
Ultimobranchial bodies Calcitonin	Gills, kidney	Regulation of Ca^{2+} metabolism
Corpuscles of Stannius Hypocalcin	Gills	Ca^{2+} homeostasis
Pancreas Insulin	All cells	Increases glucose permeability, appetite, catabolic to anabolic (as in liver)
Glucagon	All cells	Antagonist to insulin, stimulates SS release and IGF-1 & IGF binding proteins
Glucagon-like peptide (GLP-1 & 2)	Brain, all cells	Stimulates glycogenolysis, inhibits appetite
Amylin	Pancreas, gut	Decreases appetite
Pancreatic polypeptide Y (PY)	Brain	Stimulates food intake?
Neuropeptide Y (NPY)	Brain	Stimulates food intake, increases pituitary secretion LH
Peptide YY (PYY)	Brain?	Involved in regulation of metabolism?
Cholecystokinin (CCK)	Brain	Decreases appetite
Gut and stomach Ghrelin	Brain	Stimulates appetite and food intake
Glucagon-like peptide (GLP-1)	Pancreas, stomach	Stimulates insulin secretion, inhibits gastric emptying
Galanin	Brain, pancreas	Stimulates food intake
Gastric inhibitory polypeptide (GIP)	Brain, pancreas	Stimulates insulin secretion, inhitbits gastric acid
CCK	Pancreas, gall bladder	Stimulates insulin secretion?

Table 9.1 *Continued*

Site and hormones produced	Target organ	Effects
Vasoactive intestinal peptide (VIP)	Gut	Control gut motility and secretions
Secretin	Pancreas	Stimulates acinar at low pH
Bombesin, neurotensin, enkephalin, somatostatin and other peptides	Gut and elsewhere, stimulates other hormones	Many and varied
Adipocytes Leptin	Brain	Inhibits appetite
Gonads Relaxin	Uterus, brain	? but in sharks and rays relaxes uterine muscles
Testosterone, Estradiol-17β, Progessterone	Brain, genital tracts, and various tissues	Sexual behaviour and secondary sexual characteristics
Kidney juxtaglomerular cells Renin	Kidney, chromaffin tissue	Osmoregulation euryhaline fish, blood pressure regulation
Heart Atrial natriuretic peptide (ANP), C-type natriuretic peptide (CNP) from the atrium, VNP from the ventricle	Gills, kidney, and intestine. Rectal gland in sharks and rays	NaCl extrusion at gills, reduces gut uptake NaCl
Interrenal Corticosteroids	Gills, kidney	Stress response, osmoregulation
Chromaffin tissue Adrenalin	Circulation	Gill vasodilation and systemic vasoconstriction
Noradrenalin	Circulation	Increases heart rate, and blood glucose
Brain Many neuropeptide families and hormones, brain natriuretic peptide (or B-type natriuretic peptide, BNP)	Many	Varied
Pineal Melatonin	Pituitary	Links photoperiod and pituitary

organs have been recognized, we need to look earlier: to amphioxus or ascidian tunicates. Here, there are no direct equivalents of the endocrine organs of fishes, but there are intriguing morphological and biochemical hints of precursors. For instance, the amphioxus endostyle (which secretes mucus for filter feeding) takes up iodine and couples it to tyrosine, then forming thyroxine (Thorpe and Thorndyke (1975)) just as in the vertebrate thyroid (Figure 9.4). There is therefore a direct link with the mucus-secreting endostyle of the lamprey ammocoete larva which gives rise to the adult thyroid on metamorphosis. Again, the tunicate neural gland and its ciliated duct open to the pharynx are reminiscent of the way that the anterior part of the vertebrate pituitary (the adenohypophysis, see p. 265) forms during embryonic development from an upgrowth of the roof of the pharynx (Rathke's pouch).

The difficulty has been to find in these protochordate "anlagen" of vertebrate endocrine organs, equivalents of the hormones they produce in vertebrates, or

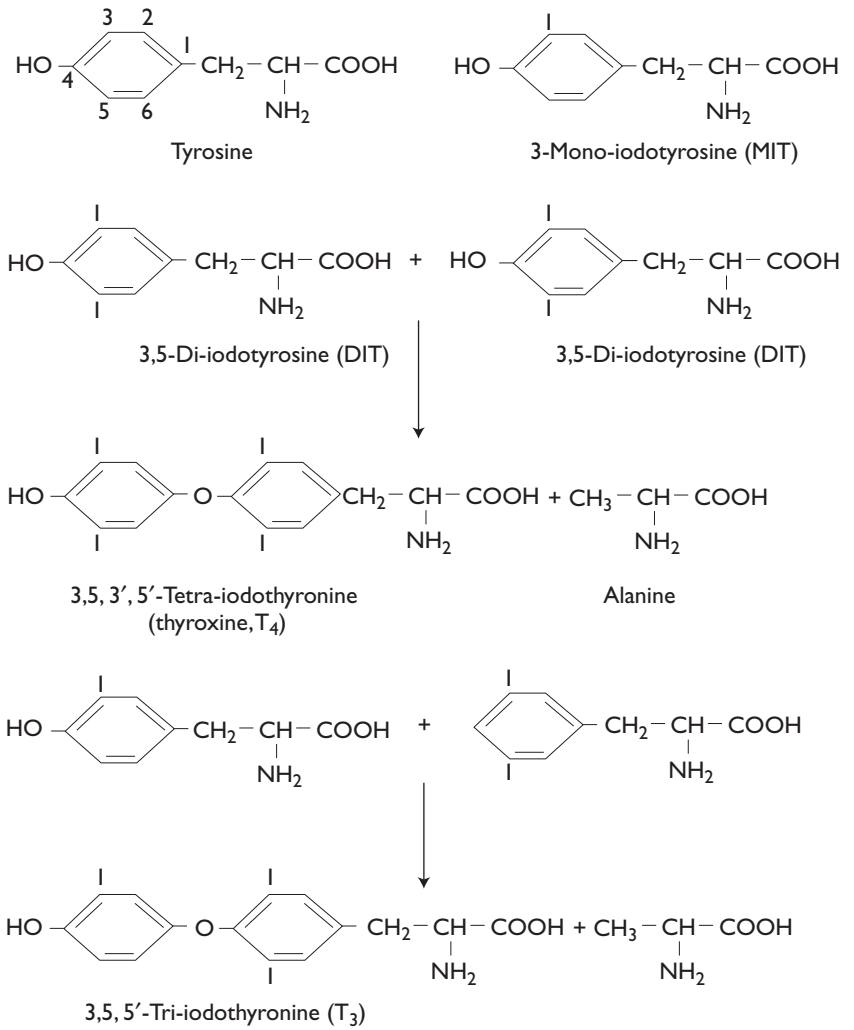

Figure 9.4 The derivation of the two thyroid hormones, triiodothyronine and thyroxine from tyrosine. After Barrington (1964).

if such are found, to know what role they might perform. Curiously, we have no idea what thyroxine may do in amphioxus, nor what might be the function of the cells in the ascidian neural gland and brain containing hormonal peptides (shown by immunocytochemical studies using heterologous antisera to vertebrate peptides). Again, there is an insulin-like peptide (ILP) in amphioxus which is the product of a single copy gene, possibly representing the ancestral gene from which vertebrate insulin and insulin-like growth factor (IGF) genes were derived. But what ILP may do in amphioxus is unknown. Recent reviews of protochordate endocrinology by Sherwood *et al.* (2005, 2006), dealing mainly with the ascidian *Ciona*, where the genome is completely known, concludes that tunicates seem to have some, but not all, of the necessary molecules to develop a vertebrate-like pituitary or thyroid system. What *has*

emerged from such immunocytochemical studies, though, is that in both protochordates and vertebrates, there is similar peptide hormone activity in gut cells and in brain neurons.

The brain–gut axis

The development of sensitive immunocytochemical methods for hormonal peptides shows that such peptides as those of the gastrin/cholecystokinin family are not only found in the endocrine cells of the gut, where they were first discovered (by less-sensitive techniques), but are also present in neuron cell bodies and their processes in the peripheral and central nervous system. There is the same duality in nervous system/gut neuropeptides in most invertebrates (molluscs, arthropods, and annelids, for example), but, in cnidaria, neuropeptides such as FRMFamide are only found in the nervous system, and this is why it is supposed that these neuropeptides originally arose in the nervous system and only later became products of gut cells. So the concept of a brain–gut axis for neurohormonal peptides arose; the same peptide hormones present in the gut and the brain may have had their origin in neurons of the ancestral invertebrate central nervous system.

The very large variety of neuropeptides found in both today seems correlated with both nervous system evolution and the diversity of their functional roles. The multi-functional tachykinins, found from ascidians upwards (Satake *et al.*, 2004) are perhaps the prime example. Obviously, although the structure of peptides of neurons and gut cells in fishes may be similar, at different sites they are likely to have very different functions and target organs. For example, the remarkable modulator neurons of nerve 0 (the *nervus terminalis*) in teleosts contain one of the gonadotropin releasing hormones (GnRHs), functioning as a neurotransmitter. This presumably had modulating functions on neuronal activity in the central nervous system, but a related GnRH is produced by the pituitary neurohypophysis to act on adenohypophysial cells to release gonadotropins.

9.4 The Urophysis

In elasmobranchs and teleosts, large neurosecretory neurons (Dahlgren cells) at the caudal tip of the spinal cord send axons ventrally to end in palisades along capillary walls. The neurons are more condensed in teleosts and other gnathostome fish, sending their axons down an urophyseal stalk, exactly analogous to the hypophyseal stalk of the pituitary, making in some teleosts a conspicuous ventral swelling at the end of the cord (Figure 9.5). The whole arrangement is similar to that in the neurohypophysis of the pituitary, albeit on a simpler scale, and naturally led to studies of the properties and structure of the hormones it was presumed to secrete. A remarkable sustained effort by Lederis and his colleagues at Calgary in southern Alberta (see Lederis 1977) heroically processing large numbers (over 250 000) of urophyses of the white sucker (*Catostomus commersoni*), showed that two principal peptides were released, urotensins I and II, together with less well-known urotensins III and IV. These were first distinguished by their rather diverse effects on mammalian preparations. Urotensin I (UI) has hypotensive actions in mammals, and in fish, is involved in stress responses, in vasorelaxation, and in osmoregulation. Urotensin II (UII)

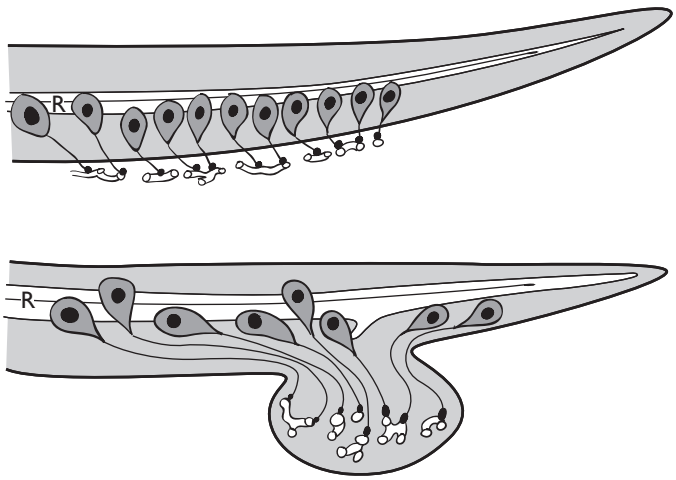

Figure 9.5 The urophysis of the elasmobranch (above) and the teleost. Note association of swollen axon terminals of the neurosecretory neurons with blood vessels, and Reissner's fiber R, in the central canal of the cord. After Fridberg and Bern (1968).

has smooth-muscle stimulating actions, and in fish stimulates the smooth muscle of the reproductive tracts. It also is involved in sodium exchange. Not much is known of the functions in fishes of urotensins III and IV. Following an earlier finding that in plaice (*Platichthyis flesus*) two kinds of Dahlgren cells could be distinguished electrophysiologically, Cioni *et al.* (2002) have suggested that UI secretion is modulated by nitrergic Dahlgren cells, whereas a second non-nitrergic population of these cells secretes UII.

Subsequent immunocytochemistry established that another peptide, urocortin, with close sequence identity to UI, was found in mammalian brain neurons. The mammalian corticotropin releasing factor (CRF) is the primary regulator of the vertebrate stress response, and is very similar in structure to urocortin. It thus seems clear that the original suggestion by Enami, that the teleost urophyseal hormones are concerned in osmoregulation, was right, despite initial failures to confirm his experiments. Bern (1985) has given an interesting account of the struggle to understand the urophysis. In elasmobranchs, the neurosecretory Dahlgren cells are scattered along the hinder part of the spinal cord instead of grouped as in teleosts, they secrete urotensins involved in water balance.

The importance of the urophysis is denoted by its rich descending cholinergic, noradrenergic, peptidergic, and serotinergic innervation, as well as by its conservation from lampreys to lungfish. Although we do not have a urophysis at the end of our spinal cord, UII occurs in our spinal cord neurons.

9.5 The Pituitary

The pituitary is the most complex endocrine gland in the body. It controls the secretory activity of three other endocrine glands as well as producing hormones of its own (for example, the melanocyte stimulating hormone,

MSH) that act directly on effector tissues. In addition, it is the chief link between the nervous and endocrine systems. The basic structure of the pituitary (hypophysis) is essentially the same in all vertebrates; it consists of two parts of different structure, function, and embryological origin. The nervous part (pars nervosa or neurohypophysis) is a downgrowth from the floor of the diencephalon under the hypothalamus (p. 366), and the epithelial part (the adenohypophysis) is an upgrowth from the roof of the pharynx, arising as Rathke's pouch in development. In the less-advanced elopomorph teleosts such as the milkfish (*Chanos*) and the tarpon (*Megalops*) the connection with the pharynx is still retained. Between these two regions, there is a complex system of blood vessels. In lampreys and hagfish, these vessels are more simply arranged than in other craniates, for with the exception of these agnathans, the vascular link between the two divisions of the fish pituitary (as in higher vertebrates) forms a portal system transporting blood and hormones from the neurohypophysis to the distal part of the adenohypophysis. Although the basic plan of the pituitary is the same in all fishes (see Schreibman, 1986), there are differences in the arrangement and relative sizes of the different pituitary regions, as seen in Figure 9.6. The different divisions of the adenohypophysis were first named in tetrapods, and those in fishes have been homologized with them, based upon the same hormonal content of the secretory cell types (reflected in their differential staining reactions). So, for example, the pars distalis is characterized by cells secreting thyroid stimulating hormone (TSH), PRL, adrenocorticotrophic hormone (ACTH), gonadotropins, and growth hormone (GH). Some idea of the complexity of the mechanisms controlling hormone release from pituitary cells is given in Figure 9.7, which

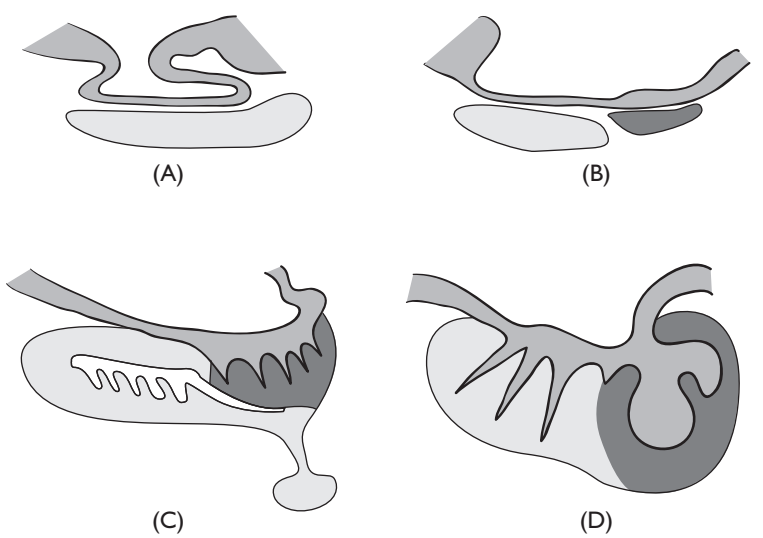

Figure 9.6 Schematic mid-sagittal sections of the pituitary in different fish: (A) hagfish; (B) lampreys; (C) elasmobranchs; (D) teleosts. Medium shading: nervous tissue; light shading: pars distalis of the adenohypophysis; dark shading: pars intermedia of the adenohypophysis. After Ball and Baker (1969, p. 1).

shows the feedback loops involved in GH synthesis and secretion in the teleost pituitary.

How did this complex multifunctional organ arise in chordate evolution? As we have seen, the ascidian neural gland is in some respects a possible *morphological* precursor of the adenohypophyseal part of the pituitary, but no trace of the neurohypophysis has been found in the ascidian brain. In amphioxus, the infundibular organ in the ventral part of the "brain" vesicle has been suggested as equivalent to the neurohypophysis, and the development of the link with Hatschek's pit equivalent to the adenohypophysis (Gorbman, 1999; Lacalli, 2004). Several immunocytochemical studies on amphioxus have been undertaken in an attempt to demonstrate the presence of vertebrate neuropeptide hormones in these structures, but the results so far have not been entirely convincing, and any functional role remains enigmatic. One difficulty, which has now been resolved by recent continuing work on the ultrastructure of the larval amphioxus head and the expression of homeobox genes (p. 351) in amphioxus, is that homology has now more or less clearly been established between the different regions of the amphioxus cerebral vesicle, and those of the vertebrate brain. So, although amphioxus seems to show hints of the origin of the chordate pituitary, it is in hagfish and lampreys that we first see the pituitary clearly.

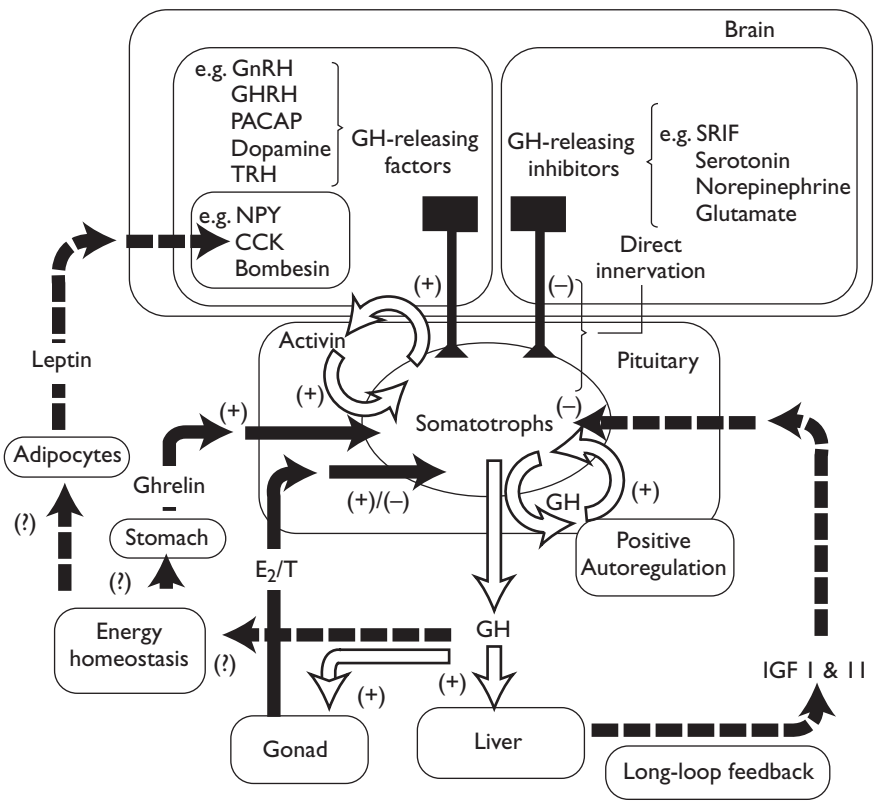

Figure 9.7 Pituitary feedback loop. Wong *et al.* (2006).

The pituitary in hagfish and lampreys

In lampreys, as seen in Figure 9.6, the adenohypophysis is divided into three regions, termed the anterior and posterior pars distalis and the posterior pars intermedia (although the homology of these regions with those in other chordates is uncertain). They are linked to the neurohypophysis by an extensive capillary network, but there does not seem to be the same portal system carrying neurosecretory material from the neurohypophysis as is seen in gnathostomes. In hagfish, the two parts of the pituitary are separated by a thick sheet of connective tissue. In other words, it seems that in both lampreys and hagfish, the nervous control of hormonal release by the adenohypophysis (such a striking feature of pituitary function in other chordates) is absent. The neurohypophysis receives neurosecretory axons from the pre-optic nucleus and hypothalamus. Not a great deal is known of pituitary function in hagfish, while in lampreys hypophysectomy has provided evidence for a melanophore stimulating hormone (MSH) since the lamprey becomes pale. Recent work has shown that the hormones of the pituitary–gonadal axis are present in the lamprey adenohypophysis, just as in vertebrates. In the neurohypophysis, arginine vasotocin (AVT) has been demonstrated (see Table 9.2), although involved in stress responses, its other roles are unclear (see Balment *et al.*, 2006). Warne *et al.* (2002) have given a useful review of the roles of the related arginine vasopressin in mammals and compared this with what was then known of AVT in fish.

Table 9.2 Vasotocin and oxytocin family in different vertebrates, note variety in elasmobranchs

Vasopressin-like peptides	
Arg-vasopressin	Cys-Tyr-Phe-Gln-Asn-Cys-Pro-Arg-Gly(NH_2) mammals
Lys-vasopressin	Cys-Tyr-Phe-Gln-Asn-Cys-Pro-Lys-Gly(NH_2) pig, macropodids
Phenypressin	Cys-Phe-Phe-Gln-Asn-Cys-Pro-Arg-Gly(NH_2) macropodids
Arg-vasotocin	Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Arg-Gly(NH_2) all nonmammals
Oxytocin-like peptides	
Oxytocin	Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly(NH_2) ratfish, mammals
Mesotocin	Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Ile-Gly(NH_2) dipnoi, amphibians, reptiles, birds, marsupials
Seritocin	Cys-Tyr-Ile-Gln-Scr-Cys-Pro-Ile-Gly(NH_2) *Bufo regularis*
Isotocin	Cys-Tyr-Ile-Ser-Asn-Cys-Pro-Ile-Gly(NH_2) bony fishes
Glumitocin	Cys-Tyr-Ile-Ser-Asn-Cys-Pro-Gln-Gly(NH_2) rays
Valitocin	Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Val-Gly(NH_2) spiny dogfish
Aspargtocin	Cys-Tyr-Ile-Asn-Asn-Cys-Pro-Leu-Gly(NH_2) spiny dogfish
Asvatocin	Cys-Tyr-Ile-Asn-Asn-Cys-Pro-Val-Gly(NH_2) spotted dogfish
Phasvatocin	Cys-Tyr-Phe-Asn-Asn-Cys-Pro-Val-Gly(NH_2) spotted dogfish

After Hyodo *et al.* (2004).

The pituitary in elasmobranchiomorpha

In elasmobranchs and holocephalans, the pars intermedia is very large and closely interdigitated with the neurohypophysis, and the pars distalis is elongated with a unique ventral lobe in the floor of the chondrocranium linked to the rest of the pars distalis by a long stalk (Figure 9.6). In holocephali, the equivalent of the ventral lobe is a group of follicles entirely separate in the adult from the rest of the pituitary. So the pituitary does not look much like the pituitary of other fishes. Unlike lampreys and hagfish, there is an abundant and complex vascular bed including a portal system between neuro- and adenohypophysis, and palisades of neurosecretory terminals from the pre-optic nuclei lie closely adjacent to the capillaries, in just the same manner as in the teleost urophysis. In addition to this "indirect" hormonal link some nerve fibers pass directly to innervate cells in the adenohypophysis. The arrangement means that hormone release is under the control of direct feedback systems as well as by hormonal feedback loops, such as that whereby GH in the blood evokes the release of insulin-like growth factor 1 (IGF-I). Because of the cartilaginous chondrocranium, experimental hypophysectomies are easier in elasmobranchiomorphs than in bony fishes (although in salmonids, an approach via the orbit has been successful), so that it is possible to remove different regions of the adenohypophysis separately to examine the effects produced.

Thus, for example, removal of the ventral lobe in dogfish has shown that various reproductive hormones are secreted there, such as follicle stimulating hormone (FSH) and gonadotropin (or gonadotrophin) releasing hormone (GnRH), while incubation of ventral lobe extract with thyroids increases thyroxine release indicating secretion of TSH. Other experiments have shown that MSH is secreted by the intermediate lobe, acting to expand the melanophores and darken the skin, and PRL by the distal lobe, acting (in the euryhaline stingray *Himantura*) to control plasma osmolarity, and sodium and urea retention. Although the ventral lobe is not directly linked to the neurohypophyseal-hypothalamic neurosecretory axons, the control hormones they release reach the ventral lobe via the general circulation to regulate the secretion of the ventral lobe hormones.

The neurohypophysis produces a number of hormones which are all nonapeptide rings formed by disulfide bridges at the two cysteins (Table 9.2). These belong to two families, the vasopressins, such as AVT, and the oxytocins (OT), perhaps derived from the duplication of a single gene in agnathans, since lampreys only possess AVT. The distribution of neurohypophyseal hormones in different fish is curious and interesting. Most teleosts have AVT and isotocin, while lungfish produce AVT and mesotocin (as do most terrestrial vertebrates. The OT family and its roles have been reviewed by Gimpel and Fahrenholtz (2002). In elasmobranchs and ratfishes, as well as oxytocin itself, several hormones of the OT family have been identified, differing in their amino acid sequence (Table 9.2). Not much is yet known of the function of these hormones in elasmobranchs, although there is immunocytochemical evidence for OT-like receptors in liver and gills of trout. When injected into rats they have antidiuretic, lactational, and oxytocic (parturition-inducing) effects. Remarkably enough, even within the same species of elasmobranch, different individuals may have different members of the oxytocin family hormones.

What seems a plausible view of this remarkable diversity is that urea-based osmoregulation has relieved chondrichthyes from the strict restraints on AVT structure of other fish (Acher, 1996). Apparently "random" amino acid substitutions can take place so long as the functional region(s) of the hormone remain conserved, and it is certainly very striking that in another hormone, relaxin (p. 279) Callard and his colleagues (1989) emphasize that on present data, there has been almost as much amino acid substitution and sequence change within the elasmobranchs, as there has been between elasmobranchs and mammals.

The teleost pituitary

In teleosts, the pituitary looks rather different from that of other fishes because the neurohypophysis ramifies into all the regions of the adenohypophysis (Figures 9.6 and 9.8), sending at least two kinds of axons into intimate contact with the secretory cells of the adenohypophysis. The range of hormones produced by the adenohypophysis is similar to that in elasmobranchs, but in some areas, owing to the commercial importance of such teleosts as eels and salmonids, more is known of their actions. For example, in eels and in salmon, GH from the pars distalis acts on receptors in the liver inducing the liver to secrete insulin-like growth factors (or somatomedins), which directly stimulate chondrogenesis. Interestingly, since hypophysectomy in salmon reduces receptor number, the pituitary itself regulates the hepatic growth hormone receptors.

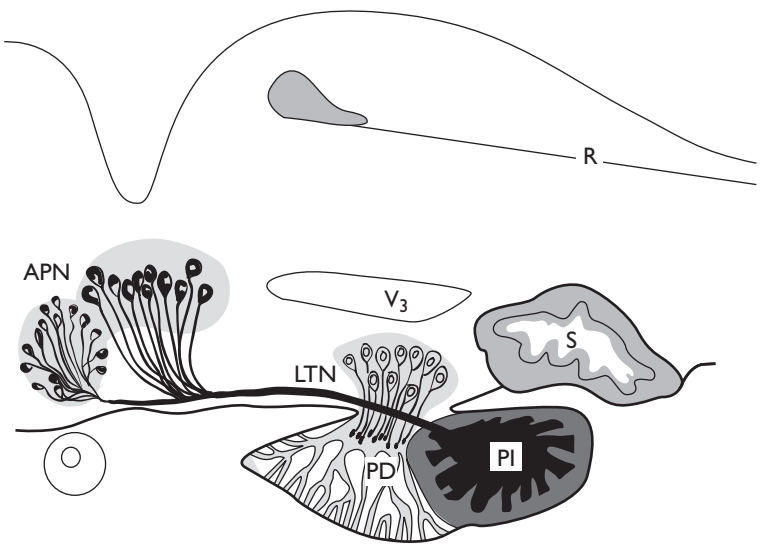

Figure 9.8 Sagittal section of the pituitary of the eel, *Anguilla*, showing the nerve tracts from the anterior preoptic nucleus (APN, divided into two parts with large and small neurosecretory neurons), and from the lateral tuberal nucleus (LTN). The infundibular organ dorsally secretes Reissner's fiber (R) which passes down the spinal cord. V_3: third ventricle; S: saccus vasculosus; O: optic chiasma; PD: pars distalis; PI: pars intermedia. After Knowles and Vollrath (1966, p. 311) and Olivereau (1967, p. 286).

Teleost pituitary hormones

Prolactin (PRL)

A rather wide spectrum of actions for prolactins has been observed in teleosts, for example lipid metabolism and the control of steroidogenesis (in *Fundulus*), but its main role in freshwater seems to be the regulation of water and ion permeability of gills, kidney, gut, and bladder (Sakamoto and McCormick, 2006). In euryhaline teleosts, plasma prolactin levels decrease when entering seawater, the reverse on entry to freshwater from the sea.

Growth hormone (GH)

Growth hormone has many actions, e.g. osmoregulatory, adrenocorticotrophic activity (thus being potentially diabetogenic), thyrotropic, and possibly immunological and reproductive effects (Figure 9.9). The present considerable interest and effort in producing transgenic salmonids using growth hormone transgenes to obtain larger fish, might perhaps be better devoted to the use of IGF-I transgenes. The effects of GH on skeletal muscle (the required end product in farmed salmon) is mediated by IGF-I from the liver. In contrast to elasmobranchs, teleost melanophores are innervated by autonomic nerve fibers, and, although in some species MSH is present and darkens the skin if injected; in others it has no effect, perhaps in the latter being overridden by the nervous control mechanism. There may be several varieties of MSH derived from the same pro-hormone peptide that also gives rise to ACTH.

The neurohypophyseal hormones of teleosts include arginine vasotocin (as in other fishes), and two different members of this family, isotocin and mesotocin (Table 9.2). The role of these is still unclear, although arginine vasotocin probably has a variety of effects including peripheral vasoconstriction, and oviducal smooth-muscle contraction. Recent work on male Beaugregory damsel fish (*Stegastes leucostictus*) using AVT and its receptor antagonist, Manning compound, has shown that AVT modulates aggression levels against other males (Santangelo and Bass, 2006).

9.6 The Thyroid

In contrast to the pituitary, the thyroid is of simple design, and the hormones it produces are of the same relatively simple structure in all vertebrates. The evidence for the evolutionary origin of the thyroid is clear. Not only is there a direct morphological link between the protochordate endostyle and that of the lamprey ammocoete, see Wright and Youson (2005), but also the thyroid hormones are found in protochordates. Nevertheless, we have no inkling (not through lack of effort) what the thyroid hormones of protochordates do. In adult lampreys the thyroid gland is a series of follicles scattered along the pharyngeal floor. It arises in ontogeny from the larval ammocoete endostyle which opens into the floor of the pharynx (Figure 1.17). In lampreys and in all fishes, the thyroid consists of a series of unicellular follicles, which are usually more scattered than in higher vertebrates. These take up iodine and produce the thyroid hormones. Both the ammocoete endostyle and the adult thyroid follicles take up iodine, and iodinate tyrosine adding one or two iodine atoms to make mono- or di-iodotyrosine. These then condense in

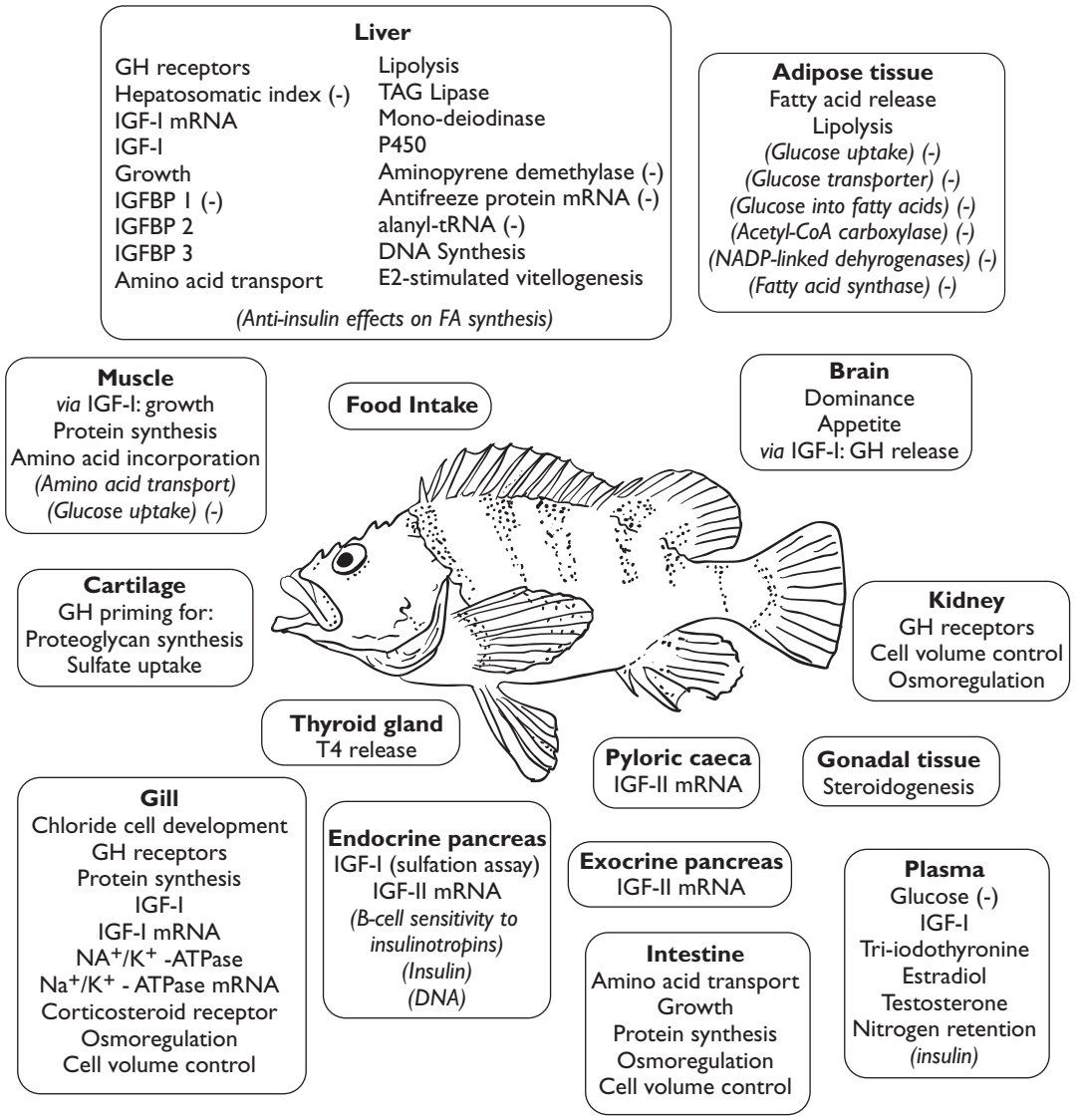

Figure 9.9 The multiple targets for growth hormone action in teleost fish. After Mommsen (1998).

pairs, losing an alanine residue (Figure 9.4) to make the two thyroid hormones tri-iodothyronine (T_3), and thyroxine (T_4). At the periphery of the thyroid follicles, these hormones are bound to the glycoprotein thyroglobulin which has a similar amino acid composition to the thyroglobulins of higher vertebrates. The hormones are unbound by protease hydrolysis of the thyroglobulin within the follicle cell, and then released into the circulation. Thyroxine levels in ammocoete blood are around 8.0 μg%, compared to 0.5 μg% in adult lampreys, but surprisingly, in ammocoetes blood levels decline markedly at metamorphosis and goitrogens induce metamorphosis! This

unexpected situation is discussed by Youson and Sower (2001), but is not yet well understood.

What is the function of the thyroid hormones in gnathostome fishes, and how is their release controlled? Curiously enough, although there is good evidence for TSH thyroid regulation by the pituitary, in fishes the role of the thyroid hormones is rather confusing, and different from that in other vertebrates. In birds and mammals, the thyroid hormones are calorigenic, stimulating oxidative metabolism, but this function has not been shown in poikilotherms such as fishes and amphibia. Though T4 stimulates the dramatic flounder metamorphosis, as it does in amphibia, it certainly also plays a central role in smoltification (as it is inelegantly called), the later transformation of the salmon parr to the smolt, prior to the latter going downriver to the sea.

This involves all kinds of changes in body pigmentation, visual pigment, silveriness, carbohydrate and lipid metabolism, and in osmoregulatory ability. Silvering of the skin increases when parr are treated with thyroxine (since there is increased deposition of reflecting guanine platelets in the scales; see p. 327). Blood thyroxine levels in *Oncorhynchus* species reach a peak at the time of the new moon in the spring, before the downstream migration, and although recent work has shown that this peak does not synchronize with peaks in tissue concentration, it provides an excellent "timer" for release of young salmon from hatcheries. In brown and rainbow trout (*Salmo trutta* and *Oncorhynchus mykiss*), T_3 is essential for seawater adaptation. Pituitary hormones such as cortisol, PRL, GH, and IGF-1 are also involved in smoltification and osmoregulation. The present situation seems best summarized (as Matty (1985) points out) by the view that the fish thyroid aids adaptation of the fish to environmental changes such as temperature and osmotic stress, and to the rapid internal changes during growth and sexual maturation. One interesting observation which has yet to be fitted into any general scheme of fish thyroid function, is that thyroxine levels are high in the yolk of teleost eggs (derived from the maternal blood supply to the ovary), and may be important in regulating development.

9.7 Calcium Homeostasis

Several different hormones and receptors interact in maintaining extracellular Ca^{2+} levels, which need to be tightly regulated. PRL and GH are *hyper*calcemic, while calcitonin and stanniocalcin are *hypo*calcemic. Central nervous system levels of Ca^{2+} are apparently controlled by the Dahlgren cells of the urophysis, which contain *parathyroid hormone related protein* and where there are calcium-sensing receptors. Receptors for serum Ca^{2+} levels are found in the gills. The parathyroid glands of tetrapods derive from the ultimobranchial glands budded from the last gill pouch. They often cease activity for a short while after human thyroidectomy, and occasionally remain inactive for longer, when the patient then has to take Ca^{2+} supplements, and may suffer from tingling and cramps in fingers and toes.

The ultimobranchial gland

Although lacking in hagfish and lampreys, in other fishes paired or unpaired outgrowths from the last branchial pouch migrate during development to lie over the pericardium. Originally described in elasmobranchiomorpha, where

the tissue is follicular, like the thyroid, in teleosts it is solid; but in both groups the ultimobranchial gland contains high concentrations of the straight-chain peptide calcitonin. In mammals, calcitonin is secreted by the parathyroid and is a potent hypocalcemic factor, inhibiting bone resorption. Experimental studies in fishes have shown in teleosts that calcitonin increases the renal output of Ca^{2+}, and increased efflux but decreased influx of Ca^{2+} across the gills. Further, in female salmon, serum calcitonin levels rise until spawning when there is a dramatic decline. Thus it seems that calcitonin in fishes is involved in Ca^{2+} metabolism, together with stanniocalcin from the corpuscles of Stannius, and prolactin produced by the pituitary. An interesting finding by Girgis and her colleagues (1980) is that a calcitonin very closely similar to human calcitonin occurs in the nervous system of amphioxus and the hagfish (*Myxine*), where it presumably functions as a neurotransmitter. These workers suggest that this human calcitonin-like molecule was the parent brain peptide from which were derived the later members of the calcitonin family found in the ultimobranchial bodies and in the human thyroid.

The corpuscles of Stannius

These are small spherical bodies lying on, or embedded in, the kidneys of bony fishes, first described in teleosts by Stannius (1839). Each is well vascularized and innervated, and its cells have the fine structure typical for protein-secreting cells. Some fish have only a few corpuscles, others, such as the bowfin (*Amia*), may have hundreds. As the distinguished French physiologist Fontaine showed in 1964, removal of the corpuscles produces an immediate rise in plasma Ca^{2+} while, conversely, injection of extracts of the corpuscles into normal eels results in a rapid fall in plasma Ca^{2+}. The glycoprotein hypocalcemic hormone of the corpuscles, stanniocalcin (STC), acts on unidentified target cells in the gill, kidney, and intestine. STC release from the corpuscles is triggered by a rise in serum Ca^{2+} and is important in Ca/Phosphate homeostasis in teleosts. A closely similar hormone STC1 was later found in mammalian tissues, particularly in neurons (Zhang *et al.*, 1998) where it seems to have widely diverse roles, possibly because it regulates local Ca^{2+} uptake in different cell types.

9.8 The Gastro–Entero–Pancreatic Endocrine System

The pancreas

As Epple (1969) has pointed out, the structure and location of the endocrine pancreas varies in different groups of fishes (Figure 9.10). In some teleosts, such as *Cottus* and *Lophius*, relatively large lumps of endocrine pancreatic tissue (Brockmann bodies) can be isolated, and their hormones extracted, to see the effects of pancreatomy. The endocrine portion of the pancreatic tissue contains various secretory cell types staining differentially with dyes like paraldehyde-fuchsin, in a similar way to those in the mammalian pancreas, and producing similar hormones. Epple (1969) reviews these.

In mammals, the level of blood glucose is controlled by two pancreatic hormones: insulin and glucagon. Glucose enters cells only in the presence of insulin, which thus lowers blood glucose levels; glucagon raises blood glucose levels by stimulating glucose production from stored glycogen. So blood glucose levels result from the balancing of two hormones with opposite actions.

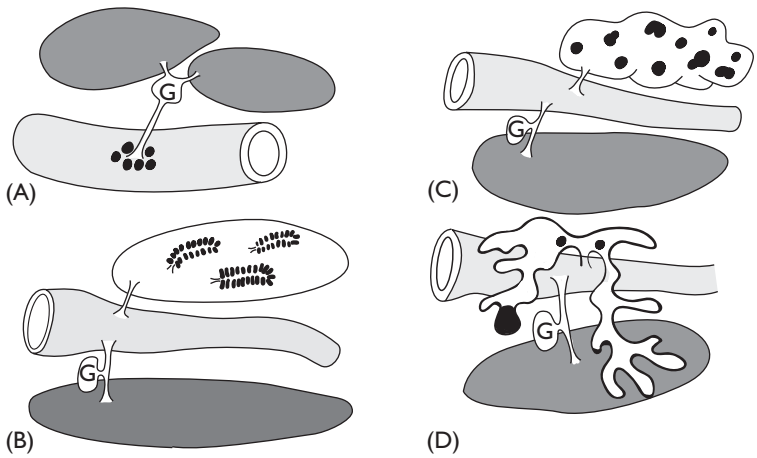

Figure 9.10 The pancreas in different fishes: (A) *Myxine*; (B) elasmobranch; (C) compact teleost type (*Anguilla*); (D) ramified teleost type. Islet tissue black, liver dark gray shading, gut: light stipple. G: gallbladder. After Epple (1969, p. 275).

Not surprisingly, the two are produced by different cell types in the pancreas. In fishes as in mammals, the A cells produce glucagon, the B cells insulin, and the D cells somatostatin (SS). SS was first found in sheep brains in 1973, and studies in fish have largely contributed to the present knowledge of the SS signaling system (Nelson and Sheridan, 2005). The large somatostatin family regulates a wide variety of processes, linked with many receptor types. SS in hagfish and *Anguilla* inhibits insulin release. As well as the three pancreatic hormones mentioned above, immunocytochemistry has shown cells containing other regulatory peptides of the neuropeptide Y family, such as pancreatic polypeptide, neuropeptide Y itself and peptide YY. Some of these may be colocalized in the same cells as the more familiar pancreatic hormones such as pancreatic polypeptide and glucagon (Jonsson, 1993). A similar peptide Y, (but without a terminal amide) has been found in the pancreas of the angler fish (*Lophius*). What is the function of this variety of different hormones in the fish pancreas? In lampreys, there is good evidence that insulin regulates blood sugar, but why blood insulin levels vary seasonally is not understood. In the holocephalan *Hydrolagus*, and in elasmobranchs, glucagon evokes a rapid but transient hyperglycemia, while insulin evokes hypoglycemia. In addition to these expected effects, however, in many bony fishes glucose homeostasis may not be a major role of insulin, which seems more to be concerned with amino acid metabolism.

Gut hormones

The many different polypeptide hormones released from the gut cells themselves and the "neurohormonal" substances released from the autonomic nerve fibers innervating the gut, have two main functions: (1) to control secretory activity, and (2) to control gut motility. Some also act on other organs in the body. There is a strikingly long list of regulatory polypeptides recognized in the fish gut, belonging to two main families: *gastrin/cholecystokinin*, and

secretin/glucagon/vasoactive intestinal peptide. There are also many others, such as substance P (found also in the dorsal horn of the spinal cord, see p. 355), bombesin, enkephalin, somatostatin, and neurotensin, and (in elasmobranchs, and perhaps also in *Latimeria*) rectin controlling Cl^- secretion by the rectal gland. The functions of most of these gut peptides are still poorly known (Buddington and Krogdahl, 2004). This topic is still something of a minefield, for it is exceedingly hard to devise critical experiments to distinguish the individual functions of, say, secretory cells and intrinsic neurons in what is, after all, a piece of internalized skin with the gut cells facing the changing external environment of the gut contents. Nevertheless, much has been established. The peptides of the *cholecystokinin* (CCK) family share the highly evolutionarily conserved common C-terminal pentapeptide amide sequence (-Gly-Trp-Met-Asp-Phe-NH_2). It seems that Cionin in ascidians (p. 283) was the 'original' parent of the family, and gave rise to CCK which has long been involved in stimulating digestive enzyme secretion (as it does in ascidians), only later as new target organs appeared, taking over the roles of stimulating gall-bladder contraction, and stimulation of pancreatic enzyme secretion. In bony fishes, CCK from cells in the intestine inhibits acid secretion by the oxyntic cells of the stomach, which are stimulated to secrete by bombesin from stomach cells as the meal enters. In mammals, acid secretion is evoked by gastrin release, but in fishes this peptide has apparently not yet diverged from CCK. Gut motility in fishes is controlled by the autonomic nervous system via the cholinergic, adrenergic, and aminergic supply to the gut musculature, but this control is modulated by gut hormones. Thus bombesin greatly potentates the effects of acetylcholine, and substance P, and enkephalin stimulates gut muscle contractions.

9.9 Chromaffin Tissue, and the Interrenals

In higher vertebrates, the adrenals lie next to the kidneys, and consist of an inner medulla and outer cortex, producing different hormones (the medullary catecholamines adrenaline and noradrenaline, and the cortical corticosteroids) so that they are really two endocrine glands in one. In fishes, however, they are separate: the medulla represented by chromaffin cells innervated by spinal autonomic fibers, and the interrenals by yellowish patches or lumps near the kidneys and posterior cardinal veins (Figure 9.11).

Chromaffin tissue

The release of adrenaline and noradrenaline into the blood from chromaffin tissue in response to stress is rapid and dramatic. Handling trout, for example, causes catecholamine levels in the circulation to rise several *hundred* times. This release not only increases heartbeat amplitude, and gill vessel vasodilation (hence lowered vascular resistance), as well as systemic vasoconstriction, but also induces hypoglycemia, changes in gill ionic permeability and sometimes increased lipolysis. These varied stress responses are under the control of the autonomic nervous system, and, like the stress responses of mammals, evidently fit the fish to withstand disturbance by external factors. They are of obvious importance in aquaculture where monitoring circulating catecholamines has enabled stressful procedures to be avoided.

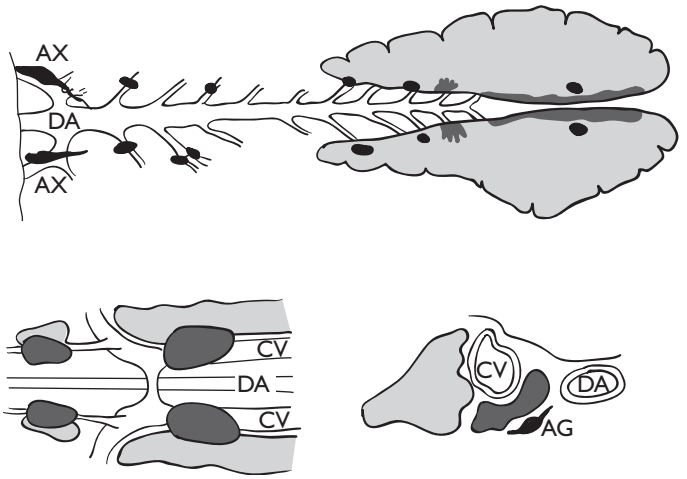

Figure 9.11 Chromaffin and interrenal tissue. Above: In an elasmobranch *Raia*. Below left: a teleost, *Anguilla*, anterior to left. Kidneys, light gray; interrenals, dark gray shading; chromaffin tissue, black. Below right: transverse section of kidney and interrenal. DA: dorsal aorta; CV: cardinal veins; AG: autonomic ganglion; AX: axillary body. After Vincent and Curtis (1927, p.110), and Vivien (1958).

Interrenals

The interrenals produce corticosteroids (such as cortisol) under the regulation of corticotrophin (ACTH) from the adenohypophysis. These hormones have several functions. In elasmobranchs, a unique corticosteroid (rectin) is involved in control of NaCl secretion by the rectal gland, thus the interrenal may be involved in osmoregulation, as it certainly is in teleosts, where blood cortisol levels rise when euryhaline fish are transferred to seawater, and where cortisol injections alter gill ionic and water permeability. In teleosts, stress such as handling or cold shock elevates blood cortisol levels, acting synergistically to the immediate catecholamine response, and these also change during the reproductive cycle.

9.10 Kidney Hormones, and the Renin–Angiotensin System

In mammals, the juxtaglomerular cells of the kidney secrete renin which, in the blood, acts on the plasma precursor of the polypeptide angiotensin. Angiotensin causes a rise in blood pressure, and stimulates corticosteroid secretion by the adrenals, resulting in kidney sodium retention. In fishes, juxtaglomerular cells have not been found in agnatha and elasmobranchs, but they are seen in other fish groups, including *Latimeria*, and renin pressor activity is seen when kidney extracts are incubated with the blood and then tested in mammals. However, what renin does in fishes and how its release is regulated are not yet clear, although the system probably plays some part in water and ion regulation, at least in euryhaline fishes. In lungfish, the renin-angiotensin osmosystem seems to be important in the normal control of blood pressure.

Hormones from the heart, natriuretic peptides

The fish heart is very sensitive to volume enlargement and to mechanical load. These stresses induce defensive endocrine activity from the heart, and increase production and secretion of several vasorelaxant natriuretic peptides which act to unload the heart. The first discovered of these important hormones (in rat heart, 1981) was atrial natriuretic peptide (ANP), and then C-type natriuretic peptide (CNP) from the atrium, and ventricular natriuretic peptide (VNP) from the ventricle (Loretz and Pollina, 2000). Brain natriuretic peptide (or B-type natriuretic peptide, BNP) was also found in some fish, and *all* tetrapods. In mammals, these peptides act on the kidneys, in fish they primarily act to excrete NaCl at the gills and rectal gland and limit gut intake as the fish drinks. Warne *et al.* (2002) have compared the actions of the structurally similar hormones in the mammalian kidney and reviewed what was then known.

The family of natriuretic peptides is not equally distributed in all fish (BNP is absent in some teleosts, and hagfish and sharks have only one natriuretic peptide), and although long supposed to have arisen from a neuromodulatory, CNP-like brain peptide, the situation now seems more complicated (Inoue *et al.*, 2003). Figure 9.12 shows the scheme they propose.

In contrast to mammals, where natriuretic peptides act through natriuresis and diuresis to bring about long-term reductions in blood volume and blood pressure, in fishes the primary action appears to be the extrusion of excess salt at the gills and rectal gland, and the limiting of drinking-coupled salt uptake by the alimentary system.

Natriuretic peptides seem to be seawater-adapting hormones with appropriate target organs including the gills, rectal gland, kidney, and intestine, with each regulated via, predominantly, either A- or B-type (or perhaps C- or

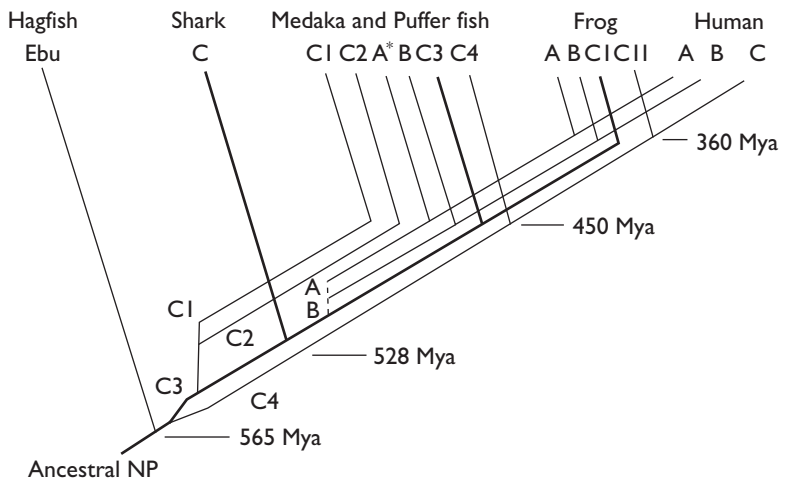

Figure 9.12 Lineage of natriuretic peptide (NP) genes. Different hatchings indicate different lineages. A, ANP; B, BNP; C, CNP; C1-C4, CNP-1–CNP-4 of teleosts; CI, and CII, CNP-I, and CNP-II of the frog, respectively; Ebu, hagfish NP. Time of divergence is indicated in Mya The dotted line indicates unspecified timing of divergence. *, Medaka ANP has not been found. From Inoue *et al.* (2003).

D-type?) natriuretic peptide receptors. Natriuretic peptides act both directly on ion-transporting cells of osmoregulatory tissues, and indirectly through increased vascular flow to osmoregulatory tissues, through inhibition of drinking, and through effects on other endocrine systems. Weybourne *et al.* (2005) have shown in the euryhaline flounder (*P. flesus*) that AVT receptors are located in the kidney blood vessels rather than in the tubular walls.

9.11 Gonadal Hormones and the Regulation of Reproduction

In all vertebrates, the hormones controlling the production of gametes and the sex steroid hormones from the gonads have been known to be very similar since the 1980s (Figure 9.13). Those in gnathostome fish are homologous to the glycoprotein follicle-stimulating (FSH) and luteinizing hormones (LH) of mammals. More recently, after a prolonged effort, Kawauchi, in Japan, and his colleagues in the USA and Canada succeeded in identifying a glycoprotein hormone in lamprey pituitary, suggesting that subsequent duplications gave rise to the gnathostome LH, FSH, and also TSH (Kawauchi and Sower, 2006).

So the pituitary control of reproduction has been long-conserved, arising prior to the split of the agnathan clade from the gnathostomata. The hypothalamic-pituitary-gonadal (HPG) axis (Figure 9.14) begins with GnRH neurons in the brain and ends with the sex hormone testosterone in both ovaries and testes, (in the ovaries, testosterone is secondarily converted to progesterone). The common signaling GnRH has two or three different forms in fish, and these forms may or may not be segregated in neurons in particular brain nuclei. In lampreys, as Youson *et al.* (2006) describe, GnRH functions may change during ontogeny. Interestingly, not only are there receptors in the pituitary (not all in pituitary cells producing gonadotropins, Parhar *et al.*, 2005)

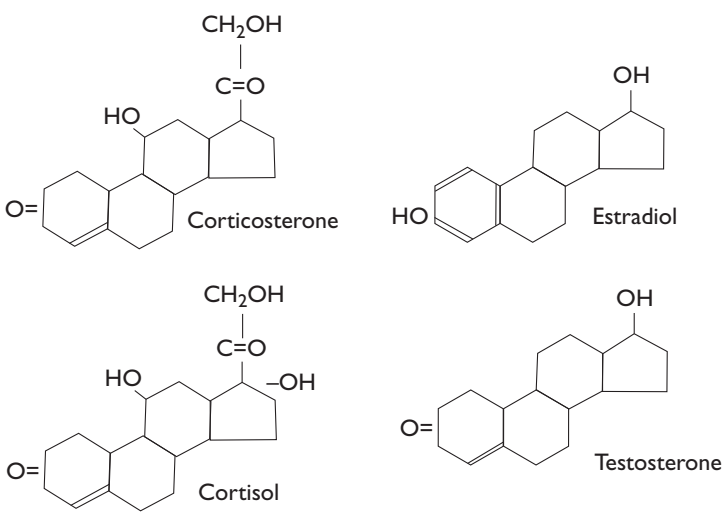

Figure 9.13 The structure of two gonadal steroid hormones (right), compared with two interrenal steroids. After Barrington (1964).

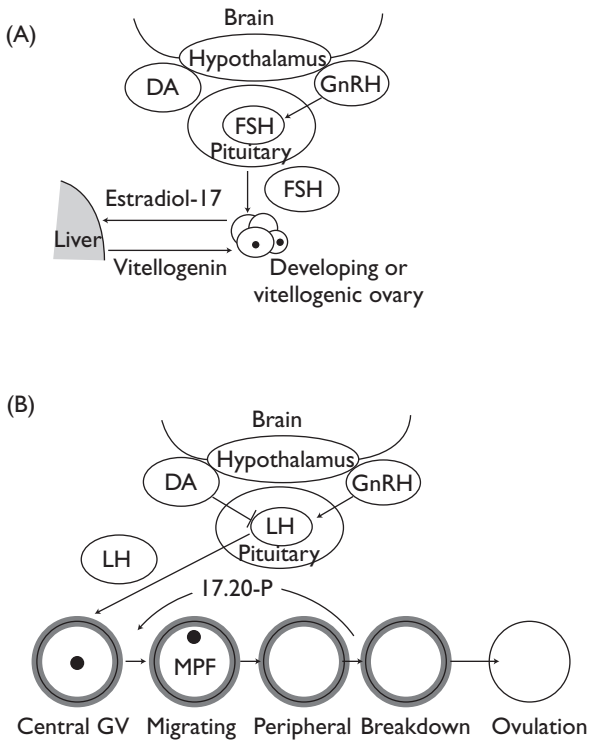

Figure 9.14 GnRH and the ovary. Ovarian endocrine regulation of a cyprinid fish, during vitellogenesis (A), and in final oocyte maturation (B). In (A) FSH release stimulated by GnRH, promotes follicular secretion of estradiol evoking vitellogenesis. In (B) LH release is stimulated by GnRH, and post-vitellogenic follicles respond by secretion of the maturation-inducing steroid 17α,20 β-dihydroxy-4-pregnen-3-one. From Yaron *et al.* (2003).

but GnRH actions are not limited to the central nervous system and the pituitary gland. Further work is needed to see whether their suggestion that because GnRHRs are expressed in peripheral tissues related to the senses, reproduction and homeostasis, GnRH is involved in these functions.

Knowing the intricacies of the control and effects of mammalian sex hormones, it should be no surprise to find that in fish the HPG axis is also changed by social interactions. For instance, in the cichlid *Astatotilapia burtoni* Au *et al.* (2006) showed that expression levels of GnRH receptors in the pituitary were linked to male territoriality.

Elasmobranchs

As we might expect, with their wide repertoire of reproductive strategies (p. 233), the hormonal control of elasmobranch reproduction has attracted much attention. Elasmobranchs provide excellent models for the regulation of reproduction in higher vertebrates as Cimini *et al.*, (1989) pointed out. The development of the gonads and sexual behavior, and their seasonal changes, are controlled by GnRH from the neurons of the pre-optic nuclei, released into the systemic circulation from the neurophysis, which then regulates

gonadotropic hormone secretion by the ventral lobe of the adenohypophysis. Strikingly, GnRH is present in the neurons of the terminal nerve (nerve 0) in elasmobranchs, which, as in teleosts, is linked to many brain regions, including those controlling sexual behavior and physiology. Gonadotropic hormone evokes increased androgen and estrogen production from the gonads. These steroid hormones (testosterone and estradiol-17β) are found in the serum, and in the ovaries and testes. The relatively slight differences in the structure of the different steroids (seen in Figure 9.13) testify to the specificity of the receptors for which they are designed. Estradiol-17β is produced in the ovary by cells of the follicle walls, while progesterone is produced by the corpora lutea (post-ovulatory follicles). In rays, progesterone is involved in the sequence of events of vitellogenesis, egg capsule formation, and oviposition, while estradiol is required for the development and maintenance of the reproductive tract. Ovarian steroid secretion signals the proper timing of egg laying or parturition.

Not all gonadal hormones in elasmobranchs are steroids however. The ovaries in viviparous sharks like the sand tiger (*Odontaspis*) and the oviparous ray (*Raja erinacea*) contain the peptide hormone *relaxin*, a member of the insulin-like growth factor hormone family, which all share the α- and β-chains linked by disulfide bridges familiar in the insulin molecule itself. Relaxin acts on the lower part of the female reproductive tract causing it to expand to permit the exit of the egg case or fetus, and, as in mammals, the greatest effect is seen after priming by estradiol. It also seems to regulate uterine contractions in conjunction with progesterone.

Teleosts

In general terms, the gonadal hormones of teleosts are similar, and play similar roles to those of elasmobranchs, although since relatively few teleosts are viviparous, control of the female reproductive tract is less striking than in elasmobranchs. More is known in teleosts about the sites of origin of hormones in the gonads, and of the hormonal control of behavior, including sex changes. These last are of considerable importance in aquaculture, where ripening of fish by pituitary injection (not necessarily from the same species) enables fish farmers to produce fry at all seasons of the year. But it is the production of single-sex batches of fish that has stimulated much fairly recent interest in teleost endocrinology, chiefly in salmonids and tilapias. The aim is to farm only the sex with the better conversion efficiency and growth rate. In *Tilapia aurea*, all male stocks are produced by treating the young newly hatched fry with methyl testosterone. Male tilapias are more appreciated than females, but with salmonids the reverse is the case, and all-female stocks are produced by various estradiol treatments. The effects of androgens and estrogens on the secondary sexual characters of elasmobranchs is not very dramatic (increase in clasper size in immature males, for example), but in teleosts, there may be striking "nuptial" color changes and elongation of intromittent organs (as in the bitterling (*Rhodeus*) which lays its eggs in freshwater lamellibranchs). There may also be behavioral changes of an equally notable kind in such reproductive activities as nest-building, and male parental behavior. There are, however, quite different effects of castration and steroid injection in different species, and it seems that different pathways regulate sexual behavior in different fishes.

9.12 The Pineal

The pineal and parapineal arise as dorsal evaginations from the roof of the diencephalon (see p. 365), and, although the parapineal is present in adult lampreys, in other fishes it disappears or is much reduced during ontogeny. The presence of receptor cells, pigment, and associated nerve fibers in connection with the posterior commissure makes it quite clear that the pineal has a sensory function (see p. 325) but, as in mammals, there are dense-cored secretory vesicles in the sensory cells, and the pineal is obviously a photosensitive endocrine organ. In lampreys and teleosts (little is known of the elasmobranchiomorph pineal) the pineal contains the hormone melatonin and its precursor serotonin, both of which show marked diurnal changes, rising at night. In trout plasma, melatonin levels rise at night to around 150 pg ml^{-1}, twice the level seen during the day (Figure 9.15). What is the significance of these marked diurnal changes in pineal and plasma levels? In goldfish, removal of the pineal abolishes or changes diurnal variations in liver glycogen, and in other fish the usual circadian rhythm (see Meier *et al.*, 1992) of color change is eliminated by pinealectomy. On the whole, it seems that melatonin secretion by the pineal provides the link between photoperiod and hypothalamic-pituitary function, and between photoperiod and seasonal gonadal development.

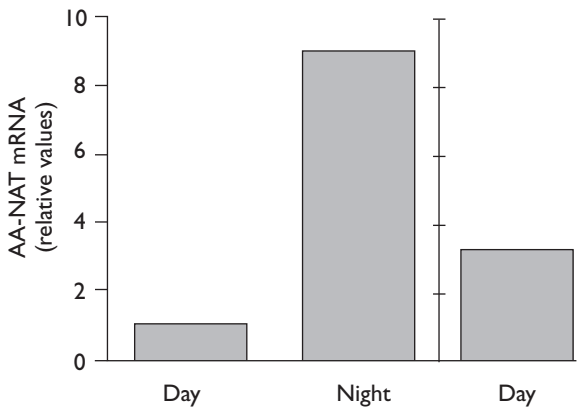

Figure 9.15 Pineal and RNA circadian cycle. Expression of melatonin synthesis genes is controlled by a circadian clock in the pike (*Esox lucius*) pineal organ. Relative expression of the mRNA encoding the melatonin synthesis enzyme serotonin *N*-acetyltransferase (AA-NAT) which is controlled by a clock. Coon *et al.* (1998).

9.13 Origin and Evolution of Fish Hormones

The origin and evolution of fish hormones raise intriguing questions. For example, when and whence did the different hormones arise in phylogeny, and what were their original functions? For example, the approach by Hoyle (1999) where the nucleotide sequence of mRNA or cDNA encoding many of these peptides has been determined, which has allowed evolutionary distances to be estimated based on the DNA mutation rate has considered the neuropeptides of the oxytocin/vasopressin family, the growth hormone releasing factor (GRF) superfamily and the substance P/tachykinin family

which have been isolated from many vertebrate classes. Does information about the amino acid sequences of hormonal peptides in living forms make it possible to reconstruct the structure of ancestral hormones? Naturally, such questions are linked to similar questions about the evolution of hormonal receptors and their associated intracellular signaling G-proteins. It seems fairly clear that while hormones evolve, so too can their functional roles.

Origin

Because of its medical importance, insulin has been much studied since its discovery in mammals in 1922, and it was soon realized that it was present in animals over a wide phylogenetic range, such as mollusks and insects (Figure 9.16). No insulin genes have been found in the yeast genome, nor has insulin been found in sponges (as earlier incorrectly reported) so it seems that genes for insulin appeared with the arrival of the metazoa (Chan and Steiner, 2000). Insulin is supposed to have arisen from an ancestral proinsulin-like protein, perhaps a serine protease, whose original functions may have been in food processing and digestion.

We might suppose that the insulin molecule was inherited by agnathan fish from protochordate ancestors, and then underwent changes from the ancestral precursor, giving rise in later fish and higher vertebrate groups to the insulin superfamily of related peptides (insulin, IGF-I and -II, relaxin, and the Leydig insulin-like peptide Ley-IL). These all contain six highly conserved cysteine residues, structurally similar, and it seems that the genes for insulin, IGF-I and -II have been derived from a common ancestor, although relaxin and Ley-IL genes came from a different precursor gene (see Conlon, 2000b).

Hagfish have undergone a long evolution from the ancestral forms speculatively placed in evolutionary trees like that of Figure 1.1, perhaps diverging some 400 million years from ourselves, yet their insulin shares 61% of amino

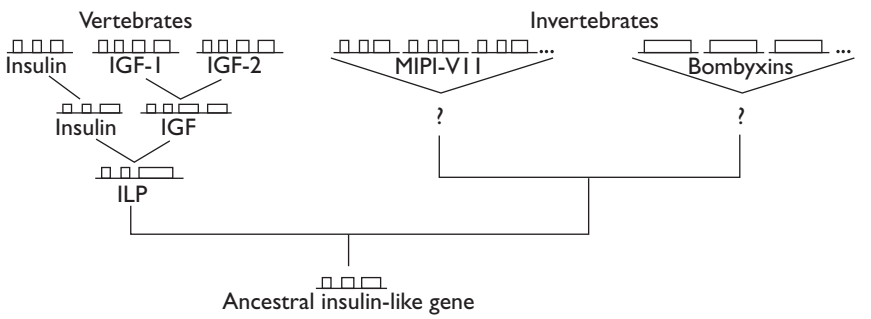

Figure 9.16 Phylogeny of vertebrate insulin, IGF-I, IGF-II and invertebrate insulin-like peptides. It is assumed that an insulin-like gene is the common ancestor of vertebrate insulin\IGF and invertebrate insulin-like peptides. Amphioxus ILP may be an extant representative of the ancestral gene which subsequently duplicated to form insulin and IGF found in early agnathan vertebrates. Further gene duplication resulted in distinct IGF-I and IGF-II genes found in all gnathostomes. Multiple independent gene duplication events have also occurred during the evolution of invertebrate insulin-like peptides from the original insulin-like gene. After Chan and Steiner (2000).

acids with our own. Like other aspects of their organization, the structure and functions of their hormones today presumably exhibit both "ancient" and "modern" components. One might reasonably suppose that the highly conserved region was the active site, and that random neutral substitutions took place in the amino acids in other positions.

If we assume (rather implausibly perhaps) that such neutral changes took place at a constant rate (i.e. that the molecular clock ran steadily), then the degree of similarity would indicate the time elapsed between the two hormones in different animals. Unfortunately, despite the evidence for a constant rate of neutral changes for certain proteins, such as albumin, it has been observed that molecular clocks based on insulin sequences frequently tell the wrong time. A constant rate is not necessarily correct for hormonal peptides that are under stringent functional constraint. It is not always easy to reconcile phylogenetic trees for hormones based on sequence comparisons with those based on the properties of the hormones. Figure 9.17 shows such a comparison for the calcium-regulating hormone calcitonin. Note that an ancient gene duplication is held to have occurred at the base of the fishes, and that a more recent duplication took place in teleosts. There are two calcitonin-like molecules in birds, supporting the idea of a primitive duplication, but the difficulty is that the artiodactyls seem to have been separated for a remarkably long time from the rest of the vertebrates, and a more recent rapid evolution of the artiodactyl calcitonin molecule seems more probable. We still need more sequence and pharmacological data before a less-speculative tree can be produced.

Despite doubts that may be felt about the constant rate of neutral sequence changes, nevertheless, the striking differences between elasmobranchiomorph

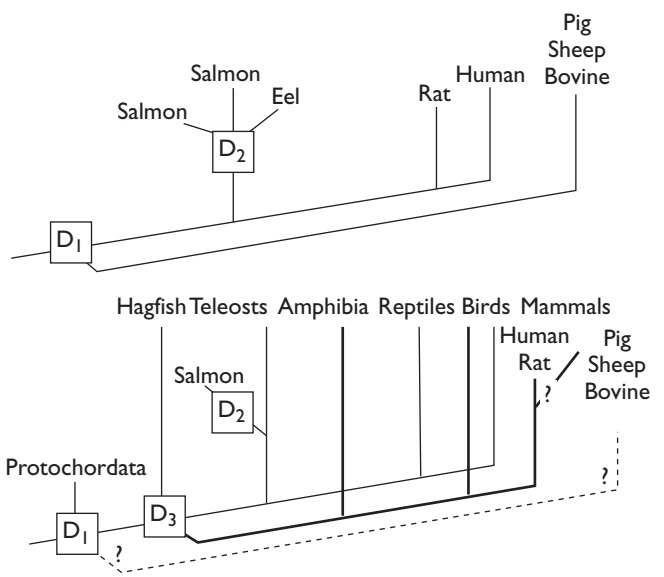

Figure 9.17 Evolution of calcitonins according to "molecular" data (above) and to data on various properties of the calcitonins in different groups. Gene duplications are assumed to have taken place at D1–D3. Modified from Fontaine (1985).

and other fish hormones of the same families (for example the neuropituitary hormones of the arginine vasotocin family) clearly indicates a long separate history, even if we are unsure when the divergences took place.

Changes in function

A striking feature of vertebrate peptide hormones, as we have already seen, is the existence of families of hormones sharing sequence homology to a considerable degree (e.g. Table 9.2), but which nevertheless may have quite different functions. It seems reasonable to suppose that the members of these families have arisen by gene duplication, followed by subsequent divergence resulting from substitutions and deletions. An example of a relatively recent duplication is given by work on the two forms of GH in the chum salmon (*Oncorhynchus keta*) where the two forms have conserved a core of invariant amino acid residues (presumably representing the active site) and differ little elsewhere. Interestingly, in the same fish, the growth hormone variants share with PRL a 24% homology along the 188 amino acid chain, but there is no identical conserved core. In other words, it looks as if a more ancient gene duplication took place, and that subsequent changes led to different active sites and the evolution of two hormones with different active sites, but still retaining nearly a quarter of the ancestral amino acid sequence.

Change in function need not necessarily, however, involve changes in the active sites. It may involve recruitment of new receptors and new target organs. For example, Cionin, of the cholecystokinin family, appears in ascidian tunicates (Sherwood *et al*., 2006), where it is involved in control of intestinal enzyme secretion (as well as being widespread in brain neurons). In hagfish, intestinal enzyme secretion is again its function, but in other fishes, it has recruited new target organs, and has become involved in control of stomach acid secretion as well as gall bladder secretion.

Like the hormones themselves, the receptors probably belong to families associated with the different families of related hormones, and like them also these related receptor types probably arose by gene duplication and subsequent modification to change their specificity and sensitivity. See Moncaut *et al*. (2005) for the variety of GnRH receptors and their possible origins. In teleosts and the South American lungfish *Lepidosiren*, AVT injection evokes blood pressure increase and diuresis, whereas in mammals the equivalent hormone is antidiuretic. This puzzling difference seems most easily explained by the existence of two related receptors, one set in the pre-glomerular circulation, and the other in the peripheral vasculature. In fishes the latter seems to be more sensitive or much more abundant, hence AVT produces a pressure diuresis. In mammals, by contrast, the receptors of the peripheral circulation are either less sensitive or they are much less abundant than the pre-glomerular receptors, hence the result of injection of the hormone is antidiuretic.

Envoi

In some ways, fish endocrinology is too large a subject to attempt in a single chapter: all we can do is give a very cursory account of why it is so interesting as well as practically important, and urge the reader to remember that fish have often led the way in vertebrate endocrinology.

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10 Sensory Systems, and Communication

The remarkable sensory systems of fishes show the most fascinating adaptations to different habitats and modes of life. What other animals have been able to live in every kind of water and in some cases in air also, use highly sophisticated electrical systems for examining their surroundings, and can follow the track of their prey swimming past even after it has long gone? Such specialized adaptations are naturally found in different fishes, but there is, however, one aspect of their sensory systems where all fish seem deficient compared to "higher" vertebrates.

10.1 Proprioception

Compared with terrestrial animals, fish are very poorly equipped with proprioceptors. 75% of nerve fibers supplying cat limb muscles are sensory fibers from the muscle spindles and Golgi tendon organs, monitoring the length and tension of muscles, and are hence very important in controlling posture. These sense organs seem to be absent in fishes, and proprioceptors associated with locomotor muscles are known only in *Myxine* and rays. Although ray proprioceptors (in parallel with the slow locomotor muscle fibers of the pectoral and pelvic fins) are much simpler than spindles, their responses to ramp stretches are very similar to those of spindles (Ridge, 1977), and seem able to provide the same kind of information for locomotor control as do muscle spindles. There is a gradation in batoids between those that swim by flapping such as the manta *Rhinoptera* (and rhinobatoids which flap to assist myotomal locomotion), and those that send undulatory waves across the pectorals, like dasyatid stingrays. As we might perhaps expect, it seems that proprioceptors are most abundant in the latter, in particular in the small muscle fiber bundles that link the tips of the fin cartilages. In sharks, there are no proprioceptors among the muscles of the fins, but coiled corpuscular pressure receptors (a little like mammalian Pacinian corpuscles) under the skin act as proprioceptors. Probably the rarity of proprioceptors, except in special cases such as batoid wings, the extraordinarily flexible barbels of goatfish, and the anterior finrays of gurnards (*Trigla*), is related to the relatively insignificant role of gravity for aquatic organisms of similar density to water, and to the damping of movements by the medium.

10.2 The Acustico-lateralis System

The acustico-lateralis system enables fish to respond to three kinds of stimuli: water-borne vibrations, gravity, and angular accelerations. To do so, they are equipped with receptor hair cells that have a very particular structure in all vertebrates. A single kinocilium lies on one side of the top of the cell (providing a convenient marker for the scanning microscopist), next to the cuticular plate which bears a graded series of elongate stereocilia or stereovilli linked at their tips (Figure 10.1). At the bases of the hair cells in the lateral line there are both afferent and efferent synapses, the latter perhaps acting to dampen responses resulting from the movements of the fish itself. In the ear itself, however, as in the mammalian cochlea, there are two types of hair cells, one of which does not receive basal efferent innervation. In the lateral line, in free neuromasts, and in the sensory maculae of the semi-circular otic canals, the apices of the hair cells project into gelatinous cupulae, while in the lower part of the ear, they are overlain by the membranes of the three otoliths (Figure 10.2).

Mechanical stimuli deflect the stack of stereovilli, and this opens tension-gated channels near their tips, which permit mostly K^+ and some Ca^{2+} to enter the stereovilli where aligned actin filament bundles and several types of myosin motors can be activated. Fascinating details of the critical oscillatory operation of hair cells, and how they are "tuned" for remarkable sensitivity (threshold displacement being only 1–2 nm) have been worked out in

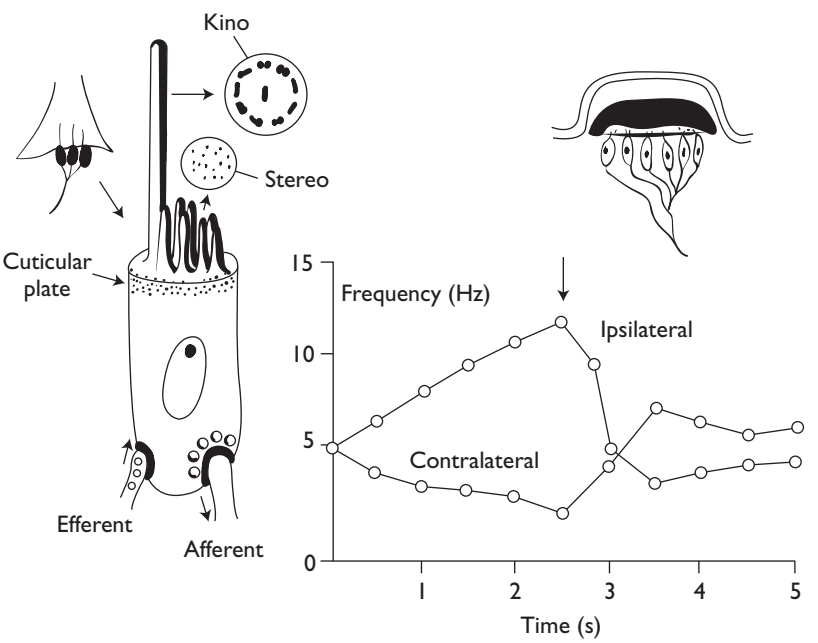


Figure 10.1 Top left: single external neuromast; top right: otolith organ. The tips of the sensory cells lie in gelatinous (cupola-like) material in otolith sulcus. The hair cell has both afferent and efferent synapses at its base. On the right: responses of horizontal semi-circular canal maculae in *Raja* showing changes in impulse frequency during rotation (stopping at arrow). After Lowenstein and Sand (1940).

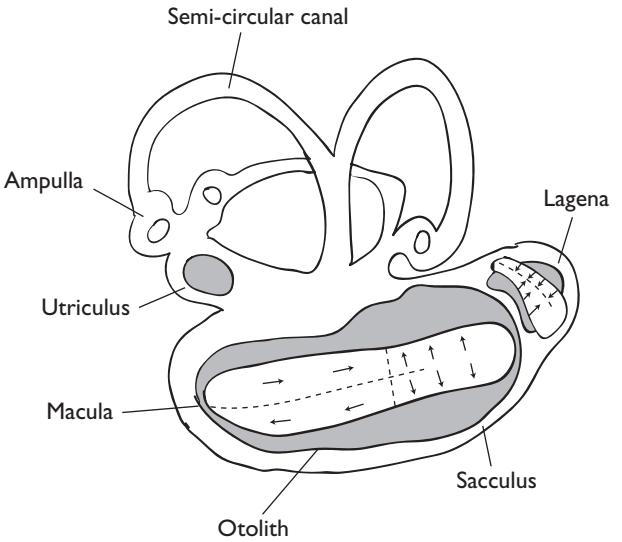


Figure 10.2 Diagram of the labyrinth of a typical teleost. The stippled areas show the extent of the otoliths overlying the sensory maculae. The polarity of the "fields" of hair cells is shown by arrows in the sacculus and lagena.

mammals and in bullfrogs (where hair cells are very large) but there is no reason to suppose that they are not similar in fish. Note that the kinocilium is *not* involved, unlike the cilia of other mechanoreceptors, and its role is unclear. It contains several kinds of myosin proteins and has been suggested to be motile in some cases.

In the ray ear (enclosed in cartilage rather than in bone, and so easier to dissect) the hair cells of the horizontal canal have their kinocilia oriented towards the utriculus, while in the anterior vertical canal, the hair cells have the kinocilia oriented away from the utriculus. This especially simple dichotomy allowed Lowenstein and Sand (1940), interrupted by war, and then Lowenstein and Wersäll (1959) to record from these two hair cell groups during rotation of the labyrinth on a special turntable (driven, in the old traditions of UK build-it-yourself physiology, by two ex-RAF 24 v motors) and thus to show that the resting discharges of these hair cells were inhibited when the stereovilli bent towards the kinocilium, and excited when bent away from it (Figure 10.1).

Burighel and his colleagues have shown recently that among the variety of ciliated sensory cells in ascidians (including some with cupulae), there are some hair cells with afferent and efferent innervation, and graded stereovilli just like those of vertebrates. Probably the system arose to detect low frequency vibrations at short range, only later in chordate phylogeny did its other functions appear. There are also rather similar hair cells in the ears of squid, so that it seems that a simple single-cilium primary sensory cell may have been transformed into a hair cell type (with stereovilli) on several occasions. So far, none of these interesting cases have been examined with recent molecular methods to detect possible evolutionary changes from sensory cells without stereocilia to those where the kinocilium is apparently inactive.

To understand how the hair cells respond to mechanical stimuli we have to understand the two kinds of stimuli produced by sound sources. Water is very incompressible (14 000 times less than air at 1 atmosphere) and sound travels at 1500 m s^{-1} compared with air at 300 m s^{-1}. A sound source produces two types of stimulus – a back-and-forth motion of the particles in the medium (particle displacement), and a sinusoidal change in pressure (sound pressure). Their amplitudes drop at different rates depending on the distance from the source. Particle displacement is more important near the source and sound pressure at a distance from the source. The region close to the source that is important for particle displacement is called the "near-field" (its radius from the source being the wavelength of the sound divided by six), and the more distant region where sound pressure is important is the "far-field." The near-field is thus more important at low frequencies; for example at 100 Hz, the wavelength λ is 15 m and the near-field 2.5 m; at 1000 Hz, λ is 1.5 m and the near-field only 0.25 m. None the less, even at high frequencies, the near-field may be important, especially so to shoaling fish such as herring, whose swimming movements generate high frequency pulses (Denton and Gray, 1993).

Obviously, to increase sensitivity, especially at high frequencies at some distance from a sound source in the far-field, a response to sound pressure is required. A variety of fish have evolved mechanisms that couple pressure-sensitive structures (which are always compressible gas-filled spaces such as swimbladders, or otic bullae) to displacement detectors (the hair cells) by pressure-movement transduction. These most ingenious devices will be considered on p. 297.

The lateral line

The lateral line receptors are either in subepidermal canals on the head and in a canal extending along the body (Figure 10.3), or in superficial neuromasts on the skin. The morphology and phylogeny of teleost canals are discussed by Webb (1989) who concludes that a branched tubule system linked to the head canals is primitive. The superficial neuromasts are hair cells in small groups lying on the scales and around the head, which bear gelatinous cupulae projecting freely into the water. In some fish they are extraordinarily abundant, in the goldfish (*Carassius auratus*) there may be 1000 around the head and six–nine on *each* scale! In the canals, the hair cells lie in small cushions spaced along the canal, their apices projecting into cupulae which deflect as seawater is displaced within the canal. Adjacent hair cells are often of opposite polarity, and so the fish can detect movements of opposite directions along the same axis. The canals are closed apart from small pores along their length, presumably this arrangement shields the hair cells from unwanted "noise" generated by currents and by the movements of the fish itself. Only in holocephali are the canals open all along their length.

The canals around the head give the fish a picture of the source(s) producing vibrations in the near-field enabling such fish as the piper (*Hyporhamphus ihi*) to detect the limb movements of the small crustaceans it feeds on, and for such surface feeding fish as the top minnow (*Aplocheilus*) to detect insects struggling on the water surface. Along the body, the arrangement of the lateral line in most fish strongly suggests that the receptors are laid out to provide a long baseline, and in several quite different sorts of fish (for instance

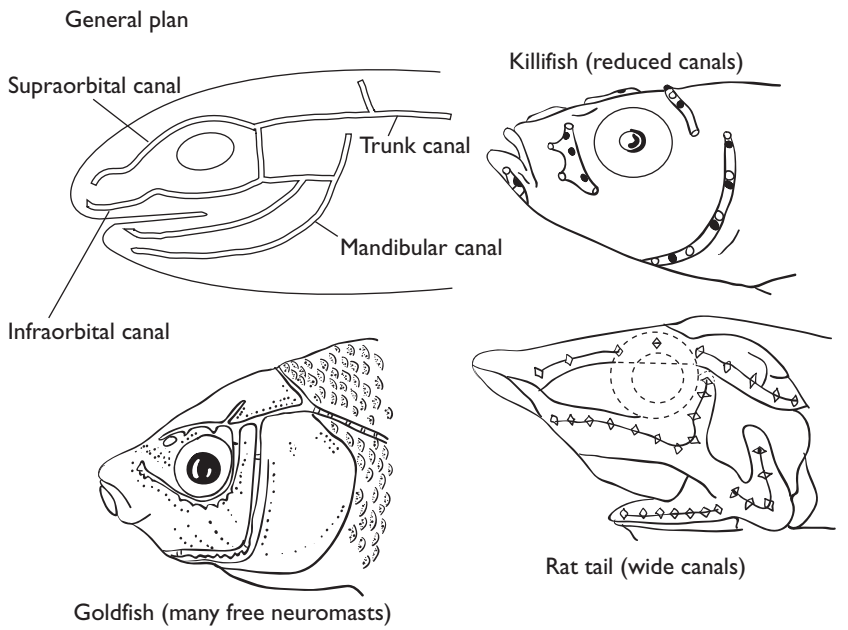


Figure 10.3 Top left: the base plan of the lateral line canals of a teleost showing modifications in different groups. The goldfish retains the basic plan with large numbers of free neuromasts over the head and body. The killifish has its canals and number of neuromast organs much reduced. The rat tail (Macrourid), a fish of deep water, has wide and deep canals covering much of the head. After Blaxter (1987); Puzdrowski (1989).

Holocephali and macrourids) there is a long thread-like tip to the tail which much extends this baseline. A similar elongation is found in many weakly electric fishes (p. 303) presumably for the same long electroreceptor baseline. It seems most probable that by integrating lateral line receptor responses along the baseline, the fish can locate the source of the vibrations received. The prey fish and squid will cause water movements, so enabling the fish to home in on the prey. Experiments with the freshwater sculpin (*Cottus bairdii*) by Coombs and her colleagues (Coombs, 1999; Coombs *et al*., 2000) indicate that both distance and direction of moving prey (or a small vibrating ball) can be determined by the fish using its lateral line alone (Figure 10.4). Not all fish have extensive lateral line systems. Figure 10.5 shows variations in its extent within different catfish genera.

Interestingly, importantly, and somewhat astonishingly, recent studies (Pohlmann *et al*., 2001) have shown that piscivorous catfish can follow prey wakes even some 10s of seconds later using their lateral lines, and particle velocimetry has traced the decay of water movements in wakes caused by the passage of other fish (Hanke and Bleckmann, 2004). Calculations by Pumphrey (1950) suggest that *Aphanopus carbo* (a 1 m black elongate midwater trichiurid, Figure 3.21 p. 87) could locate the fish it preys upon at a distance of 32 m using the particle displacement of the lateral line receptors. We have already seen that the hair cells respond to deflections of the stereovilli of

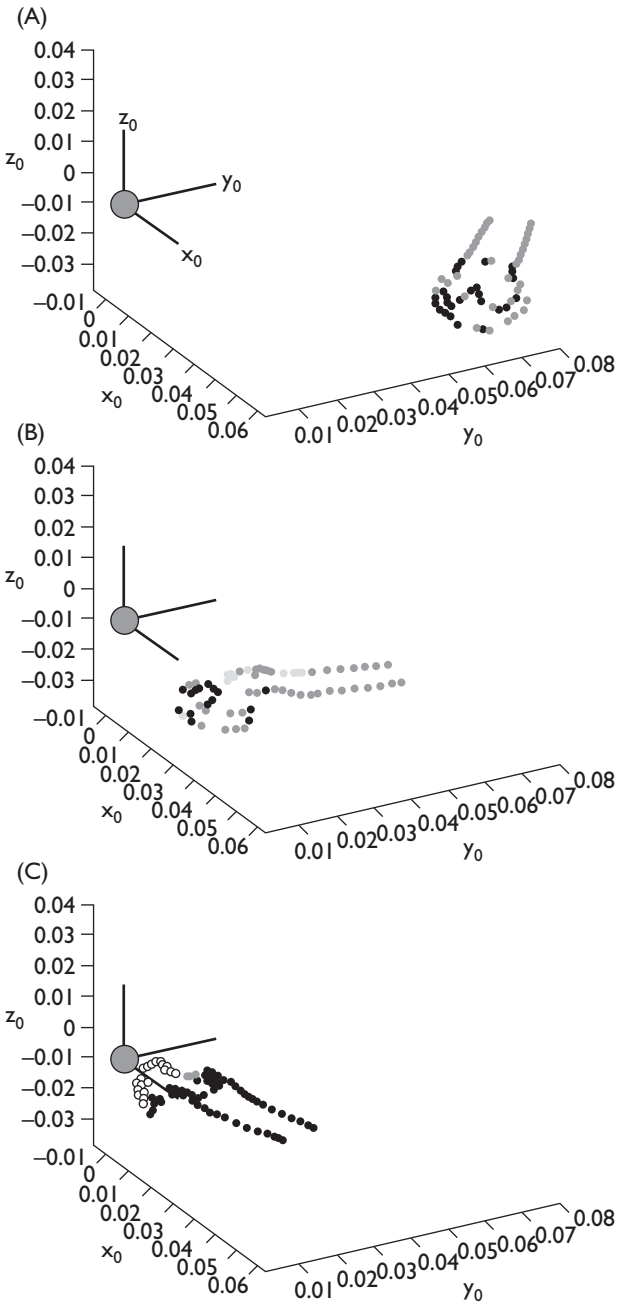


Figure 10.4 Modeled three-dimensional maps of changes in lateral line excitation on head and body of a mottled sculpin (*Cottus bairdii*) attacking a vibrating source in its tank. A–B: source begins; sculpin orients to strike, C: strikes at source. Each shaded dot represents excitatory input from time of signal onset A: to the time the sculpin strikes C: ○ & ● show maximum pressure differences along line of canal whilst midtone ◎ represents near-zero difference in opposite directions. After Coombs *et al.* (2000).

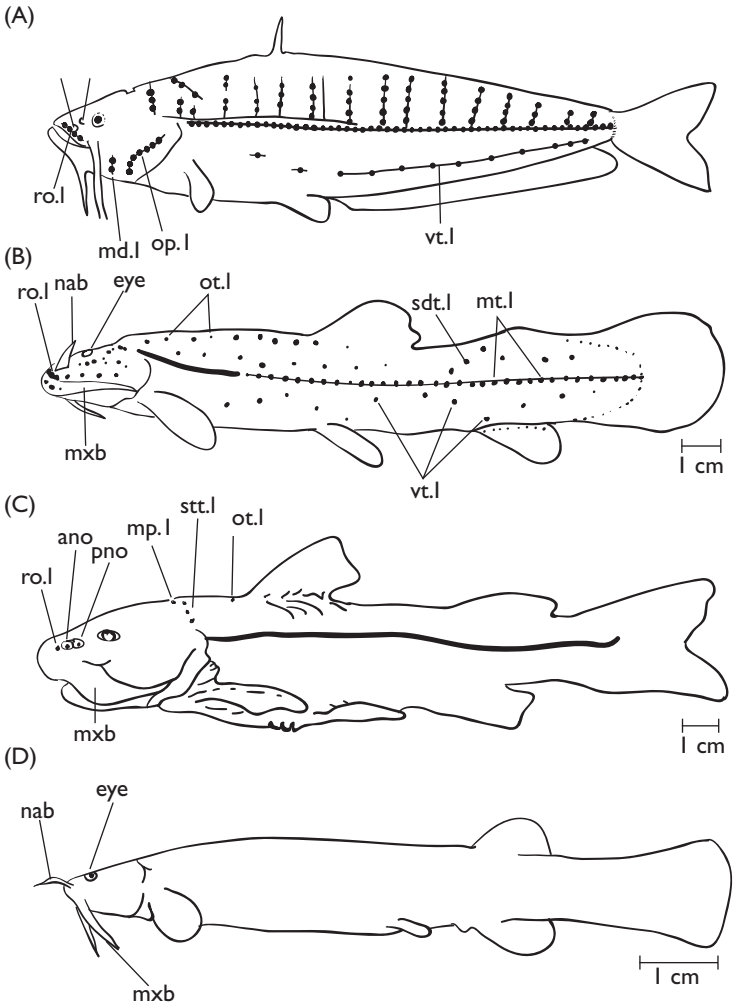


Figure 10.5 Lateral lines and lines of superficial neuromasts in various catfish. A: *Parasilurus asotus*; B: *Nematogenys inermis;* C: *Diplomystes chilensis;* D: *Silvinichthys mendosensis*. After Arratia (2005). Key: mxb, nab: maxillary and nasal barbels; ro: rostral; op: opercular; vtl: ventral; mtl: median.

only 1–2 nm, and with this kind of sensitivity any particle displacements resulting from prey movements would be lost in the noise produced by the movements of *Aphanopus* itself. The fish avoids this problem by keeping the body rigid, sculling itself along slowly using the minute forked tail (there is a flexible joint in the vertebral column just at the base of the tail). After stalking its prey in this way, to within range of the large eyes, *Aphanopus* erects its dorsal fin and swims rapidly by body oscillations. Other trichiurids, such as the silvery *Trichiurus*, have no caudal fin and the large dorsal fin is enlarged, presumably the fish swims slowly by undulating this fin. In this case, the lateral line passes along the body in an unusual ventral position, possibly to keep it as far as possible from the "noise" generated by the fin. This second method

of propelling an elongate body supporting the lateralis baseline parallels that of the freshwater weakly electric gymnotid knifefishes, where the detectors are different but the requirements the same. Trichiurids are obviously highly specialized for detection of near-field particle displacements and the acustico-lateralis lobes of the brain are enormous, but, in more normal fish, detection range is probably around 1 m, and the lateral lines are used to locate sources vibrating at frequencies up to 200 Hz or so.

Free neuromasts are very abundant on the surface of some fish, especially on and around the head, and detect the velocity difference between the fish and the surrounding water (Engelmann *et al.*, 2002). It seems that superficial neuromasts in running water can no longer locate the responses of a vibrating probe, which can still be detected by the sheltered neuromasts of the lateral line (See Engelmann and Bleckmann, 2004).

The inner ear

In fishes, the inner ear (or membranous labyrinth) lies close to the brain on either side of the head. There are, at the top, orthogonally arranged semicircular canals and a lower part comprising the utriculus, sacculus, and lagena (Figure10.2). The swellings, or ampullae, of the semicircular canals contain a sensory crista equipped with a cupula, the cristae responding to angular accelerations as the fish turns. The three parts of the lower labyrinth each contain a sensory macula covered by an otolith membrane (Figure 10.2). In teleosts this membrane invests a fairly massive single calcification, the otolith, but in elasmobranchs the calcifications consist of more diffuse otoconia and in the bottom-living *Rhina*, even of sand grains (these enter via the endolymphatic duct). The maculae respond to sound, gravity, and to linear accelerations of the fish's body. The sacculus is enlarged in most species except for clupeoids which have an enlarged utriculus. In the holostean bowfin (*Amia calva*) and some cypriniform species, the lagena is enlarged.

10.3 Sound Reception

If the head of a fish vibrates in a sound field, the calcareous otoliths overlying the maculae (Figure 10.2) make smaller movements than the surrounding tissues since their density is higher. This causes the hairs of the hair cells to bend, so firing the hair cells if their polarity is appropriate to the direction of the vibration. The hairs act as a spring to return the otolith to its resting position but it will vibrate at the same frequency as that of the sound stimulus.

In the cristae of the semicircular canals, the hair cells all have their polarity along the same axis so that excitation occurs with fluid motion in one direction and inhibition in the opposite direction. The maculae, however, are divided into "fields" of hair cells (Figure 10.2). In each field, hair cells polarized in one direction are accompanied by adjacent hair cells polarized in the opposite direction. There is some evidence that the cells are broadly tuned to particular bands of frequencies. In those fishes without accessory hearing structures, such as the swimbladder or otic bullae, the inner ear perceives only the particle displacement aspect of the sound stimulus. Fish such as cod can perceive the direction of the stimulus as well as appreciate its intensity and frequency. In fact, the cod and many other fishes have extensions of the

swimbladder close to the back of the skull so giving some opportunity for pulsations of the swimbladder (in response to sound pressure) to stimulate the inner ear via the bones of the skull. What the swimbladder and other gas-filled structures do is to enhance the particle displacement aspect of the sound stimulus by transducing sound pressure to particle displacement.

Pressure to displacement transduction should thus improve both sensitivity and the range of frequency that is perceptible. In the Ostariophysi (or Otophysi) a chain of ossicles links the sacculus to the swimbladder. These ossicles (derived from vertebrae) were described and their function understood by Weber as long ago as 1820, they are still called the Weberian apparatus (Figure 10.6), although some of his names have been changed. As the swimbladder pulsates in a sound pressure field, displacements of the swimbladder wall rock the tripus, and this movement is transferred to the claustrum via the intercalarium and scaphium. The claustrum is coupled to a sinus containing perilymph adjacent to the saccular maculae. The analogy with the ossicles of the mammalian ear is obvious.

Testifying to the advantages of sensitive hearing, a surprising number of non-ostariophysan fishes has simpler links between the swimbladder or another gas-filled space with the endolymph of the inner ear via a thin-walled part of the saccular or lagenar wall. In mormyrids, anterior branches from the swimbladder become cut off as isolated sacs, each with their own rete mirabile to fill it; sound is used at long distances, electrical communication at shorter (see Yan and Curtsinger, 2000). In anabantids an accessory respiratory chamber above the gills (p. 140) is linked to the inner ear by a thin-walled window, whilst anterior projections of the swimbladder contact the inner ear in the holocentrid soldier fish (*Myripristis*), as they do in notopterids and sciaenids (Figure 10.7). In clupeoids such as anchovy (*Engraulis mordax*), herring (*Clupea harengus*), and sprat (*Sprattus sprattus*) there is a most interesting

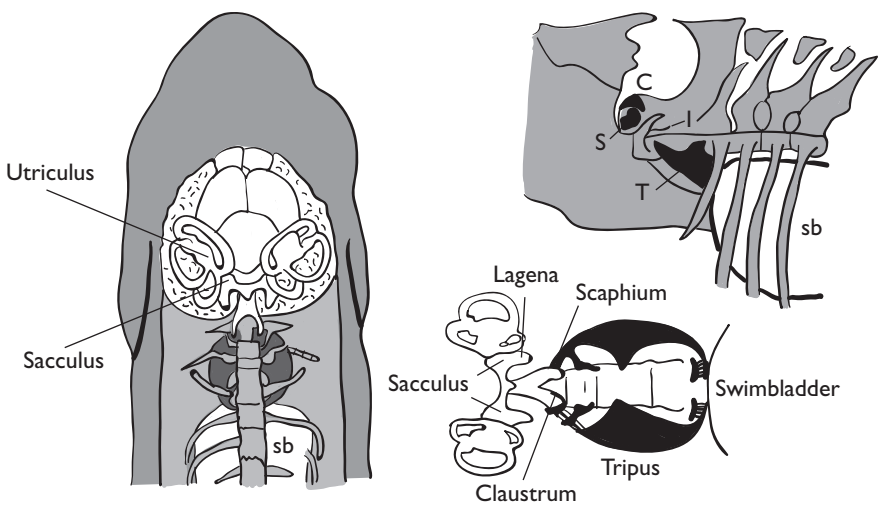
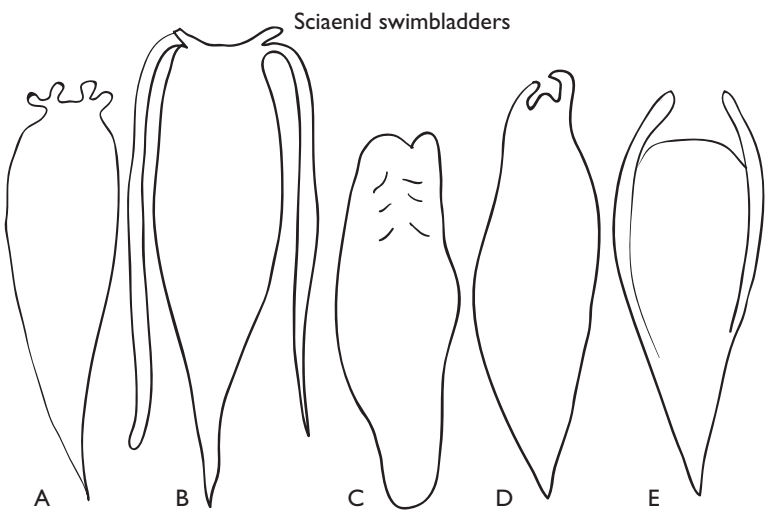


Figure 10.6 Ostariophysan ear ossicles. Left: dorsal view of goldfish. Right: detailed view of ossicles. S, scaphium; C, claustrum; T, tripus; I, intercalarium; sb, swimbladder. After von Frisch (1936).

system, carefully analyzed by Denton and Gray (1979) and their colleagues. Partially gas-filled otic bullae on either side of the head act as pressure-displacement transducers very close to the utriculus of the inner ear (Figure 10.8). The bullae contain gas in the lower part separated from perilymph in the upper part by an elastic bulla membrane. The gas-filled part is also connected to the swimbladder by extremely fine gas ducts about 8 μm in diameter. As sound waves pass, the gas in the bulla changes in volume in sympathy with the changes in sound pressure. The bulla membrane vibrates at the same frequency, forcing perilymph in and out of a fenestra or orifice in the upper wall of the bulla. The fenestra is adjacent to the utriculus and the shear of perilymph over the external surface of the utriculus stimulates the macula. (In the sprat there is even a very fine elastic ligament joining the bulla membrane to the utricular wall which may help in sensing changes of depth – hydrostatic pressure.) Nearby there is another membrane, the lateral recess membrane in the lateral wall of the skull, that also moves in sympathy with the bulla membrane. The lateral recess membrane is at the back of the sac on the external surface of the head from which all the lateral line canals radiate (Figure 10.8). Thus, changes of sound pressure stimulate not only the displacement-sensitive maculae in the utriculus but also the neuromast organs of the head lateral line, a condition unique to the clupeoid fishes.

There is a further ingenious arrangement in the clupeoids that makes this elaborate acoustico-lateralis system independent of hydrostatic pressure. The fine gas ducts connecting the bullae to the swimbladder allow the swimbladder gas to act as a source or sink of gas as the fish move up and down in the



A *Petilipinnis grunniens*
B *Pachypops foureroi*
C *Pachyurus francisci*
D *Pachyurus schomburgkii*
E *Plagroscion squamosissimus*

Figure 10.7 Sciaenid swimbladders, showing links with acoustico-lateralis system. From Casatti (2002).

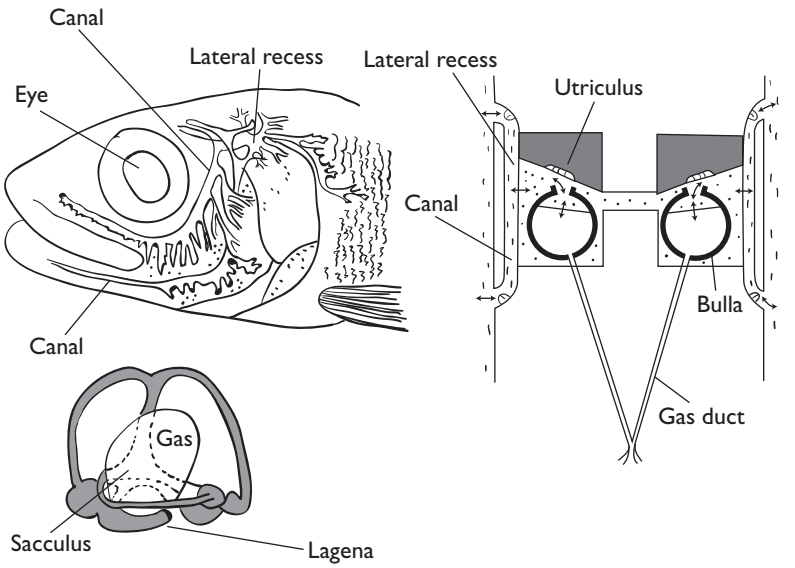


Figure 10.8 Gas-filled bullae and inner ears in mormyrids and clupeids. Bottom left: mormyrid ear showing gas bulla applied to the sacculus (the bulla has a rete supplying it with gas, not shown). Right: schematic diagram of gas-filled bullae (in dorsal view) of a clupeoid; clear areas: gas, dotted: perilymph, dark shading: endolymph, short lines: seawater in lateral line canals; top left: side view of a herring head showing the complex lateral recess. After Blaxter *et al.* (1983); Denton and Gray (1979); Stipetic (1939).

water. When the fish move down, gas is pulled forward to the bulla and when the fish move up, gas is pushed back into the swimbladder. The time constant for this is the order of 30 s which prevents changes of the higher frequency sound pressure affecting the mechanism. Without this connection to the swimbladder the bulla membrane might burst during a big change of depth (remember that hydrostatic pressure increases by 1 atm every 10 m depth increase); also the bulla membrane tends to remain in its flat resting condition where it is more responsive to sound pressure. The clupeoids make very extensive vertical migrations (see p. 114) so that the compensation mechanism described above is essential.

The auditory performance of fishes is usually shown as an audiogram (Figure 10.9). Note that the non-ostariophysans have narrower frequency responses and lower sensitivity than ostariophysans, with the herring somewhere in between (but with an especially good low frequency sensitivity). Clupeoids are regarded as hearing specialists, capable of hearing sounds up to several kHz, as can goldfish (*Carassius auratus*). At least some clupeoids such as the American shad (*Alosa sapidissima*) can detect ultrasound (Figure 10.10), and there is evidence that herring can hear the acoustic pingers (10 kHz) attached to nets to deter dolphins. Recording from brain neurons in *A. sapidissima* has shown that ultrasound is not only detected but also processed differently to sound of lower frequency (Plachta *et al.*, 2003), perhaps to localize the source better. Most hearing generalist fishes such as salmonids or *Osteoglossum* do not detect sounds above 1 kHz.

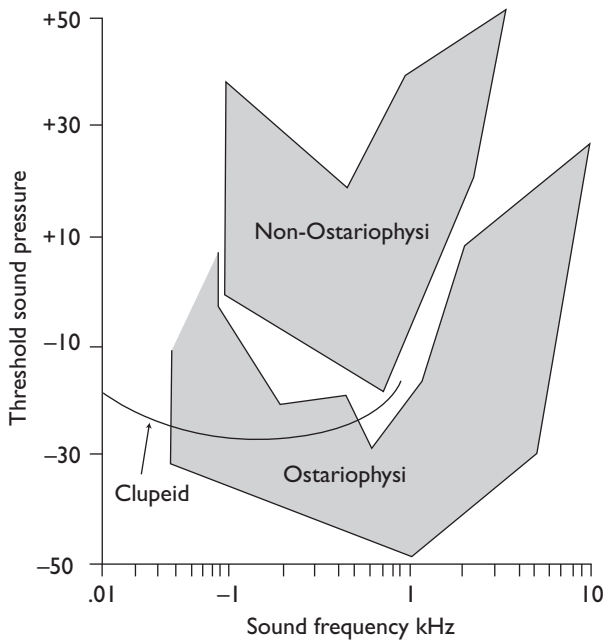


Figure 10.9A Audiograms of otophysan (ostariophysine) and non-otophysan fish compared with a clupeoid. Note that some clupeoids can detect well above 1 kHz (see text).

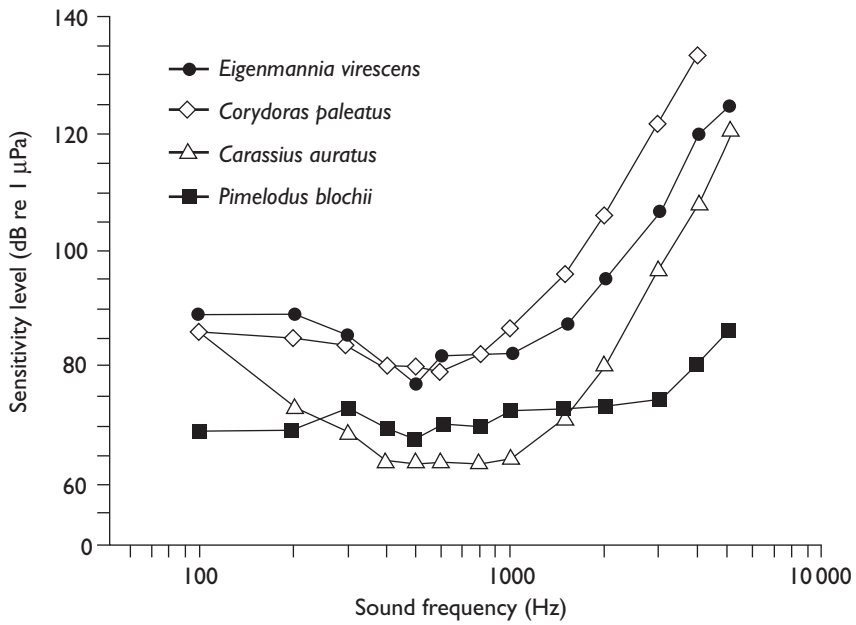


Figure 10.9B Sensitivity vs frequency of several otophysans and non-otophysans. *Eigenmannia* is a weakly electric fish. Frequency (in Hz) log scale. From Ladich (2000).

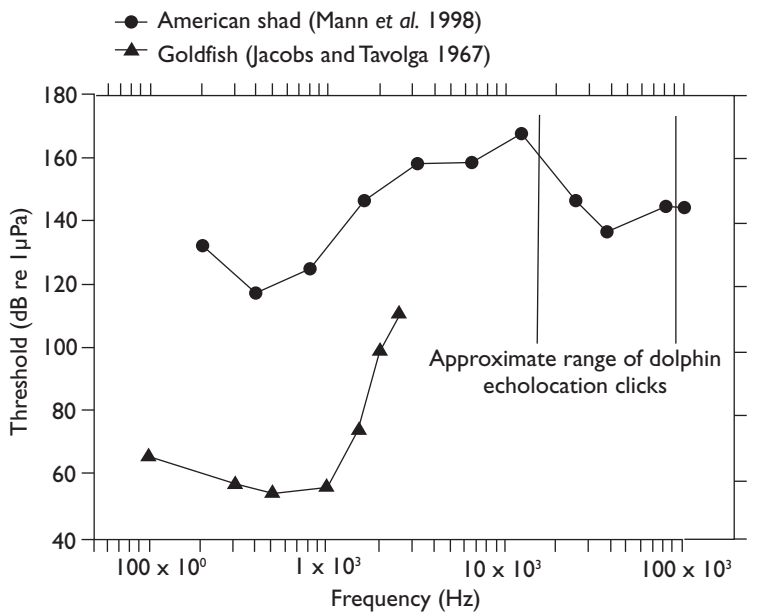


Figure 10.10 Audiograms for the American shad (*Alosa sapidissima*) and the goldfish (*C. auratus*). These are the lowest sound pressure at each frequency the fish detected. After Mann *et al.* (2001).

10.4 Sound Production

Fishes in 50 different families produce sounds in various ways, by rasping spines and fin rays, or by burping, farting, or gulping air. Some of these mechanisms may involve the swimbladder acting as a resonator, while, in some, special drumming muscles vibrate the swimbladder wall so that it acts as an internal loudspeaker. Ladich and Bass (1998) have listed some of the sound-producing mechanisms in several teleost families; some catfish even have two different mechanisms. Cottids (sculpins) lack a swimbladder, but nevertheless produce a variety of sounds.

Bass and McKibben (2003) have surveyed the neural mechanisms and behaviors of teleost sound communication. Many teleosts communicate vocally, and most sounds are involved in social behavior within a species and less commonly in communication between species. Perhaps the most-studied fish has been the plainfin midshipman (*Porichthys notatus*), which produces grunts and moans and (what are mysteriously known to non-residents of the US as) boat whistles. But more recently mormyrids have come to the fore in studies of sound in social communication. We have seen that some mormyrid species are hearing specialists with swimbladder extensions linked to the inner ear (Figure 10.8), and it is indeed remarkable that as well as having to take in and analyze the results of their complex electrical communication systems, they can also concentrate (as one might say) on receiving and making a variety of calls! A female *Pollimyrus isidori* emits a series of electric organ discharges (EODs) as she enters a male's territory, evoking from him an acoustic

display of moans and grunts. If she leaves his territory, he growls (Figure 10.11). The situation for the fish is simplified since it is electrically silent except during growls when normal frequency is reduced. Gurnards (Triglidae) grunt when disturbed by a predator but also display visually, perhaps helping them to escape predation. Sounds are relatively common during courtship, as for example in courting haddocks (*Melanogrammus aeglefinus*) p. 243. In the batrachoid toadfish (*Opsanus tau*) the males occupy areas of the sea bed and make boat whistle calls apparently as a means of delineating their territory, or attracting mates, since the rate of calling often increases when a female approaches.

10.5 Electroreceptors, and Electric Organs

The powerful electric organs of the electric catfish (*Malapterurus*), and electric ray *Torpedo* were known to the ancients, and while curious receptors of unknown function had long been described in elasmobranchs and some teleosts, it was not until the early 1950s that Lissmann (1951, 1958) showed that the African teleost *Gymnarchus niloticus* emitted weak electrical signals. A live *Gymnarchus* was a wedding present to him, and in the Cambridge Zoology Laboratory was found to be continuously emitting pulses at 250–300 Hz, much to the astonishment of his friends from Physics (see Moller, 1995).

This was a most striking discovery. Not only was this a "new" sensory modality, easily monitored directly by electrical measuring instruments, but, as it soon turned out, electroreceptors were widely distributed in most groups of fish, and in aquatic amphibia. Rather less sensitive electroreceptors were more recently even found in the platypus (*Ornithorhynchus*). Probably the two most fascinating aspects of electroreception and electric signal production have been the extraordinary sensitivity of some electroreceptors, and the

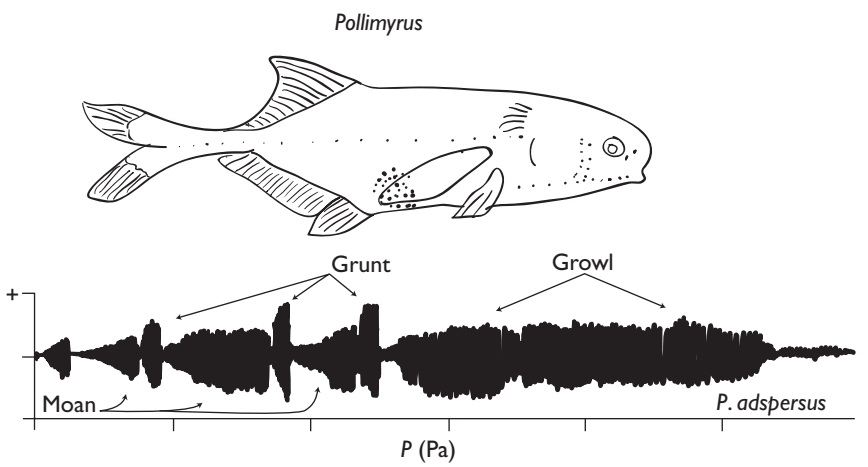


Figure 10.11 The weakly electric fish *Pollimyrus* with a sonogram of the moans, grunts and growls it makes. After Crawford and Huang (1999).

gradual unraveling of the ways in which different electric fish survey the waters around them with weak electrical signals.

After Lissman's work, electroreceptors were subsequently also found in lampreys, all elasmobranchiomorph fishes, in a variety of teleosts, in sturgeons, *Polyodon*, and in Dipnoi; they probably also occur in *Latimeria*. These respond to low frequency signals (~10 Hz or less) and to d.c. fields. Electroreceptors of a different kind, responding to high frequencies (and insensitive to d.c. fields) are found in several families of freshwater teleosts – Gymnarchidae, Gymnotidae and Mormyridae – which also have electric organs.

Ampullary (tonic) receptors

First found in sharks and rays in C17 by Lorenzini, whose name they bear, the function of these receptors remained unknown for two centuries, until Kalmijn (1971) showed that they were electroreceptors used to detect prey (Figure 10.12). In rays and sharks, they are groups of jelly-filled tubes each leading to an ampulla in whose wall a group of sensory cells is embedded (Figure 10.13).

Although these are modified neuromasts, the sensory cells have no efferent innervation (p. 306). The apex of each cell bears microvilli and/or a single kinocilium protruding into the lumen of the ampulla, which is full of low-resistance jelly similar in ionic composition to seawater. Since the canal wall has a high resistance (30–100 times that of the nerve myelin sheath) the canals act as ideal "submarine cables" with zero leakage conductance and inductance, ending in open circuit at the ampullary end of the canal (Waltman, 1966). The ampullae are collected together to lie in capsules (Figure 10.14), so that the cell bodies of the sensory cells are as near isopotential as possible yet their apices are linked to canals which may open far from each other on the skin surface. In this way, the fish can compare accurately the differences in

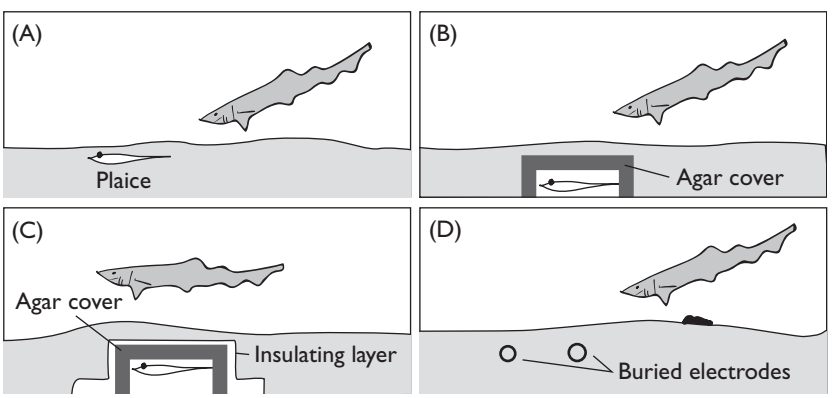


Figure 10.12 Hunting by electroreception. A: The dogfish can detect the buried plaice (*Pleuronectes*) by the d.c. field it generates. B: Even when sheltered under an agar cover, because agar is "transparent" to current, the plaice is detected. C: When the agar is covered by an insulating plastic layer, the plaice is not detected. D: Buried electrodes producing a field like that of the plaice are attacked. From Kalmijn (1971).

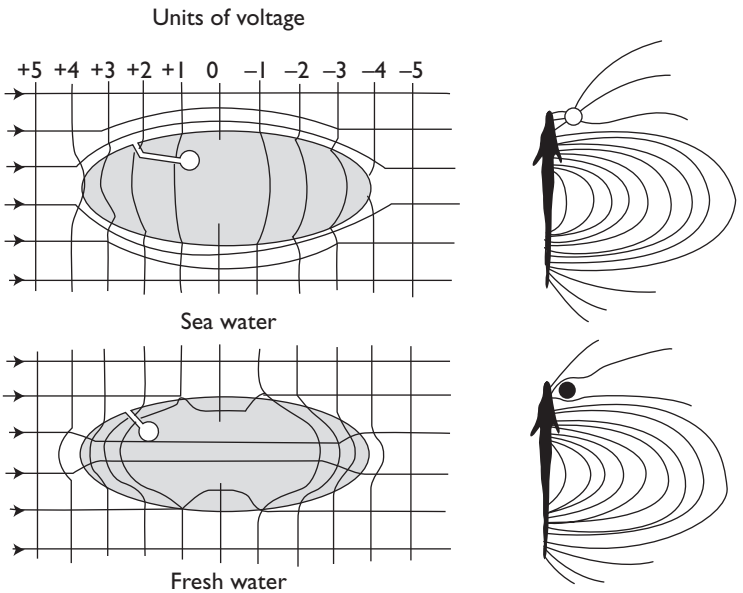


Figure 10.13 Passive and active electroreception. Left: fields around marine and freshwater fish with ampullary organs. The freshwater fish has high skin resistivity and requires only short canals, whereas the marine fish has lower skin resistivity and needs only short ampullary canals. Right: current lines of the fields generated on one side of a freshwater electro-locating fish. In the upper diagram the field is distorted by an object of high conductivity (such as living prey), below by an object of low conductivity (such as a rock). After Bullock (1973); Lissmann and Machin (1958).

potential at different sites around the body. The ampullae of Lorenzini in sharks and rays are tonic receptors giving a long-lasting response to very low frequency (8–10 Hz) or d.c. stimuli. Their sensitivity is remarkable, rays responding to voltage gradients of 0.01 $\mu V\ cm^{-1}$, corresponding to 1 $mV\ km^{-1}$! Any reader who has struggled to record such gradients with high quality electronic equipment will appreciate this performance. Sensitivity is quite enough to permit stingrays to orient statically in small tanks to the earth's field, and indeed, for sharks and rays to detect the fields that arise as they swim through the magnetic field of the earth and so to navigate across the ocean.

Similar ampullary organs connected to the surface of the skin are found in many freshwater fish, although here, the canals are much shorter. In the sea, body fluids have a higher resistivity than seawater, for they are more dilute (p. 162) and the skin is only a moderate insulator. In freshwater, the converse is the case, for the skin has a high resistance, retaining ions from osmotic loss to the very dilute environment, and the body tissues and fluids are much more concentrated than the surrounding water. The consequences are that in a uniform electric field in freshwater little current will enter the fish body (which is at isopotential), and the voltage will develop across the skin, hence the receptor cells need not have a long canal to the outside. In contrast, the marine fish with lower skin resistance needs a long canal to obtain a detectable voltage gradient between the canal opening and the ampulla (Figure 10.13).

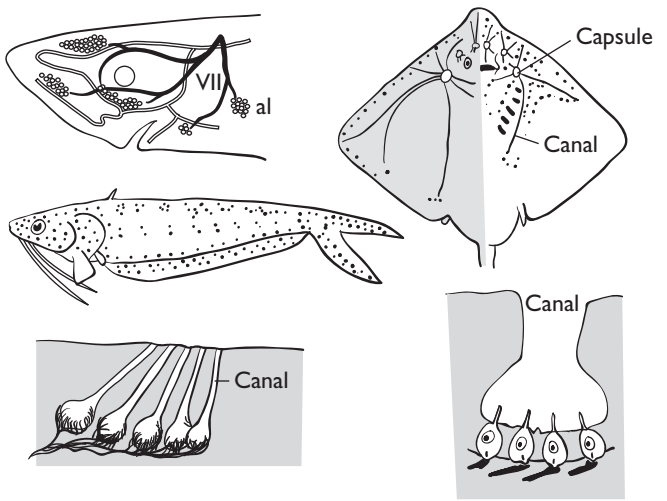


Figure 10.14 Ampullary electroreceptors. Upper left: groups of ampullae (al) on head of a shark. Mid-left: distribution of ampullary organs on the catfish (*Kryptopterus*). Bottom left: groups of ampullary organs from shark snout. Upper right: upper (L) and underside (R) of ray showing openings of ampullary organs, and capsules into which canals collect. Only a few canals are indicated. Bottom right: ampullary organ from sturgeon snout, the sensory cells are ciliated. After Murray (1967); Jorgensen (1980); Bennett (1971).

Freshwater elasmobranchs (such as the stingrays that live in the Amazon basin, (p. 181) have (as the reader will have guessed) short canals to their ampullae of Lorenzini.

In gymnotids and mormyrids, and in the siluriform catfish *Ictalurus* (*Amieurus*), and *Kryptopterus*, the ampullary organs are arranged in longitudinal rows or scattered over the head and body at high density. This widespread distribution of the ampullae allows the fish to compare potentials over a considerable area, no doubt improving their ability to detect the direction of the source of an electric stimulus (just as the long baselines of the lateral line canals assist in directional perception).

Tuberous (phasic) receptors

A second general type of tuberous electroreceptor is also found in the weakly electric gymnarchids and mormyrids, this varies somewhat from family to family, mormyromast organs being rather complicated with two differently tuned receptors (Figure 10.15). Some of these receptors are involved in electrolocation, others in intra- and extra-specific communication: the responses from the sensitive knollenorgans of mormyrids play a very particular role in communication. The knollenorgans are *phasic* receptors, tuned to the timing of EODs, and less good at determining their amplitude. They are insensitive to d.c. fields, but respond to high-frequency stimuli (100–20 000 Hz). Central links between the command nucleus firing the EOD of the fish, and the knollenorgans, effectively prevent the fish hearing its own EOD, so that it can detect those of

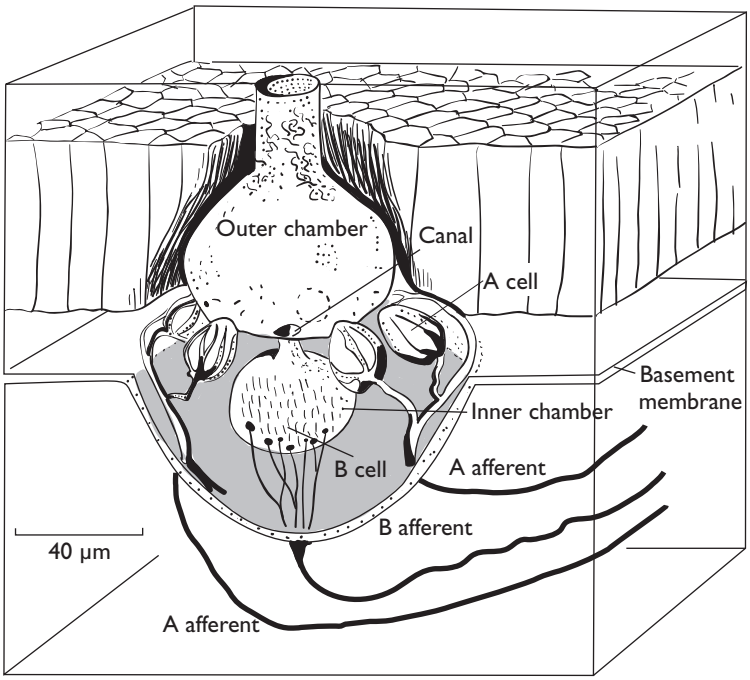


Figure 10.15 Reconstruction of mormyromast tubular receptor. Note short canal. From Bell *et al*. (1989).

other fish around it, and distinguish not only its own species from others but also the sex of its neighboring EOD emitter.

Neurophysiologists have made considerable progress in working out the central processing of electroreceptor information, (see Coombs *et al*., 2002), to which much of the mormyrid brain is devoted (Chapter 11). There are some striking parallels with the treatment of afferent information in the mammalian brain.

The signals emitted by these weakly electric fish, can be classed either as pulse-type signals (where the inter-EOD interval is longer than an individual EOD), or wave-type signals, where the inter-OED interval is similar to the length of the EOD (Figure 10.16). The South American gymnotid *Apteronotus albifrons* emits continuously all its adult life wave-type EODs at the staggering frequency of 1700–1800 Hz. Two wires in the tank of such a fish linked to a speaker would produce a continuous high-pitched humming note between A and B flat in the fifth octave. Perhaps even more remarkably, the related *Eigenmannia* is able to detect minute time intervals of as little as 400 ns between receptors on different parts of its body (see p. 375). Curiously, the fish that Lissmann first examined, *Gymnarchus niloticus*, is the only wave type electric fish in African freshwaters; it is also the largest, at up to 1 m.

In the tropical lakes and rivers where electrolocating fish live in large numbers, they are obliged to cope with EODs emitted by others as well as their own, and, as we have seen, mormyrids can block reception of signals other than their own. Both species using wave-type EODs and pulse-type EODs are found to change their frequency whenever another fish with a frequency

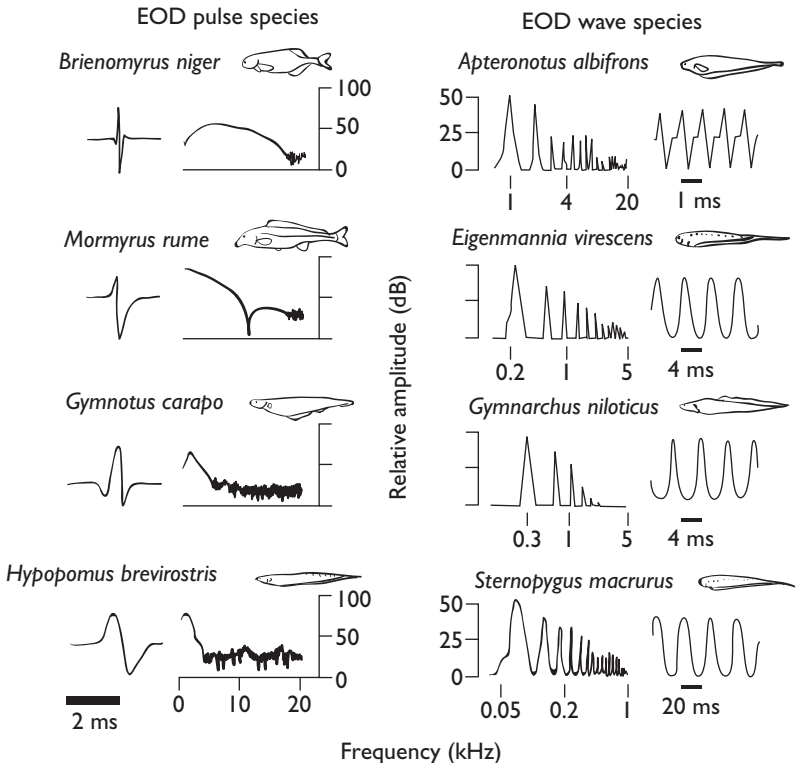


Figure 10.16 Pulse and wave-type electric organ discharges (EODs) of various weakly electric fish. From Moller (1995).

within 1–2% comes within range. This jamming avoidance response (JAR) always operates to increase the difference between the frequency of the intruder and the fish's own frequency, allowing a number of such fishes to electrolocate successfully when in close proximity.

Stoddard (2002) in an interesting article, has pointed out, *inter alia*, that emitting electric signals has its dangerous side if an electrodetecting fish eater is lurking around; this could either be another gymnotiform or a catfish. Some gymnotids specialize in eating other smaller species whole, while some just bite off the tails, a sensible strategy since the tails are quickly regenerated.

Most fascinating accounts of the ingenious behavioral experiments which gradually unraveled what the fish *might* learn about its surroundings from such weak electric signals have been given by Kramer (1994) and by von der Emde (1999, 2004). The even more challenging task of understanding which particular features of the EODs they emit and then detect *are* used to determine *which* of the features of their environment is still in progress. These experiments have provided good evidence that the fish can not only detect objects within a distance around its length, but that they can also determine the distance of such objects from them, and measure several electrical properties such as their impedance. In this way they can distinguish between living and non-living material. They can also determine the shapes of objects, and just as we (visually) can, they recognize a given shape even if is presented at

different sizes and angles. Space does not permit a full discussion of the remarkable similarities between the way we analyze visual images and weakly electric fish analyze electrical images, but the reader is urged to delve more deeply into one of the most intriguing facets of fish sensory systems. Some necessarily brief remarks about electrosensory maps in the cerebellum are made on p. 374.

10.6 Electric Organs

In some gymnotid species, the electric organs are derived from modified nerve axons but in all other fish the electric organs are modified striated muscle fibers, consisting of stacks of flattened cells innervated on one side (Figure 10.17). This arrangement, like batteries in series, sums the small (mV) electric potentials from membrane depolarizations so giving rise to much larger external potentials. In a few species such as the marine ray (*Torpedo*), the freshwater eel (*Electrophorus*) and the catfish (*Malapterurus*), the voltage generated (500 V in *Electrophorus*) is sufficient to stun prey, and certainly acts as an effective defense against predators.

The marine rays are weakly electric with caudal electric organs. They evidently do not use these for electrolocation (the discharges are infrequent and irregular) and so, perhaps, use the electric discharges for intra-specific

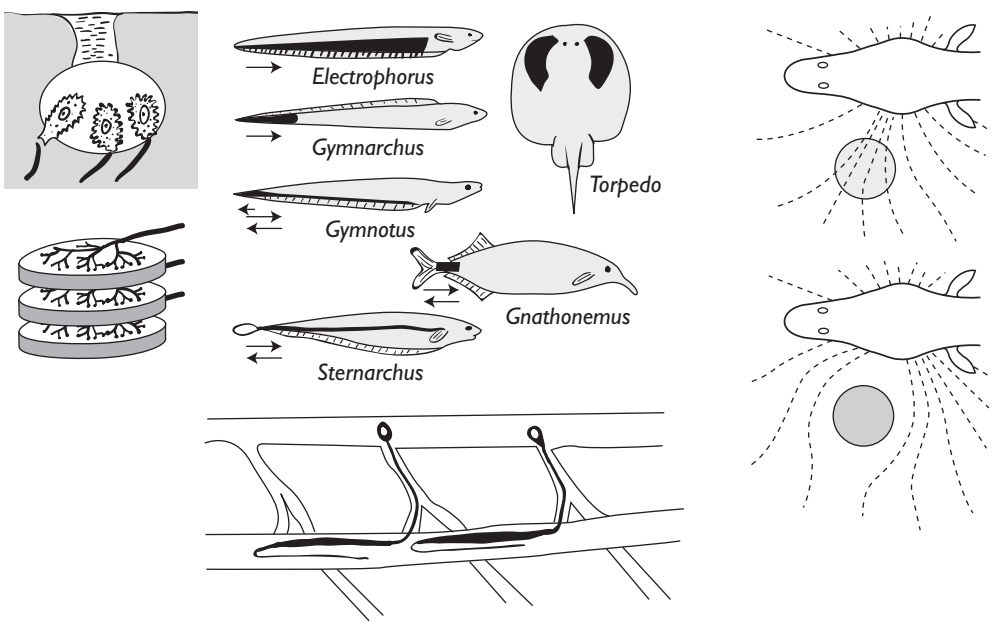


Figure 10.17 Active electroreception and electric organs. Upper left: tuberous mormyrid receptor (note large surface area of receptor cells within cavity). Bottom: the electric organ of *Sternarchus* formed from nerve fibers, upper tube spinal cord, lower, the electric organ. Lower left: stacked modified muscle fibers and their innervation in the electric organ of *Torpedo*. Center: positions of electric organs in different fishes – the arrows indicate directions of current flow. Right: lines of current flow around *Gymnarchus* with an object of the same impedance as the surrounding water (above) and a good conductor (below). From Bennett (1971); Szamier and Wachtel (1970); Lissmann (1963).

recognition. The bizarre electric organ of another marine group, the stargazers, also presents a puzzle. *Astroscopus*, with its electric organ modified from its eye muscles, is an ambush predator lying in wait for small prey on the sea bed. The electric discharges are insufficient to stun the prey and are not used in echolocation. During feeding, the electric organs emit a burst of high-frequency pulses for 150–300 ms followed by a train of discrete pulses lasting about 1 s. The duration of the burst, which occurs as the mouth opens, is correlated with the length of the prey (Figure 10.18). Can this be a signal to other stargazers about the size of available prey?

Electric organs and electroreceptors seem to have been "invented" independently a number of times in different fish lineages. In all, probably by modification of the closely related lateralis system. There are striking similarities in the ways that both lateralis and electrosensory information is processed (Coombs *et al*., 2002) and in both systems spatial maps are constructed in the brain from the information given by the receptors (see Burt de Perera, 2004, for lateralis mapping in the blind cave fish *Astyanax*).

10.7 Magnetic Reception

It has long been known that some animals can orient in magnetic fields of the same strength as that of the Earth, and use them as a compass, for example spiny lobsters, sharks, migratory birds, and turtles, but the difficulty with a magnetic sense is that until recently, magnetoreceptors, and, in particular, the link of putative magnetoreceptors to the nervous system, have proven elusive. The discovery of bacteria with a chain of single domain magnetite particles (like a row of small magnets) within them, that aligned them along the lines of the geomagnetic field, evoked a search for magnetite particles in migratory species, including tuna and salmonids as well as those mentioned above. Definitive success in finding fish magnetoreceptors and their innervation was finally achieved by Walker *et al.* (1997; see also Kirschvink *et al*., 2001 and Diebel *et al*., 2000) who found magnetite particles in special sensory cells of the olfactory lamellae in the rainbow trout (*Oncorhynchus mykiss*). These were innervated by the opthalmic branch of V. Interestingly, Scherbakov *et al.* (2005)

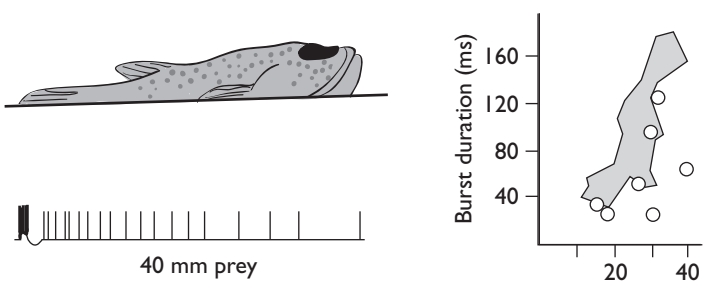


Figure 10.18 Electric discharges in the only marine electric teleost, the stargazer *Astroscopus*. Left: the fish half-buried in the sand above a record of a discharge burst. Right: relationship between lengths of discharge and of prey. After Pickens and McFarland (1964).

have shown with training experiments that the non-migratory zebra fish is magnetosensitive, raising the possibility of genetic examination of magnetoreception. Magnetoreception in different vertebrates (including fish) is reviewed by Wiltschow and Wiltschow (2005). The remarkable navigational ability of blue fin tuna (*Thunnus thynnus*) presumably mainly (if not entirely) due to magnetoreception, is seen in Figure 10.19, where a tuna was tagged with an archival tag and followed into the Gulf of Mexico to its spawning ground, and then back out again to forage in the Atlantic. The long saga of this blue fin crossing the Atlantic to its spawning site in the Mediterranean each year for 4.5 years was viewed similarly and is seen in Figure 10.19.

10.8 Vision, and Photophores

Very few fishes live in stygian darkness. Even in the depths of the oceans, the natural darkness is relieved by flashes and glows of bioluminescence from the photophores of both fish and invertebrates. Light is attenuated in water both by absorption and scattering, and falls off with depth at a logarithmic rate. In the clearest oceanic water the specially adapted eyes of deep-sea fish can still operate down to about 1100 m in daylight and perhaps 600 m at night. It is

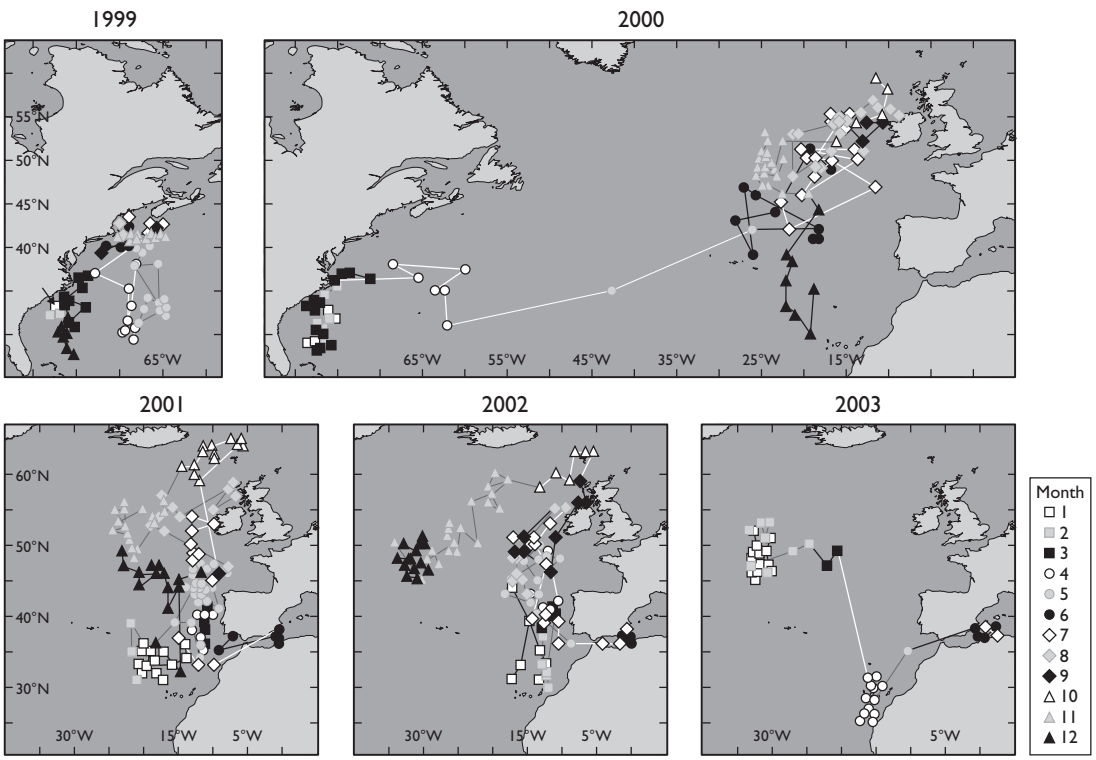
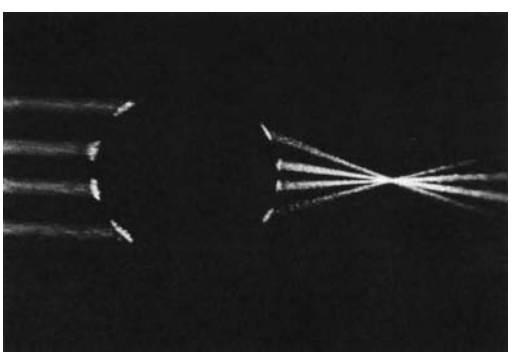


Figure 10.19 Transatlantic movements of a blue fin tuna, recorded by archival tags. From its release on 17 January 1999 (arrow) to its recapture on 2 July 2003. After Block *et al.* (2005).

often remarked that only in caves is the visual system useless, where some 40 species of blind fishes are known. However, this omits weakly electric mormyrids and gymnotids which have eyes, but these cannot be used to seek prey at night in the muddy substrates of the freshwaters where they live, and so, for nocturnal feeding, the visual system is replaced by electrolocation.

Water absorbs different wavelengths of light selectively, some being transmitted more readily than others. The wavelength (λ) that is best transmitted, λmax, is 470–480 nm in the deep ocean, 500–530 nm near the coast, and 550–560 nm or longer in many freshwaters. This change from blue toward yellow is caused by the presence of yellow pigments, mainly breakdown products of chlorophyll and humic acids in freshwater, and in the run-off near the coast. This has important implications for the fish eye in terms of its performance in light of different wavelengths (p. 321).

Although, in essentials, fish eyes (Figure 10.20) are built to the same design seen in all vertebrates, they are more varied than those of terrestrial animals because of the variety of light regimes where they are used (Nicol, 1989). Unlike us (except metaphorically) and most other terrestrial vertebrates, almost all fish are stiff-necked so modifications of the eye are needed to ensure a wide binocular visual field with overlap of the images of the outside world in each eye. The exception is the little salamander fish, *Lepidogalaxias salamandroides*, (see p. 145) which *can* bend its neck sideways and downward, and here the economical eye modification is what we might expect, loss of the eye muscles.



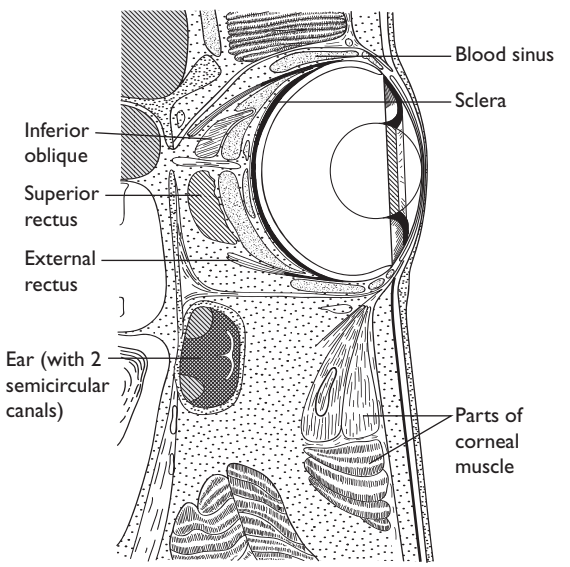


Figure 10.20 Left: section through teleost eye. Right: part of horizontal section through lamprey head showing at mc1 and mc2. Left: excised cichlid lens focusing four argon laser beams. (Photograph by kind permission of Dr R. Fernald, Stanford University.)

Near the surface, because of refraction, fish see the whole horizon above the surface compressed into a solid of angle of about 98°, called Snell's window (Figure 10.21). Refraction presents problems for fish feeding above the surface such as the archer-fish (*Toxotes*) or the large osteoglossid Arapaima (*A. gigas*), because they need to make corrections for distorted images.

Optics

Light passing into the eye is brought to a focus on the retina. Because the retina is "inverted," light rays pass through the transparent associated nerve cell layers of the retina (the bipolar and ganglion cell layers) before reaching the rods and cones at the periphery of the eye. In some fishes there are reflecting layers in the retina or chorioid (see "Reflecting tapeta") that cause incident light to pass back again through the visual cells.

The lens is unlike those in our eyes, for it is rigid and usually almost spherical with a short focal length: in teleosts about 2.55 × the radius of the lens (Matthiessen's ratio). In our eyes, the lens contains some 34% protein, in fish where there is no help in focusing from the cornea, since the corneal refractive index is close to that of the water it has to refract more, around 50%. There is little spherical or chromatic aberration; try looking at a piece of graph paper in a shallow dish full of water through a glass marble and a fresh fish lens, and you will be as astonished at the quality of the fish lens as was the physicist James Clerk Maxwell in 1853, who deduced its general properties. Not until a century later did Fletcher *et al.* (1954) work out the optics in detail. The refractive index varies across the diameter of the lens so that the rays follow a curved path. A number of fishes, for example the yellow perch (*Perca flavescens*), has yellow corneas and lenses which act as filters cutting off the shorter wavelengths and so reducing chromatic aberration.

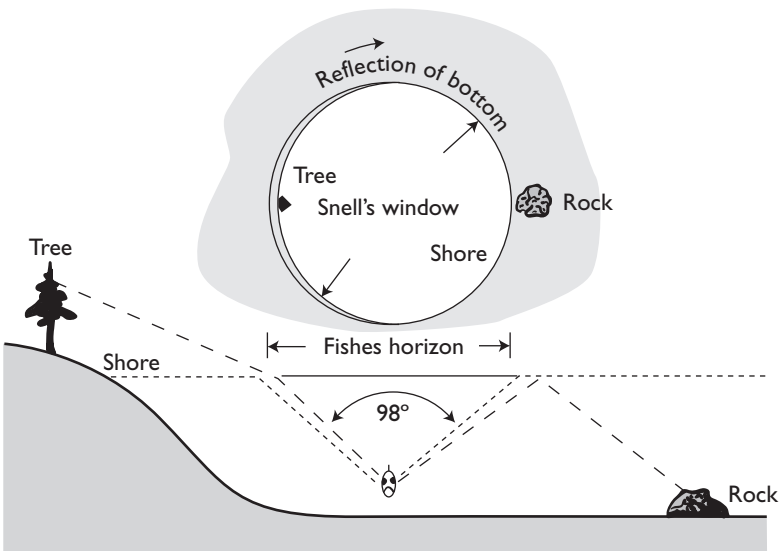


Figure 10.21 Diagram of Snell's window. In calm water a fish will see the whole horizon above the surface subtended by an angle of about 98 degrees. After Walls (1963).

Accommodation

Surprisingly, there is still some doubt how fishes accommodate to examine close or distant objects and whether at rest the eye is focused close to, or far from, the cornea. We accommodate by changing the focal length of the lens (altering its radius of curvature, by permitting it to bulge). As we age, the lens becomes stiffer and less easy to change, so even if we enjoyed both reading and birdwatching without spectacles as students, as more mature (in years at least) individuals, our internal mechanism of accommodation practically disappears so we have to resort to expensive multifocals or an irritating series of spectacles. Before such visual aids, most elderly people were practically blind. No wonder Sir Thomas More in his portrait in the Granet museum at Aix en Provence has carefully stuck his cracked spectacle lenses together. But almost all fish accommodate quite differently, by moving the lens back and forth along the optical axis, so changing the distance between the lens and the retina. The teleost lens is moved relative to the retina by the retractor lentis muscle, usually toward the tail of the fish.

Why is this strange? If we had to accommodate in this way we would have to move the lens toward the midline, into the eyeball and towards the center of the retina. But in fish, the retina is not always concentric with the lens and it as been suggested that the resting eye is short-sighted anteriorly and far-sighted laterally (Pumphrey, 1961). As the lens is moved posteriorly, the lateral view changes very little but the fish would become able to focus distant objects anteriorly (Figure 10.22). In some marine fish, where the lens moves back and forth obliquely, Somiya and Tamura (1973) calculated that objects between infinity and half the length of the fish could be focused. Because the waters of lakes and rivers are generally turbid, and the limit of useful vision is around 2 m, freshwater fish accommodate less than marine fish living in oceanic waters, where the limit may be up to 30 m.

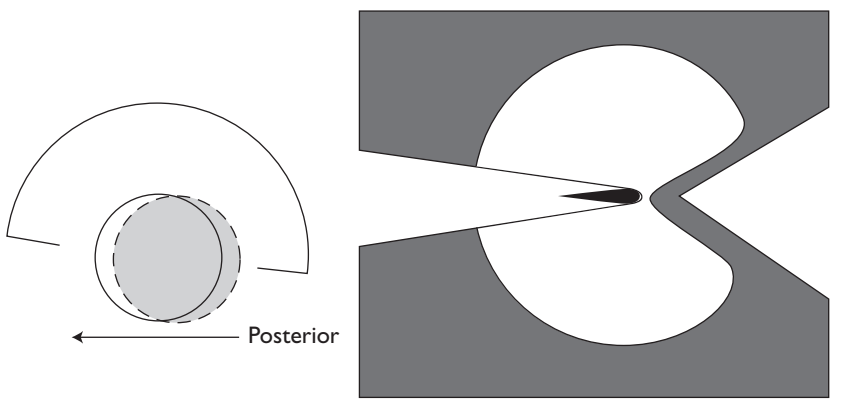


Figure 10.22 Accommodation in fishes. Left: trout eye showing lens movement from rest to fully accommodated (stippled). Right: horizontal visual field (eyes unaccommodated). The fish can only focus on the **dark** area. Behind the fish is the shadow of the body, on either side the white areas are within the locus of the near point, and ahead, the white area is beyond the far point. After Pumphrey (1961); Somiya and Tamura (1973).

Lampreys accommodate by an extraordinary and unique mechanism, for the cornea is attached to an external circular muscle (of myotomal derivation) that contracts to flatten the cornea (Figure 10.20), so pushing the lens toward the retina to focus distant objects. In elasmobranchs, the accepted view was that the eye was normally hyperopic (long-sighted) and to see more closely, the lens was moved toward the retina by contraction of a protractor muscle. But histological examination of the supposed muscle has not demonstrated muscle fibers, and electrical stimulation experiments have yielded negative results, so it is at present unclear whether elasmobranchs *can* accommodate. The most recent studies on lemon sharks (*Negaprion*), using infrared video retinoscopy through the side of an aquarium, suggest that at rest, the shark eye is emmetropic (focused near to it). In some stingrays, the distance between the retina and the lens varies around the eye because the lens is not quite spherical. Perhaps different regions of the retina are used to focus near and far objects so that accommodation is a static rather than a dynamic process. At all events, no one who has been in the water close to an inquisitive pelagic shark such as *Lamna nasus* would doubt that some elasmobranch eyes can be used to inspect prospective prey!

The posterior part of the eye is often important for binocular vision because it is on the posterior retina that overlapping images of the world to the front of the fish are brought to a focus. Binocular vision is especially important for determining the range of objects around the fish, which must move the whole front part of the body to inspect its surroundings (except in *Lepidogalaxias salamandroides* with its flexible neck). Different parts of the retina may be usable for different purposes. Unlike our eyes, with which we monitor a rather limited part of our surroundings at any one time, a fish can probably see some parts of the environment with binocular vision and other parts, to the side, with lateral vision using each eye independently. Some fishes, such as the mudskipper (*Periophthalmus*) have protuberant eyes which can move independently just like those of chameleons. A forward-directed optic axis is sometimes found in which objects to the front of the head are brought to a focus on a specialized part of the retina in the posterior part of the eye (p. 319). It is likely that this part of the retina is often most important for behavior (such as feeding) where binocularity improves the judgment of distance, while the anterior part and center of the retina monitor the side and rear of the fish for the approach of predators or the presence of conspecifics.

In ourselves and most vertebrates, the retina is vascularized, but it is not in fish, and so special measures are needed to provide oxygen for this tissue which has a high metabolic rate (as we know to our cost if even a small part of the retinal vessels become blocked). Blood supply to the fish eye in the ophthalmic artery comes from the efferent pseudobranchial artery (Figure 10.23), which forms a capillary rete (as in the swimbladder blood supply – see p. 119 for the earlier origin of the choroid rete), before reaching the choriocapillaris that oxygenates the retina. It seems likely that this rather complex arrangement operates by the retina releasing a small amount of acid to cause a Root effect release of O_2 (p. 117) from the choriocapillaris. This has to be carefully done, however, since blindness will result from retinal damage if acidification goes too far.

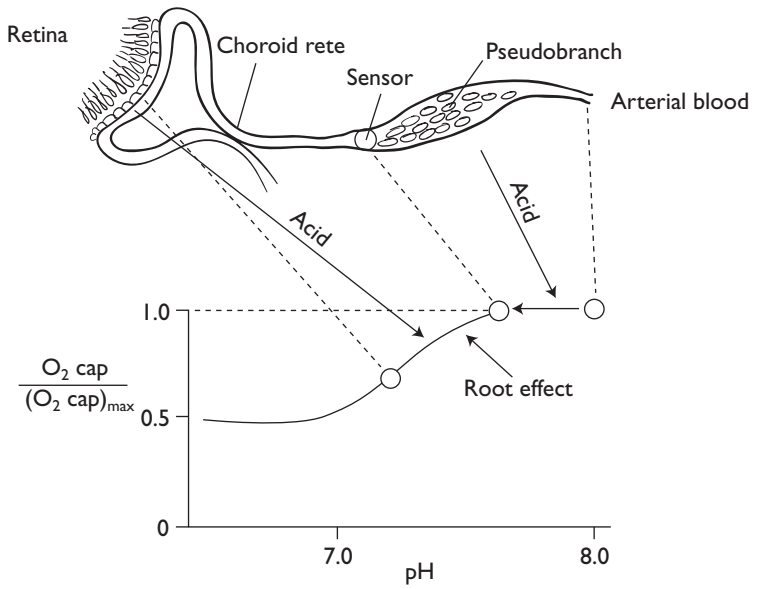


Figure 10.23 Choroid capillary rete unloading O_2 to retina from arterial blood passing into rete from pseudobranch. From Pelster (2001).

Tubular eyes

The teleost eye has been strikingly modified in many mesopelagic fishes. Eleven families, including the hatchet fish (sternoptychids, see Figure 10.36), and giganturids, have independently evolved tubular eyes pointing upward and forward with their optical axes more or less parallel (Figure 10.24). These tubular eyes were first supposed (perhaps not unnaturally) to act as telescopes, but the distance between the lens and the main retina is the same as in the normal fish eye. Such eyes have a number of advantages: they allow the fish to achieve good binocular vision in one direction; they allow the eye to have a large lens, with good light-collecting properties, without taking up too much space in the head; some are also positioned so that the fish can look predominantly upward to see their prey in silhouette against the vertically down-welling light (p. 327). One problem with a tubular eye is that the peripheral parts of the retina cannot be brought into focus because they are too near the lens; they are probably only useful for unfocused movement detection. Some species have developed an accessory retina or accessory refracting devices to obtain focused images from light entering the eye outside the main axis (Figure 10.24, right). The argentinoid *Dolichopteryx* has an accessory retina illuminated by lateral light which has not passed through the lens but through the side of the eye, then being reflected by the argentea into the accessory retina. Scopelarchids and evermannellids have no accessory retina but lateral light is focused on to the main retina by accessory refracting lens pads and ocular folds. Evidently inefficient lateral vision is a high price to pay for efficient binocular vision, hence these extraordinary elaborate modifications of the tubular eyes. Owls also have tubular eyes, which *cannot be moved in their sockets even with pliers* (Walls, 1963), and compensate for poor lateral vision by having extremely mobile heads.

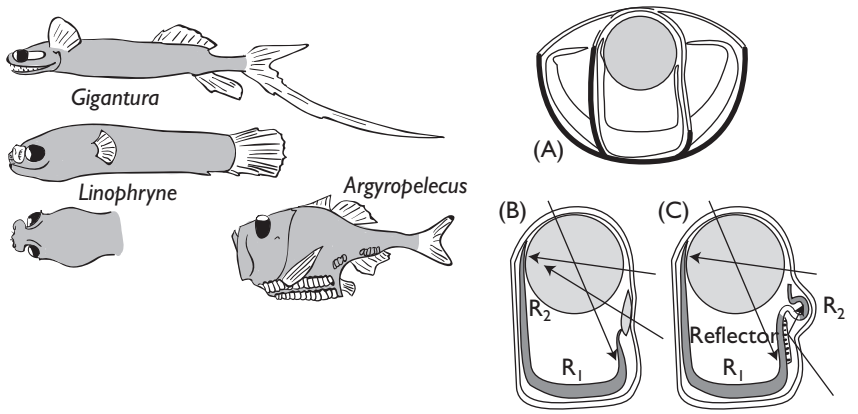


Figure 10.24 Left: mesopelagic fishes with tubular eyes. Note that *Linophryne* looks through its transparent olfactory organ. Right: modification for lateral vision. (A): tubular eye of the sternoptychid *Argyropelecus* superimposed upon normal fish eye; (B) *Scopelarchus* showing accessory scleroid lens; (C) the opisthoproctid *Dolichopteryx* with reflector and accessory globe. R1, R2: main and accessory retinae. After Munk (1966); Locket (1977).

Aerial vision

Quite different modifications have arisen in teleosts that need to see in air. The intertidal mudskipper, *Periophthalmus*, has a flattened lens for vision in air but vision is probably poor under water. The "four-eyed" fish, *Anableps*, swims at the water surface with the upper part of each eye exposed to the air (Figure 10.25). The lens is elliptical with the longer axis refracting light from below the surface and the short axis refracting light from above the surface. Light can thereby be focused on a divided retina. In air, the curved corneas of all fishes become refractive making the fish short-sighted. Flying fish and some shore-living clinids ingeniously get around this by have two flat corneal "windows" that enable them to look downward at the water surface. The most complex visual situation is posed for the osteoglossid *Pantodon*, which lives just below the water surface, and has to sort out what it sees in air, and under water, both internal reflections from the surface and direct light from deeper sources. Saidel (2000) explains how this complex system operates, with a double retina, a falciform process, and varying cone orientation.

Reflecting tapeta

In most elasmobranchs, in Holocephali, sturgeons, *Polypterus*, the lungfish *Neoceratodus*, and in *Latimeria*, light passing through the retina is reflected back by a tapetum at the back of the eye. When illuminated, the eyes of these fishes shine, as do those of cats or moths; the eyes of deep-sea squaloid sharks shine brightly with a superb greenish-blue color when hauled aboard ship. The tapetum is a specular reflector, consisting of layers of reflecting cells in the chorioid layer packed with thin platelets of guanine, like those found in the scales of silvery teleosts. The platelets are arranged at suitable angles to reflect light back into the retina along the long axis of the visual cells (Figure 10.26).

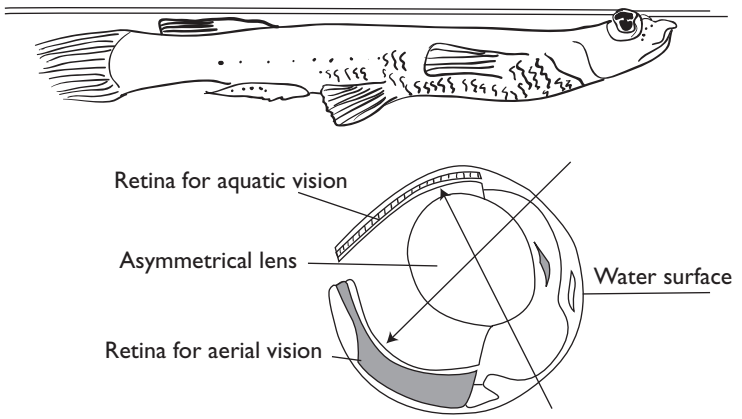


Figure 10.25 View of *Anableps* at the water surface showing how the asymmetrical lens allows it to focus objects above and below the surface on to the retina. After Walls (1963).

Reflectors of this kind are adjusted to reflect certain wavelengths by appropriate thickness and spacing of the guanine crystals; those of deep-sea sharks reflect best at the λmax that penetrates best in deep water (~475 nm).

Teleosts also show eyeshine, but the reflecting layer is instead usually in the retina, often consisting of tiny reflecting spheres, and light is backscattered in a more diffuse manner than with specular reflectors. Chorioidal tapeta are quite rare in teleosts, only being found in midwater fishes such as myctophids and the large castor oil fish, *Ruvettus*. However constructed, these reflectors all reflect light back through the visual cells, so enhancing sensitivity. It has also been suggested that tapeta reduce "noise" from the spontaneous breakdown of visual pigment since fishes with tapeta need less visual pigment for a given sensitivity. The inevitable snag is, however, that some spreading of the

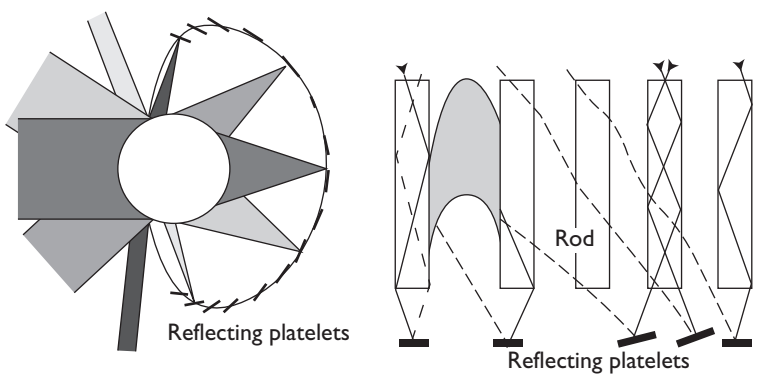


Figure 10.26 Reflecting tapeta. Left: orientation of reflecting platelets in *Squalus* tapetum. Right: paths of light rays channeled by rods and reflected by platelets in sturgeon tapetum. Pigment cell stippled. After Denton and Nicol (1964); Nicol (1969).

reflected light occurs and this may reduce acuity, (see p. 325) since sharp boundaries in the image become more diffuse as more visual cells are stimulated by the reflected light.

Reflecting layers in the eye have another potential disadvantage, for they could make fish very conspicuous when illuminated by daylight. Many sharks can occlude the tapetum by migration of black masking pigment over the tapetal surface on transfer to light, a process that takes 60–90 min; unmasking in the dark takes a somewhat shorter time. Masking pigment movement is also found in teleosts but here it is related to the movements of the visual cells, the rods and cones (Figure 10.27).

The receptors

Rods and cones are as well differentiated in most fish retinae as they are in our own. As we should expect, rods, which contain much more visual pigment than cones and so are much more sensitive, are present in all fish retinae examined, but cones with less pigment need higher light intensities, and are absent in fish living in dim light. Deep-sea squaloid sharks such as *Centrophorus*, chimaerids, rays, and some deep-sea teleosts have a pure rod retina. In many larval fishes there is a pure cone retina at hatching and the rods develop progressively during ontogeny. In the adult eye, rods are vastly more abundant than cones. Both rods and cones are held in position by an external limiting membrane, and share a common plan: an outer segment containing visual (photosensitive) pigment, an ellipsoid packed with mitochondria, an extensible myoid or footpiece, and a nuclear region (Figure 10.27). Typically, rods are longer and thinner with cylindrical outer segments and ellipsoids, while in cones the outer segment is conical, and the ellipsoid rather bulbous. The ellipsoids and outer

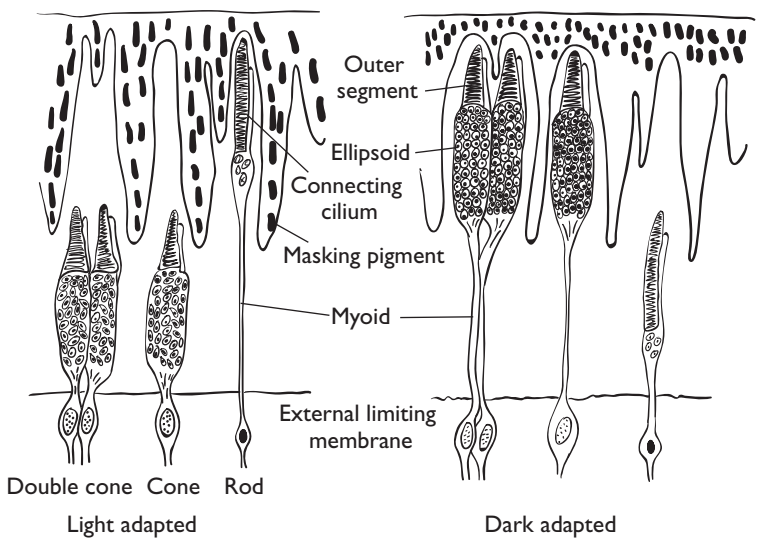
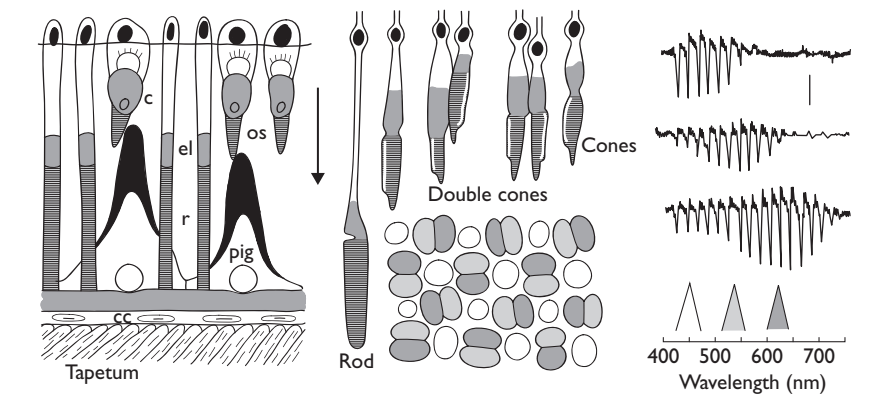


Figure 10.27 Diagram of a light-adapted (left) and dark-adapted retina (right) of a typical teleost. Note the changes in position of the retinal masking pigment and the cones and rods.

segments are joined by an eccentrically placed connecting cilium with the characteristic nine tubule pairs.

Cones are often present as pairs and generally the visual cells are disposed in beautiful regular mosaics when viewed in tangential section (Figure 10.28). In many fishes there are specialized regions. For example, the area temporalis consisting of a patch of closely packed cones (with few or no rods) is appropriately placed to receive light along the main axis of feeding, where high acuity would be an advantage. In pelagic feeders, such as herring and horse mackerel, looking upward and forward to feed, the area is postero-ventral. In horizontally feeding fish, such as the sailfish (*Istiophorus*), it is posterior, and in bottom feeders, such as the sea bream (*Sparus*), the area is postero-dorsal on the retina. The amphibious mudskipper (*Periophthalmus*) has a horizontal band of cones placed to perceive objects near ground level where both food and predators might be present. A fovea or depression with a high density of cones (as in our eye) is found in seahorses (*Hippocampus*) and pipefish (*Syngnathus*), which live by delicately sucking up passing plankton individually.



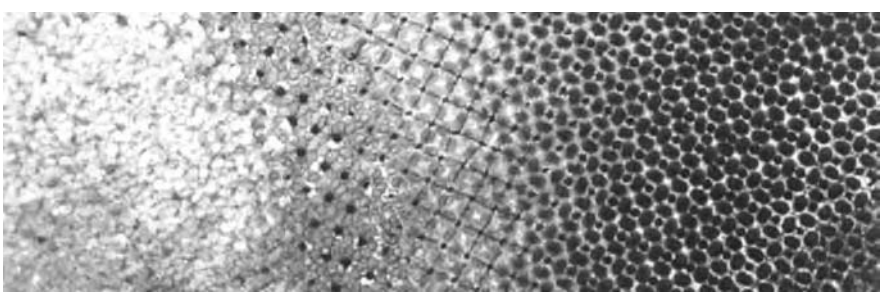


Figure 10.28 Retinal organization and function in actinopterygians. Left: sturgeon retina showing visual cells, pigment cells and tapetum cc: chorio capillaris. Middle: cell types in the teleost retina, cone mosaic below. The cones are distinguished by the wavelength their pigments respond to: white = blue; fine stipple = green; coarse stipple = red. Right: response spectra of single carp cone types when illuminated by 0.3 s light flashes 20 nm apart across the spectrum. Scale bar 20 mV. The three peaks below are absorption maxima found by microspectrophotometry. The retinal mosaic at bottom is an oblique toluidine blue 3 micron resin section at the level of the cone outer segments. From Hawshryn (1998).

Rods and cones are less distinct morphologically in lampreys, and the retina has degenerated in the hagfish. While double cones are extremely common in most fishes, triple cones sometimes occur in the brown trout, and quadruple cones in the minnow. In notothenioids there are square and row mosaics. The function of the multiple cone design, and indeed, of the mosaic retinal pattern, Engstrom (1963) remains to be established, although Wagner (1976) made an interesting beginning.

Both rods and cones are connected to the brain via bipolar cells and ganglion cells whose axons pass along the optic nerve to the optic lobes. The greater sensitivity of the rods with but a single visual pigment is enhanced by the convergence of many rods to one bipolar cell, so that their responses are summated. In some deep-sea fishes and the cusk eel (*Ophidium*) the retina is composed of a number of tiers of rods, presumably so arranged to absorb all the limited light that enters the eye, possibly also conferring some kind of colour vision (Denton and Locket, 1989). Cones, on the other hand, are divided into "populations," each with a different visual pigment (p. 319); they do not summate at the bipolar cells and confer high-acuity color vision (where this has been rigorously tested) in brighter light.

In teleosts there is a change in the eye from dim light (scotopic vision) to bright light (photopic vision). Light or dark adaptation is accompanied by retinomotor or photomechanical movements of the visual cells and melanin masking pigment (Figure 10.27), akin to the occlusion of the tapetum. In dim light, the masking pigment is well retracted toward the outside of the retina, the rod myoids are short and the cone myoids are long, so that the rod outer segments are near to the external limiting membrane and the cones are not impeding this penetration of light to the rods. As the eye light-adapts, the cone myoids shorten, bringing their outer segments toward the external limiting membrane; the rod myoids lengthen, taking their outer segments away from the cones and into the advancing masking pigment, which protects them from the brighter light. This process takes 20–30 min and (obviously) occurs around dusk and dawn. In larval fishes, before the rods develop, and in adult fish without cones, retinomotor movements do not take place during the transfer from dark to light.

These intricate adaptations of the fish retina are found only to a limited extent in Amphibia, and are absent in terrestrial animals. The amount of light entering the eye is instead controlled by rapid changes in pupil diameter. In elasmobranchs (which usually have rod-dominated retinas) variation of pupil diameter is important in species that may be subjected to changes of light intensity, but in deep-sea selachians and chimaerids, the pupils are immobile. With some exceptions, the teleosts have relatively immobile pupils. Most teleosts seem to have iris muscles or some form of sphincter but the variations of pupil diameter in different light levels are nothing like as dramatic as in elasmobranchs.

10.9 Visual Pigments

Visual pigments consist of a protein (opsin) linked to an aldehyde of vitamin A_1 (retinal) or vitamin A_2 (dehydroretinal). For a given opsin protein, A_2 visual pigments absorb at longer wavelengths (λ) than A_1 pigments. The A_1 pigments are sometimes called rhodopsins, the A_2 pigments porphyropsins. Depending

on its precise chemistry, each pigment has a characteristic light absorption as measured by a spectrophotometer. The pigments are usually referred to by the wavelength of light at which such absorption is maximal (the λmax). Because the pigments are photosensitive and break down in light, they are most sensitive (i.e. capture most photons) to light of wavelengths near their λmax. As they break down chemically, the pigments bleach and only regenerate to their original chemical state in the dark, a process that may take several hours to complete. The visual pigments are located in the outer segments of both rods and cones, and after bleaching some of the breakdown products migrate into the epithelium surrounding the visual cells where part of the regeneration process occurs.

Fishes from different environments – the deep sea, coastal waters, or freshwater – do not always have visual pigments matched to the wavelengths of light that penetrate these waters best. This seems curious, and has stimulated a good deal of research. Certainly, deep-sea fishes have rod pigments (chrysopsins, related to rhodopsin) with a λmax at 470–480 nm matched to the clear blue water in which they live (Figure 10.29). Coastal fishes with λmax at 490–515 nm and freshwater fishes of λmax at 500–545 nm, although with visual pigments shifted in the appropriate direction, are well offset from the maximum light-transmission characteristics of their environments.

Lythgoe (1979) suggested that seeing into the distance as far as possible was not the only requirement for the eye, just as important, perhaps, was the need to see prey and predators against the background, that is, to detect contrast. Objects darker than the background, or distant objects whether darker or lighter, are best perceived by visual pigments matched to the background. But for the particular (although common) case of bright objects seen at fairly close range in shallow water, offset visual pigments are better. The further they are offset from the wavelength best transmitted by the water, the brighter the objects will seem against the background. Thus, offset visual pigments enhance the contrast between bright objects and the background, and this

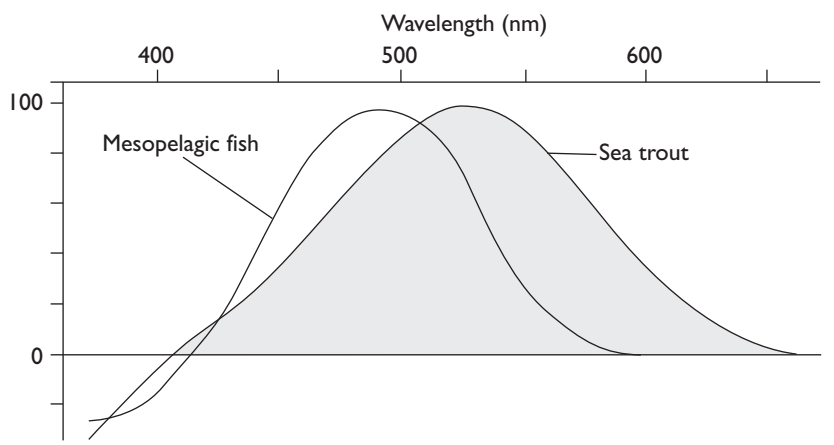


Figure 10.29 Rod pigments matched to the environment. Sea trout (shaded) compared to the mesopelagic myctophid *Diaphus rafinesqui*. After Denton and Warren (1957).

idea was supported by McFarland and Muntz's (1975) work on the visual pigments of two pelagic predatory fishes which hunt in different ways (Figure 10.30). Skipjack (*Katsuwonus*) hunt by rushing upward at fish silhouetted against the surface. They are hunting *dark* objects, and have a single visual pigment matched (at λmax 483 nm) to the background. Dolphin fish (*Coryphaena*) hunt differently, living only in the top 15 m of the water column and striking horizontally against their prey. They have three extractable pigments, (λmax 521, 499, and 469 nm). The first seems well-designed to detect bright objects (the flying fish, garfish, and crustaceans on which the dolphin fish feeds), the 499 pigment is probably in the rods, and the 469 pigment (perhaps less plausibly) may be offset for blue-green water, rich in phytoplankton.

During the transfer from light (photopic vision) to dark (scotopic vision) there is not only an increase in sensitivity to light but a change in the response to different wavelengths – the Purkinje shift – the eye becoming more sensitive to light at the blue end of the spectrum. This should not be confused with color vision. The fish may, indeed, switch from color to monochromatic vision as they dark-adapt but they may only see colors as shades of gray when both light- and dark-adapted. In nature fish can be exposed to changes of ambient illumination ranging over 10–12 log units. It is the dark–light adaptation mechanism with the populations of cones and rods with different intrinsic thresholds that allows their eyes to function over such a wide range. In many fishes, light-dependent behaviors such as feeding, spawning, and schooling lessen as the illumination falls and cease altogether when these fishes become dark-adapted. The function of the cones in dark-adapted fishes is not obvious. In some they may still allow limited feeding, avoidance of predators, or schooling (perhaps operating in conjunction with the other senses); they may be involved in controlling activity, maintaining a certain depth depending on the amount of down-welling light or in the control of vertical migration by the appreciation of an optimum light intensity (p. 422) that may be tracked at dusk and dawn.

Some of the features of fish vision (and indeed those of most vertebrates) are set out in Table 10.1 in rather a simplistic way. However, it should never be assumed that animals have evolved mechanisms that always fall into neat categories. It is both important and salutary always to remember the injunction (in another connection) of the Oxford linguistic philosopher J. L. Austin (1962): "it is essential, here as elsewhere, to abandon old habits of Gleichschaltung,

Figure 10.30 Hunting strategies and visual pigments. The dolphin fish (*Coryphaena*) hunts by striking horizontally, whereas the skipjack (*Katsuwonus*) rushes upward to prey silhouetted against the surface. After Lythgoe (1980).

Table 10.1 Summary of the physiological changes taking place as fish dark- and light-adapt

Dark-adapted (RODS)	Light-adapted (CONES)
High sensitivity	Low sensitivity
Summation	No summation
Poor acuity	High acuity
Monochromatic vision	Di-pentachromatic vision

the deeply ingrained worship of tidy-looking dichotomies." For example, there is some limited evidence that a few highly sensitive, low-threshold cones continue to function near the absolute visual threshold, and so some fishes may retain limited color vision even when dark-adapted!

When fish migrate into water of different spectral quality, as do eels and salmon, their rod pigments change. As Pacific salmon move from the sea into freshwater for the spawning run, the λmax changes from 503 to 527 nm – the rhodopsin being gradually replaced by a porphyropsin more suited to the yellower freshwater environment. The sea lamprey (*Petromyzon marinus*) changes from a porphyropsin-dominated system when migrating upstream to a rhodopsin system migrating downstream. In the freshwater juvenile of the eel, there is a pair of rod pigments absorbing maximally at 501 and 523 nm but as the eyes enlarge and the eel returns to the sea, the 523-nm pigment disappears and a new pigment develops with λmax at 482 nm.

A dual rhodopsin–porphyropsin system is found in some inshore and freshwater fishes. The relative proportions vary with the individual, its age, with the spectral nature of the environment, and with season and water temperature. Enigmatically, sometimes these variations seem to correlate with changes in the spectral quality of the water, sometimes not.

Color vision

Much early controversy raged over whether fishes had color vision, well described by Walls (1963) in his classic book *The Vertebrate Eye*. Recent experiments, in which not only absolute brightness (as determined by a brightness meter) but also the subjective brightness to the fish were controlled, have shown unequivocally that many teleosts have color vision. In view of the brilliant colors of many fishes from shallow water, especially coral reef fishes, it seems rather surprising that anyone should have supposed that such fish lacked color vision. But as Longley (1917) pointed out, whether fish see colors as we do has to be considered carefully. More recently, (Marshall, 2000) has shown convincingly that these (to us) brilliant colors and patterns may both be cryptic against the reef background, and used as specific signals against open water. Whether sharks distinguish colors is less certain. At least some species have multiple cone pigments, see Table 10.2 (Hart *et al.*, 2004). Color vision was thought to be mediated via the cones (why was this?) but it is only in the last 20 years or so that sufficiently refined optical equipment has been developed to check the mechanisms. Microspectrophotometers have been

developed that can measure the spectral characteristics of *individual* cones. Bearing in mind the low density of the cone pigments and the fact that the pigment-containing outer segment is only 5 μm in diameter, this is certainly a considerable feat, and these are tricky instruments to use. The MkV instrument illustrated (and its circuit) was state of the art in the mid to late 1960s (Figure 10.31A and B). Liebman's (1972) description of this instrument is of much interest. In the goldfish and several other species, three populations of cones each with a pigment of characteristic λmax can be identified

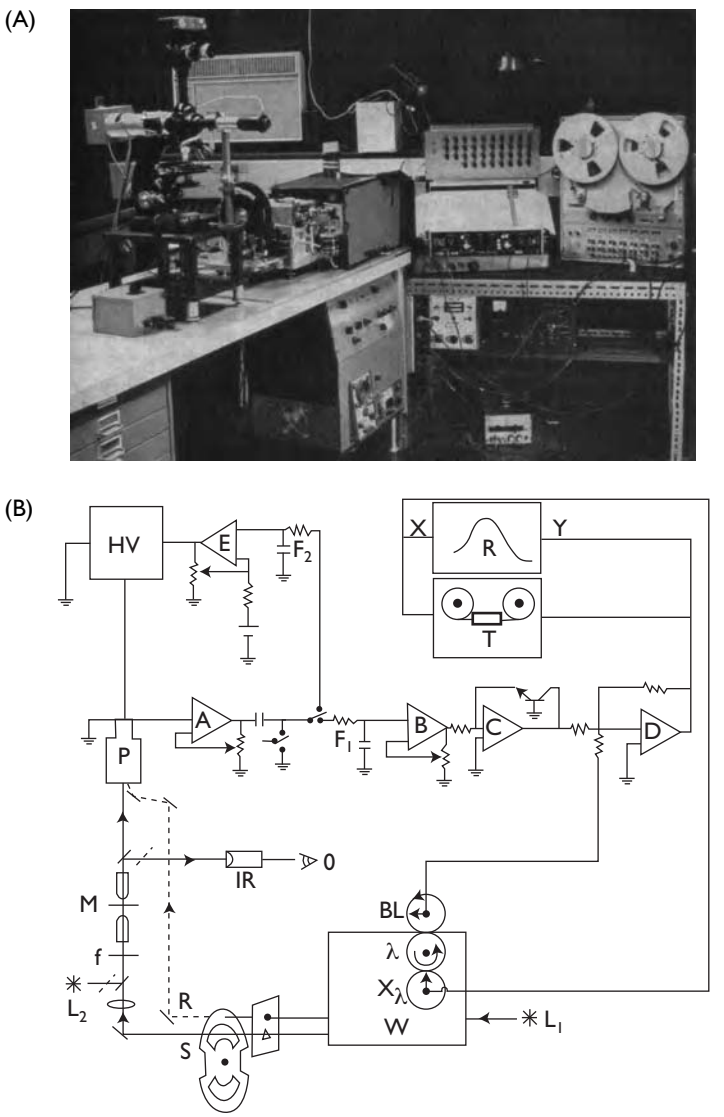


Figure 10.31 A: Liebman's microspectrophotometer. B: circuit diagram. From Liebman (1972). A–E: operational amplifiers; F_1, F_2: filters; f: infrared filter; HV: power supply; L: monochromator lightsource; M: microscope; O: observer; P: photomultiplier; W: monochromator.

Table 10.2 Multiple cone visual pigments of giant shovelnose ray (*Rhinobatos typus*) and the eastern shovelnose ray *Aptychotrema rostrata*

λ_{max} of mean prebleach spectrum (nm) given for each receptor.

Rhinobatus typus				*Aptychotrema rostrata*			
Rod	SWS cone	MWS cone	LWS cone	Rod	SWS cone	MWS cone	LWS cone
504.1	476.5	502.8	563.2	498.2	458.2	493.5	553.0

SWS, MWS, and LWS are short, medium, and long wavelength cones, probed by microspectrophotometry.

From Hart *et al.* (2004).

(Figure 10.28) to provide a trichromatic basis for color vision. Not all may persist through ontogeny (Shand, 1993), for in goatfish, for example, when the larvae leave surface waters to feed on the bottom, red sensitive cones disappear. Other fish, such as the weever (*Trachinus*), are only dichromatic with blue- and green-sensitive cones and some cyprinids are tetrachromatic. Hard for us trichromats to imagine what such fish may see!

Goldfish, some cyprinids, and *Anableps* see ultraviolet (UV) light. On coral reefs, some fish with UV patterns do not have UV-sensitive visual pigments while others do (Losey *et al.*, 1999). In brown trout, the UV sensitivity of the young disappears in the adult. In *Tribolodon hakonensis* (related to dace), goldfish, and in the roach (*Rutilus rutilus*), small single cones are sensitive to UV light and (a necessary prerequisite) the cornea and lens also transmit UV light down to 350 nm. There is a snag, however, which is that UV (λ: ~360 nm) is damaging to the retina, so yellowish lenses and corneas are often necessary filters. As we may already know or might have guessed, several online fishery firms sell UV paint to smear onto bait and this seems rather effective in increasing the catch. The interesting molecular analysis of vertebrate UV receptors by Shi and Yokoyama (2003) has shown that not all have a single origin.

Somewhat surprisingly, the pineal in lampreys contains UV-sensitive cones (Koyanagi *et al.*, 2004) with a bistable UV-sensitive pigment (λmax: 370 nm) which reverts to (λmax 515 nm when illuminated by UV light, and back to UV-sensitivity if then illuminated with visible light. Uchida and Morita (1990) earlier had obtained convincing records from pineal UV-sensitive receptors, and it appears that UV reception in fish and other vertebrates is also based on this pigment, a parapinopsin. There is some evidence of a link between UV and polarized light perception. The goldfish also responds behaviorally to the plane of polarized light, maintaining a fixed orientation to the e-vector, and rainbow trout can be trained to swim to a refuge using the e-vector as an orientation mechanism.

Sensitivity, and acuity

The larger the eye, the better its light-collecting ability and so its sensitivity but, as explained on p. 320, the sensitivity of vision also depends on the density of the visual pigment and the extent to which the visual cells summate. Adaptations to increase sensitivity such as summation and the reflection of light back through the retina by a tapetum tend to fuzz sharp boundaries and

so impair acuity. Some of the relevant features of the eyes of deepsea fish are reviewed by Douglas *et al.* (1998). Recent work on swordfish (*Xiphias*) by Fritsches *et al.* (2005) has shown that the sensitivity of their large eyes is much increased by warming as much as 15°C above ambient, using the heater tissue (Figure 10.32) described by Block (1986, 1987) and her colleagues.

The pineal body

This light-sensitive organ is present as a lobe on the upper surface of the forebrain (p. 365). In trout and tuna, for example, it lies below a transparent window but in other species, access to light is more questionable (see Falcon and Collin, 1989). In the trout the organ is most responsive to light of about 500 nm, suggesting a rod-like visual mechanism. It has been suggested that the pineal acts as a dusk detector in its tonic mode and as a shadow detector in a dynamic mode. The hormone melatonin is secreted by the pineal, causing expansion of the melanophores, and darkening of the skin in the dark (p. 280). The rhythmical secretion of melatonin is implicated in the timing of circadian rhythms, synchronizing cycles of activity with light cycles in the environment. In man, melatonin plays a role in sleep patterns and melatonin secretion at the wrong time is a cause of jet lag. Rather surprisingly, perhaps, the pineal is not the only extraocular light sensitive region, for lampreys respond to light after removal of the eyes and the pineal stalk.

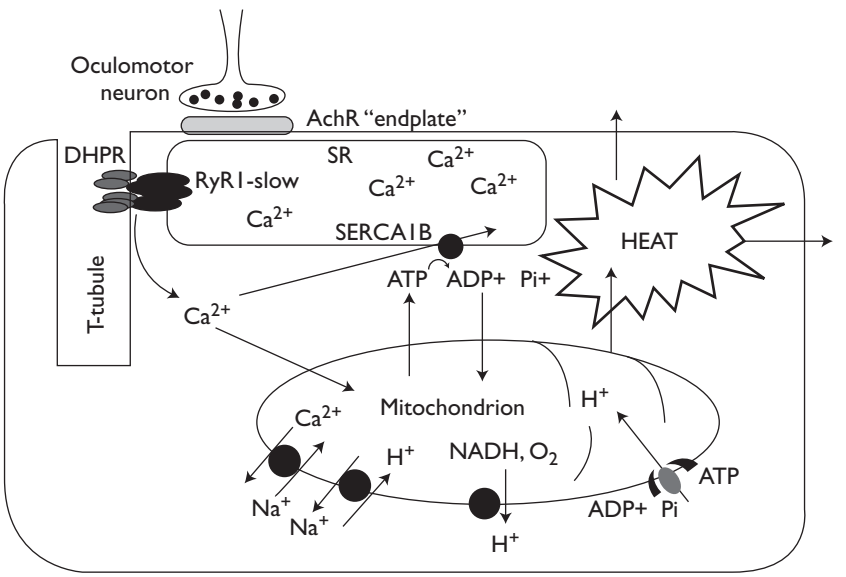


Figure 10.32 Muscle cells modified to generate heat with extensive SR and ryanodine receptors. After Block (1986). Scheme of modified muscle cell from heater tissue for the eyes of blue marlin (*Makaira nigricans*). Oculomotor innervation activates the sarcoplasmic reticulum (SR) via the T-tubular invaginations. Ca^{2+} release evokes mitochondrial production of heat. RyR1-slow are the unusual ryanodine receptors of the end feet linking T- and SR which continue Ca^{2+} pumping. From Morrisette *et al.* (2003).

10.10 Camouflage

Many benthic fishes are perfectly camouflaged against the substratum by their chromatophores. It is only too easy to step on well-nigh invisible stonefish (*Erosa*) when walking on coral reefs, and remarkable matches to their backgrounds are made by flatfishes such as *Pleuronectes*. Not only are these fishes colored like the substratum, but many have fringed margins and projections making their bodies "unfishlike" in outline. But the hardest camouflage problems are faced by pelagic fish. Countershading (inverted in the upside-down freshwater catfishes such as *Synodontis*) is common among pelagic fish such as mako sharks (*Isurus*), and in some is interrupted apparently as a lure for fish looking upward. The small cookie cutter shark *Isistius*, which has ventral green luminescence, has a dark collar around its neck, so that upward-looking fish see it as two smaller fish: larger sharks come up to attack it and are then sucked onto, and chunks carved out of their flesh (Widder, 1998; see p. 194).

Much more effective and ingenious camouflage methods are found in pelagic marine fishes.

Camouflage by reflection

Many fishes living in the upper layers of the ocean are silvery, with silvery scales (herring) or underlying silvery layers (mackerel). The silveriness results from light reflection by organized stacks of thin guanine crystals, separated by sheets of cytoplasm, as in the tapetal reflectors. Such layers of material of alternating high- and low-refractive indices operate as very efficient reflectors. Since the wavelength reflected depends upon the optical thickness (refractive index $\times$ thickness) of the layers, the highest reflectivity being when the optical thickness is 0.25, such reflectors can be (and are) "tuned" to reflect particular wavelengths. Small wonder that many silvery fish show a remarkable variety of reflected colors from deep purplish blues to greens and gold to reds, as they are held at various angles to the light.

The polar diagram of light in the sea (Figure 10.33) is symmetrical except just at the surface, and remains constant with depth, although light intensity changes. As Denton (1970) showed, this means that fishes can camouflage themselves almost perfectly by reflecting light.

Consider a flat mirror vertical in the water. An observer (a predatory fish) looking at the mirror obliquely from any angle (Figure 10.33) will see light reflected from the mirror but will be unable to distinguish this from light which would have reached its eyes if the mirror were not there, and hence will be unable to detect the presence of the mirror. Although fish are not flat-sided, they can arrange that the reflecting platelets are vertical, so achieving the same result as if the sides of the fish were flat. The bottom of the fish will be hardest to camouflage in this way, because, if looked at directly from below, it will be silhouetted against down-welling light.

Two solutions to this problem are possible: either the fish can make the ventral region compressed and knife-like, as do herring and sprat (Figure 10.33, right), or it could use photophores to generate its own light to shine down to mimic the ambient down-welling light. A moments' reflection will show that this second solution involves some taxing difficulties if it is to work properly.

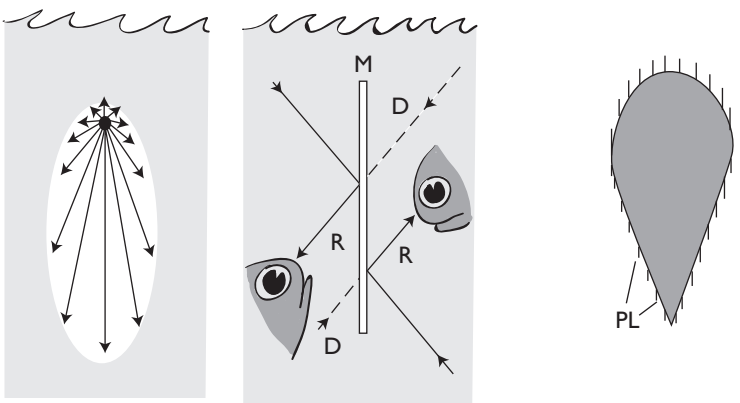


Figure 10.33 Light in the sea and camouflage with silvery surfaces. Left: radiance distribution in the sea, the length of the arrows indicating relative radiance in a given direction from their origins. Middle: predatory fish looking at a perfect mirror (M) cannot distinguish between reflected rays (R) and direct rays (D): the mirror is invisible. Right: a herring has reflecting platelets (PL) on its sides that are inclined slightly upwards, compensating for less than perfect reflection by reflecting light slightly brighter than if oriented vertically like a mirror. After Denton (1970).

Luminescence, and photophores

Light organs of different kinds are an intriguing and important feature of many mesopelagic and deep-sea fishes, and are even found in some fishes living in shallow water, such as the midshipman (*Porichthys*), although no freshwater fishes have them. Samples of fishes collected off Bermuda and in the South Atlantic showed that some 70% of all species caught had light organs, and systematic surveys have shown that around 10–15% of all marine fish genera contain luminous species. Some species use lights as lures – barbels and fishing rods with luminous tips are found in several families (Figure 10.34), while others have light organs in the mouth – or, like the flashlight fish (*Photoblepharon*) use them as headlights to illuminate their prey. Most fishes, however, use their photophores for signaling to other members of the same species, or for camouflage. In the upper 1000 m of the ocean, down-welling light will silhouette fishes looked at from below (which is why paralepids, for example, adopt a 45° head-up attitude to seek their prey). Many fish surmount this difficulty by shining light downward to match natural down-welling daylight (see Figure 10.37, below) and so make themselves invisible from below. Of course the match has to be exceptional, otherwise the fish would stand out like a dark patch or, perhaps worse, as a light patch against the background. Since it is also necessary to match the background when viewed obliquely as well as directly from below, it is hardly surprising that the most complex photophores and photophore arrays have been developed for ventral camouflage.

Fish photophores are very diverse, and their structure can only be touched on here. Many are complex, with a lens, colored filters, and a silvery reflector. There are two basic types: those in which light is produced by special photocytes, and those where symbiotic luminous bacteria are cultured in

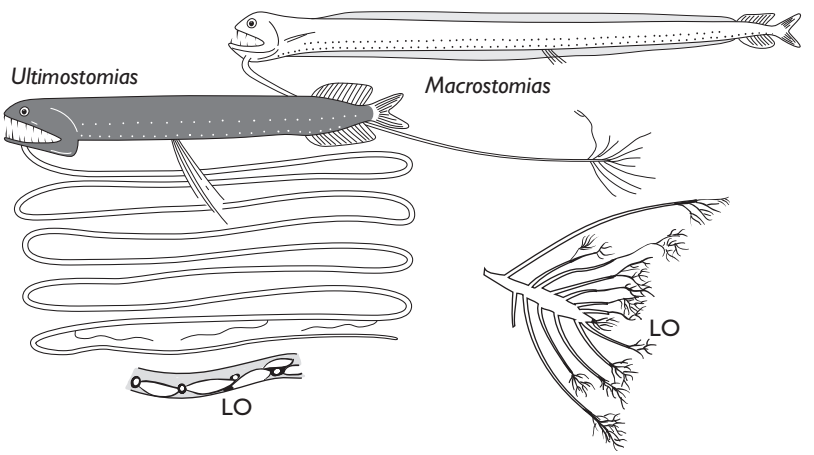


Figure 10.34 Stomiatoid barbels. *Ultimostomias* has light organs (LO) along the barbel, while *Macrostomias* has them at the tip. Note ventrally-directed photophores along the body. After Beebe (1933).

special sacs. So far, only one fish, the angler *Linophryne*, has been discovered which has both kinds.

Bacterial photophores

Bacterial light organs are either linked to the gut, or are open to the sea; the species of *Photobacterium* found in these light organs are obligate symbionts which infect the chambers during larval life. In angler fishes, analysis of their 16S rRNA genes has shown that they are specific to each host fish, and different from the free-living species. They glow continuously, so the fish can only "switch" them off by masking them with a shutter, or by rotating them into a black-lined pocket. The bacteria are in colossal numbers in these light organs. In the spectacularly luminescent flashlight fish *Photoblepharon*, the light organs contain 10^{10} bacteria cm^{-3}! *Photoblepharon* lurks in tropical reef caves during the day, flashing intermittently (by blinking the shutter over its light organs); at night it emerges to hunt copepods by the continuous light shone forwards. Some other bacterial light organs are very much dimmer, and were only discovered by a dark-adapted observer, for example the peri-anal organs of *Chlorophthalmus*; which may be used as cues for schooling. A number of fishes with bacterial light organs use them to illuminate the ventral surface, presumably for camouflage. Externally, there are no special modifications, but internally, there are remarkable specializations. The light organs are diverticula of the gut in such fishes, and are surrounded dorsally and laterally by a connective-tissue reflecting layer. Light therefore emerges downward from the light organ, and is refracted by translucent ventral muscles before passing out of the ventral region of the fish (Figure 10.35). In the extraordinary *Opisthoproctus* (Figure 10.35, left), a light organ near the anus is enclosed in black epithelium except anteriorly, where it shines into a long hyaline ventral light guide surrounded dorsally by reflecting platelets. The bottom of the fish is completely flat and light emerges evenly over the whole of this flattened

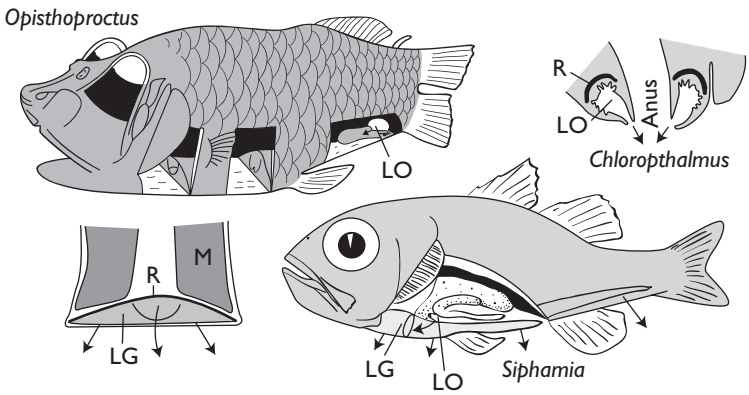


Figure 10.35 Bacterial light organs. Left: *Opisthoproctus* has a rectal light organ (LO) which shines along a light guide (LG) backed by a reflector (R) so that light is emitted along the flat "sole" of the fish (seen in transverse section below). Right top: the light organs (LO) of *Chloropthalmus* lie in the rectum and shine out of the anus. Below: the light organ (LO) of *Siphamia* opens from the gut shining into a light guide (LG) that emits light along the ventral surface to the tail. After Herring (1977); Iwai (1971); Somiya (1977).

sole. *Opisthoproctus* lives in the upper mesopelagic zone, and it seems certain that this bizarre arrangement must be used for ventral camouflage, but we do not yet know how it is tuned to cope with being seen obliquely from below.

Photophores with intrinsic light production

Perhaps partly because of problems of infection and maintenance, fishes with bacterial light organs have four at most and usually only one or two; hence they may economically make the same organ serve several purposes like the flashlight fishes, where the organs are used to illuminate prey, for schooling, and for sexual communication. Fish with photophores, where the light is generated intrinsically, on the other hand, often have many organs, and so can use different ones for different purposes. In lantern fishes (myctophids), for example, ventral series of photophores are used for camouflage while lateral ones (differently patterned in the two sexes) are evidently used for intra-specific signaling.

Sometimes different photophores emit light of different colors. This kind of photophore is often rather like an eye, for the photocytes may be backed with a reflecting layer, capped with a lens and contain color filters. They are richly innervated and under the control of the autonomic nervous system, the transmitter being adrenaline or noradrenaline. By far the most complicated photophores so far studied are those of the sternoptychid hatchetfish, *Argyropelecus* (Figure 10.36). These are arranged in groups of tubes directed ventrally along the lower part of the fish. In each group of photophores, light is produced in a dorsal chamber, lined with black pigment apart from a series of small ventral windows into the photophores. Light passes through these windows, and then through a color filter transmitting at 485 nm, and then enters the wedge-shaped photophore. This is lined with a reflective guanine layer, and the flat external surface is covered with a half-silvered mirror, again made from guanine crystals. The result of this rather complicated design is

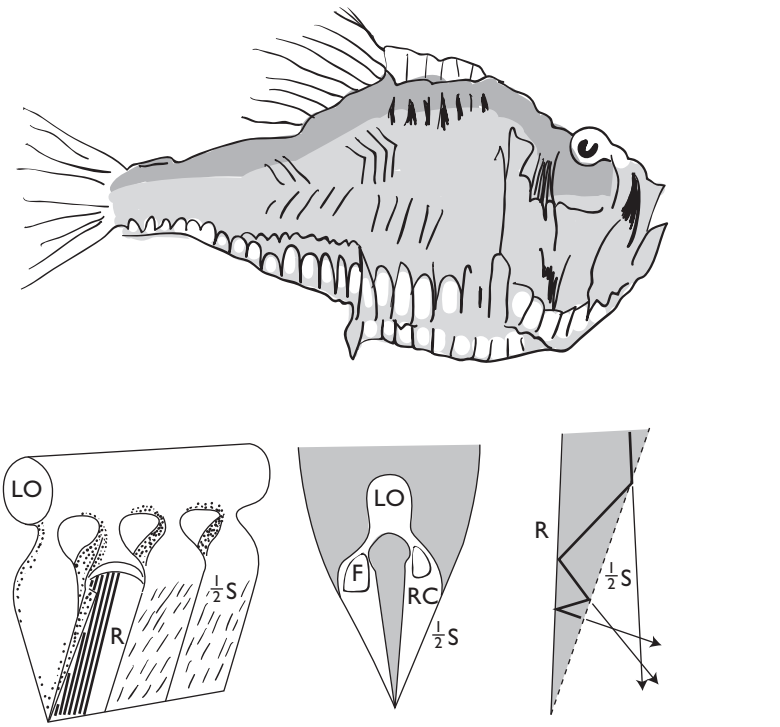


Figure 10.36 *Argyropelecus* and its photophores. Adult fish showing rows of silvery ventral photophores. Left: stereogram of system showing the dorsal light pipe (LO) connected to the ventral reflecting chambers (R) which are backed with a curved reflector, and half-silvered on the outer surface (½ S). Middle: transverse section through ventral part of body showing the light organ (LO), filters (F), and reflecting chambers (RC) with half-silvered outer surface (½ S). Right: reflections of rays emitted from light organ into reflecting chamber giving rise to appropriate radiance distribution for ventral camouflage. After Tinayre (1904); Denton (1970).

that light entering the photophores from the dorsal chamber will leave it in a particular pattern differing in intensity at different angles. Because the inner reflecting surfaces of the photophores are curved, each photophore will distribute its light in a wide arc. Denton (1970) and his colleagues (Denton *et al.*, 1972), who worked out the way in which these photophores operate, set up living hatchetfishes (at sea on board RRS *Discovery*) in a chamber in which they could be rotated while the light emitted was measured by a photomultiplier. They found that the angular distribution of the light emitted was remarkably close to that of ambient light in the sea, as also was its spectral characteristic (Figure 10.37) so making it virtually certain that *Argyropelecus* is hard to see from below!

Obviously, it is not enough for the fish to match the angular distribution and wavelength of the light it emits to the ambient light – it also has to match it in intensity. Hatchetfishes and many myctophids have small photophores which are arranged to shine into the eye (a curious arrangement at first sight); these act as reference sources to permit matching of ambient light intensity (Figure 10.37).

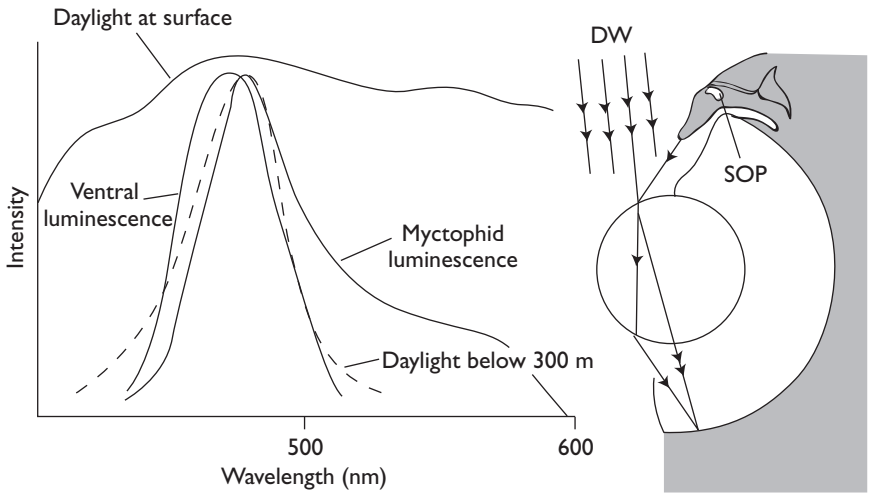


Figure 10.37 Ventral camouflage with photophores. Left: the remarkably accurate spectral match of the light emitted by *Argyropelecus* to down-welling daylight. Myctophid ventral photophores match less well. Right: down-welling daylight and the light emitted by the supraorbital photophore of the myctophid *Tarletonbeania* are compared. From Denton (1980); Lawry (1974).

Provided this little photophore is functionally coupled to the ventral photophores, the latter will be enabled to match down-welling light and so camouflage the fish if it is in a horizontal attitude.

Submersible observations, however, have often shown fish, such as *Tarletonbeania* and other myctophids and sternoptychids, in vertical and sideways attitudes, curious since they have such ingenious and complex down-welling light camouflage. Hatchetfishes produce light about equivalent in intensity to the down-welling light at depths of around 600 m (where the fish are found during the day). Much nearer the surface, fish cannot produce sufficient light for ventral camouflage, and so rely instead on transparency, or on special morphological modifications.

Yellow lenses

The adaptations of fishes are so remarkable that it should come as no surprise to find that a few fishes have devised a means of cracking the ventral photophore camouflage system, by using filtering lenses. The emission spectra of the light emitted ventrally by *Opisthoproctus* and by *Argyropelecus* are an extraordinarily close match to that of down-welling daylight, and yellow lenses (which *Argyropelecus* itself possesses) are of no help in detecting such fishes from below. But the emission spectra of myctophid photophores are much broader; so examined with an upward-looking eye equipped with a yellow lens, they will appear brighter than the background. In the clear mesopelagic zone, Muntz (1975) has calculated that their camouflage could be pierced at a distance of around 16 m. However, it seems that, usually, fish with yellow lenses employ them to *increase* the visibility of *lateral* photophores.

Red headlight fishes

An even more extraordinary special case is the use of photophores to circumvent the camouflage of the common red and dark brown animals of the mesopelagic zone. These are not visible when illuminated with blue-emitting photophores, such as those on the head of the myctophid *Diaphus*. Dragonfishes (stomiatoids) such as *Malacosteus* and *Pachystomias*, however, have large red-emitting headlight photophores underneath the eye, and their retinal pigments absorb at around 575 nm (Figure 10.38) so that they can perceive red light. Most deep-sea animals have pigments with an absorption maximum around 450–490 nm and so cannot perceive red light. With their headlight photophores, *Malacosteus* and *Pachystomias* can easily see red and brown prey, illuminating them with light of a wavelength that the prey cannot detect. Because red light is less well transmitted in the sea than blue light, *Malacosteus* has a red-reflecting tapetum and increased pigment density in the retina to make up for the inevitable loss of sensitivity. Calculations suggest that a dragonfish could detect the red light emitted by another at around 2 m, and could see its own red light reflected off its prey at about 1.3 m. The dragonfish design is obviously suited for feeding on other fish and large crustaceans. However, a single species of dragonfish, *Malacosteus niger*, is in two ways astonishingly different from all the others in the family. As Sutton (2005) has shown (by examination of stomach contents of fish from many world museums), *M. niger* seems to feed chiefly on copepods. He suggested that the fish "snacks"on copepods to keep itself going in between the rare possibility of catching something larger.

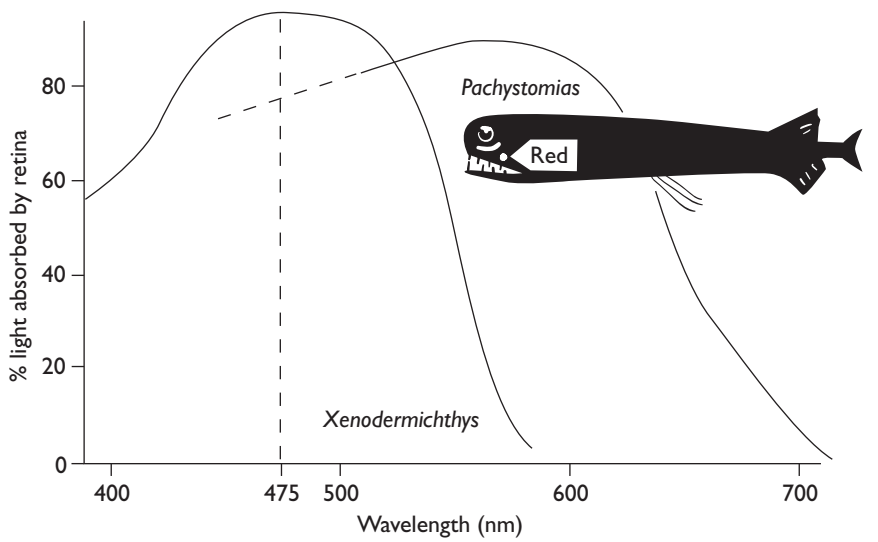


Figure 10.38 Dragonfish red photophores and visual pigments. Curves showing light absorbed by single pass through retina. The alepocephalid *Xenodermichthys* absorbs maximally at the wavelength maximally transmitted by seawater at 300 m (dashed line). The dragonfish *Pachystomias* absorbs maximally much further to the red end of the spectrum, and as it has a red-reflecting tapetum, maximum absorption of the intact eye will be greater than that of the retina alone. After Denton (1980).

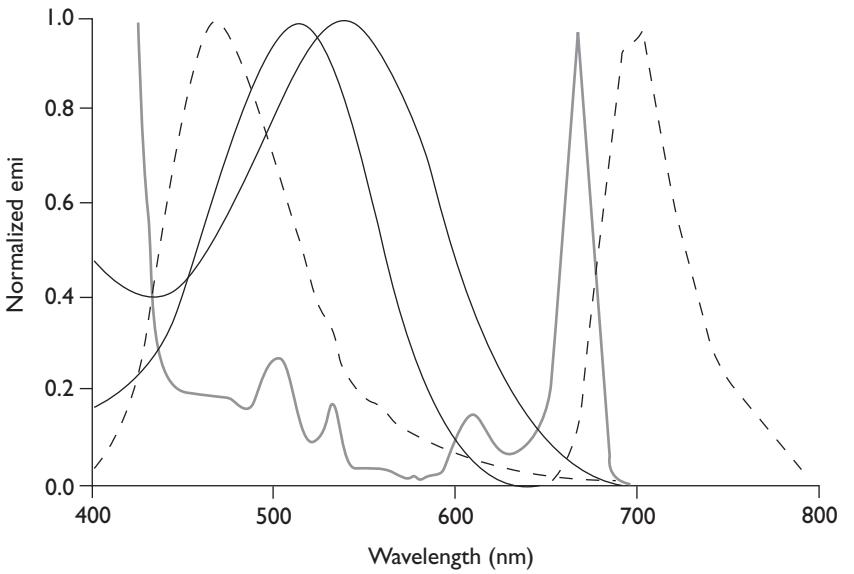


Figure 10.39 Bioluminescence and visual pigments of *Malacosteus niger*. Dashed line: light emitted by photophores; solid black lines: absorption spectra of paired visual pigments; solid gray line: absorption spectrum of the photosensitizer pigments derived from chlorophyll products in prey (see text). From Douglas *et al.* (1999).

What is more, the retina of *M. niger* only contains visual pigments that do not absorb at the longer wavelength emitted by its red photophore! The fish is therefore obliged to store a second retinal pigment (apparently based on modified chlorophyll pigments obtained from the copepods it eats) which is photosensitive and when illuminated with red light, emits a blue-green fluorescence that *M. niger*'s visual pigments *can* detect (Figure 10.39). This is surely one of the most remarkable examples of adaptation yet found in any fish, and is well-described by Douglas *et al.* (1999).

10.11 Taste, Olfaction, and Pheromones

Unlike light and sound, chemical stimuli are persistent and effectively non-directional. Although gradients of concentration may be set up around a chemical source, they are easily disturbed by turbulence caused by wind at the water surface, currents and animals. Chemicals may arouse fishes, and under certain circumstances these fish may be able to swim up a gradient of concentration. In particular, some fishes are programmed to swim up-current if they detect a chemical stimulus.

The distinction between smell (olfaction) and taste (gustation) is less clear in water than on land. Even for us, it is not so easy to distinguish between an onion and a potato if our noses are blocked.

On the whole, as Sorensen and Caprio (1998) observed, the gustatory system appears to be devoted to instinctual recognition of feeding cues, while the olfactory system responds to a much broader range of stimuli, including

geographic locations and pheromones. It is generally assumed that olfaction is distance reception, and gustation contact reception, but in fishes it is perfectly possible for the taste organs to respond to distant sources of stimuli.

In fishes, taste buds are not only found inside the mouth, as in terrestrial animals, but also on the gills, surface of the head, and on the fins and barbels (Figure 10.40). They are not usually found on the tongue.

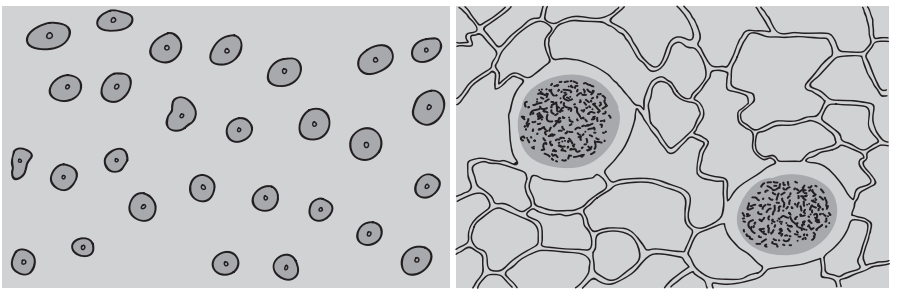


Figure 10.40 Tastebuds covering the barbels of a young reef mullet (*Upeneus*). The sensory cells lie in pits and their microvilli protrude between collar cells. After McCormick (1993).

The chemoreceptors

Taste buds contain receptor or gustatory cells with apical microvilli and supporting cells (although some controversy exists about different cell types) innervated by cranial nerves. Solitary chemosensory cells (SCCs) also exist (see Hansen *et al.*, 2006), and, although much harder to record from than tastebuds, occur sometimes in special concentrations, as in the anterior part of the dorsal fin in rocklings (*Ciliata* and *Gaidropsaurus*). This lies in a groove, and is in constant oscillatory movement, drawing water along to sample what is ahead of the fish. Kotrschal (1995) and his colleagues estimated around 10^5 SSCs mm^{-2} on the spines of this part of the fin. Interestingly, these did not respond to amino acids or other typical taste stimuli, but did respond to fish mucus and diluted saliva, suggesting that the rockling SSCs are used for predator avoidance rather than food detection.

There seems to be a neat division in the gustatory system with the VIIth (facial) nerve innervating the taste buds on the external surface and the IXth glossopharyngeal, and Xth (vagus) nerves innervating the taste buds in the pharynx. Each system plays its own part in feeding behavior, the cutaneous taste buds detecting food, the pharyngeal taste buds judging its quality inside the mouth.

Olfaction

The olfactory organ, which is usually paired, contains many thousands of receptor cells arranged in a rosette of very variable form in a chamber with incurrent and excurrent nostrils, and with olfactory tracts to the forebrain. Usually the incurrent and excurrent nares lie close together on top of the head, but in *Polypterus* and in some coral reef fishes, the incurrent apertures are at the ends of long tubes sticking up out of the head. Lampreys and hagfish only

have a single chamber and single nostril. In hagfish, there is a nasopharyngeal duct opening into the pharynx but in lampreys this duct ends blindly as a nasopharyngeal pouch. In lungfish the excurrent nostril opens into the oral cavity (as it does in tetrapods like ourselves). A pressure differential is created between the incurrent and excurrent nostrils by various means in different fishes – by forward movement, respiratory movements, or by motile cilia. In some ceratioid angler fish, in *Lophius* and gulper eels, and some deep-sea monognathid eels, the female has poorly developed olfactory organs, unlike the male where they are hypertrophied. The reader may care to think of other "deep-sea" features of *Lophius*, which in several respects seems a deep-sea fish that has moved up into shallow water.

The olfactory sensory epithelium consists of three types of receptor cells, either with several apical cilia, numerous microvilli, or an apical pit, separated by supporting cells. The cilia may have a 9 + 2 or 9 + 0 arrangement of filaments. The receptor cells are present at extremely high density – as many as 0.5×10^6 mm^{-2}. In some fish, at least, specific classes of olfactory receptors send axons to glomeruli in particular regions of the olfactory bulb, so that there is a (somewhat coarse) chemotopic map in the bulb (Laberge and Hara, 2004). Recent immunocytochemical and electrophysiological work (reviewed by Wirsig-Wiechmann *et al.*, 2002) has shown that the receptors can be modulated by GnRH in the nervus terminalis (p. 279; see also Wirsig-Wiechmann, 2001).

Like the optic cups, the olfactory organ develops very early in ontogeny. Initially it occurs as an olfactory pit, seen after hatching in many larval fishes. Later the pit sinks into the front part of the head in most species, forming a cavity, but sometimes remains open, for example in the needlefish, *Belone belone*. After a false start (see Hara, 2005) when many trials were made with particularly smelly (to us) volatile chemicals, it was realized that fish were most sensitive to *non*-volatile amino acids, responding down to thresholds of 10^{-8} – 10^{-9} M and even lower, 10^{-11} – 10^{-13} M, for bile salts, steroid hormones and prostaglandins. None of these can usually be smelt by us! Most of the data about fish olfactory thresholds were obtained by measuring electro-olfactograms (EOGs) after applying test chemicals. These are slow extracellular negative potentials across the olfactory epithelium (see Hara, 1992). Particularly low thresholds have been claimed after training for certain alcohols – when the eel *Anguilla anguilla* apparently responds to about 10^{18} M phenylethyl alcohol, equivalent perhaps to a single alcohol molecule in the olfactory chamber!

Feeding and chemoreception

Chemoreceptors are involved in feeding, predator avoidance, reproduction, and homing. It is not surprising that there is a high sensitivity to amino acids, an important component of food. Some species, such as mullets, actively flick their barbels around in the substrate, while silurid catfish trail their sensory barbels over the substratum and bullheads (*Ictalurus*) often feed on benthic invertebrates at night or in water of low visibility. The cutaneous taste buds probably trigger pick-up behavior but the food may be rejected after tasting within the mouth. There seem to be different types of sensory cells in taste-buds, which stain differently (possibly representing turnover), and at least in some fish (such as *Lepisosteus*) a few of the sensory cells receive efferent inner-

vation at their bases. Other senses such as the lateral line or electroreceptors may also play a part (p. 303). Tuna, on the other hand, have large olfactory organs although they are typically visual hunters. In experiments, chemical extracts of potential prey cause increased activity, a change of the pattern of swimming and occasional snapping movements. Thus their awareness of food nearby is increased and, after visually-mediated seizure of food, they may still reject it on the basis of its taste. Almost certainly, sharks alerted by the smell of prey (the blood of fish or humans in the water) orientate to the noise created by splashing and struggling. Probably, *Ictalurus* can make a true gradient search in stagnant water to locate a chemical source, such as liver juice, from 25 body lengths' distance. To follow odor trails to their source requires the fish to balance the intensity of the odor in its two olfactory organs or to achieve increasing odor concentration when making successive "sniffs." The fish should then be orientated towards the source.

There is naturally a great deal of interest in these topics by fishermen and fisheries scientists developing artificial baits. If the most important chemicals for inducing feeding behavior can be identified, they could be incorporated into slow-release bait blocks that might last for weeks or months, and greatly reduce the labor of rebaiting lines or traps. Amino acids seem to be the most important but the particular acid or acid combination varies from one fish species to another. Chumming, to attract fish to a locality where chemical attractants have been released, has long been established. Perforated cans of dog food, or fish blood and guts in a netting bag, are remarkably effective for pelagic sharks, but fishermen also use live bait, to provide visual and auditory as well as chemical stimuli.

Reproduction and chemoreception

Pheromones are substances secreted into the environment, and received by other individuals of the same species in which they release specific reactions, are often associated with reproductive processes. Some of the earliest work showed that the female estuarine goby (*Bathygobius soporator*) when gravid produced a chemical in its ovarian fluid that elicited courtship behavior in the male. Male goldfish use their sense of smell to identify ovulated females. For example, in a maze they preferred water containing ovulated females or ovarian fluid. Several species of oviparous fishes, including salmonids, bitterling (*Rhodeus*), loach (*Misgurnus*), ayu (*Plecoglossus*), and eels (*Anguilla*), also show behavioral responses of the male to ovulated females. It may be a more common phenomenon in freshwater where fish are more confined and chemicals less liable to dispersion. Whether hormones, excreted in urine or gradual fluids, are acting as pheromones, is now under examination, and there is accumulating evidence that steroids are involved as pheromones in stimulating oocyte maturation and milt production in goldfish. Of particular interest is how species-specific these pheromones are. Recent work on the Lake Malawi cichlid *Pseudotropheus emmiltos* (Plenderleith *et al.*, 2005), has shown that females only preferred to spawn next to conspecific males if olfactory clues were present. If these were absent, their own breed of males and males of a closely related species were not distinguishable. Some characin males have even developed a special pheromone pump to attract females on the caudal fin hence they are in the family *Glandulocaudinae*.

Homing and chemoreception

The study of homing has been confined almost entirely to salmonids – salmon, trout, and charr. Many of these are anadromous fish returning to their home stream after a period of maturation elsewhere, often in the open sea. The role of chemoreceptors in helping fish to find their way back, and to identify the home stream precisely, has been investigated over many years using a number of standard techniques. These have included comparing the homing ability of control fish and those with cut olfactory nerves, cauterized olfactory organs, or plugged nostrils. Generally fishes treated in this way show high rates of straying and some lose their homing ability completely.

The imprinting hypothesis of Hasler and Wisby (1951) proposed that streams have a characteristic odor that the juveniles such as salmon smolts learn by imprinting before they leave for the migratory phase of their life histories. (Of course, imprinting can also apply to other sensory cues.) A further hypothesis by Stabell (1984) proposes that the home-stream odor is actually a pheromone released by fish resident in the stream and specific to that fish population. This requires a two-way system of overlapping migrations – downstream of smolts, and upstream of adults.

Complex experiments, involving the transplanting of fishes at different life-history stages to foreign rivers, show that there are many inconsistent findings to explain and that it is difficult to define an all-embracing mechanism for homing by odor recognition. Generally, transplanted salmon parr and smolts return as adults to the river of release rather than their native river, but the incidence of straying is higher.

A promising line involves the artificial imprinting of salmon with low concentrations of chemicals such as morpholine and phenethyl alcohol. Coho salmon were subjected to one of these chemicals in the hatchery at the smolt stage. At an appropriate time after release, the returning adults were given the choice of streams scented with morpholine or phenethyl alcohol; 95% of the morpholine-treated fish were recaptured in the stream containing morpholine and 92% of the phenethyl alcohol-treated fish were recaptured in the phenethyl alcohol-treated stream. One of the most interesting findings is that salmonids can distinguish water which has been occupied by their own siblings. Behavior experiments in various types of flow-through tank where the fish were given a choice of water to swim in, showed that they preferred water in which their siblings had swum. The EOG in the olfactory bulb of Arctic charr (*Salmo alpinus*) is characteristic for the odor of different charr populations (Figure 10.41).

It is becoming increasingly obvious that kin recognition by chemical cues is an attribute of other fish groups, for example the recognition of young by the parents in cichlids and in the threespine stickleback (*Gasterosteus aculeatus*). Recognition of their young may allow the parents to protect them preferentially and even to avoid eating them.

Alarm substance

It was first shown by von Frisch (1938) that injured minnows (*Phoxinus phoxinus*) produce an alarm substance (*Schreckstoff*) from their skin that elicits a fright reaction in conspecifics. His standard test procedure was to cut 0.2 g of fresh skin 150 times with scissors, and dilute in 200 ml of water. Shaking for

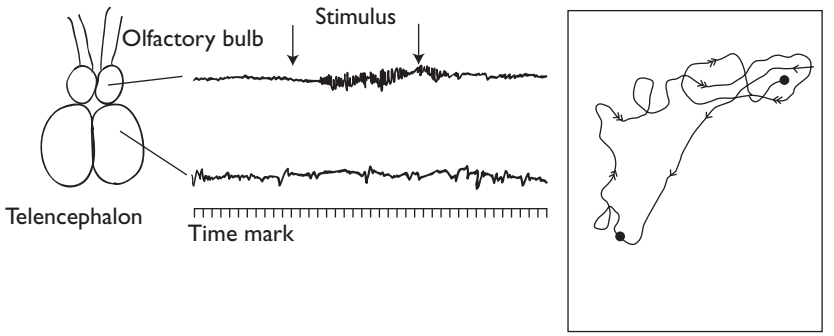


Figure 10.41 Chemoreception. Left: recordings from olfactory bulb (above) and telencephalon (below) of a charr stimulated by a water sample in which young charr of the same race had been swimming. Right: superimposed paths of intact (single arrows) and blinded (double arrows) catfish (*Ictalurus*) tracking a point of chemical release After Døving (1973); Bardach *et al*. (1969).

30 min was followed by filtration and serial dilution as required. 100 ml of the test solution was poured down the feeding tube into the tank in 45 s. Aquarium hatched minnows responded to dilutions of 1:50 000. This response is found in the Ostariophysi, although not in all species (Døving *et al*, 2006). The alarm substance is produced in epidermal "club" cells and is probably hypoxanthine-3(N)-oxide. It is not species-specific. Since the "club" cells lack ducts to the exterior, alarm substance is only released when the skin is damaged. Wisenden (2000) discusses the intriguing question of the origin of such a group protective mechanism since the injured fish derives no benefit itself, and there is a significant metabolic cost in alarm substance production. Predators produce particular odors (kairamones) during their attacks, to which prey species react.

Envoi

It is hard not to be dazzled by the extraordinary range of adaptations of the sensory systems used by fish, partly perhaps, because they employ sensory systems alien to us, but also because these systems are tuned in remarkable ways. It requires an effort of imagination to understand that the brilliant colors of tropical reef fish are camouflage to their predators, or that herring are interested in quite different features of sounds than we are. Each reader no doubt will be impressed by different sensory systems, yet the astonishing independent electrosensory systems of mormyrids and knifefish are possibly the most fascinating of all.

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11 The Nervous System

This chapter only covers just a few of the interesting topics that come to mind when thinking about the fish nervous system. The reader will have seen already (unless rashly beginning with this chapter) that very many aspects of fish biology are linked to the nervous control of different systems such as circulation, digestion, or locomotion, and an entire large volume could well be devoted to even just one of these. So in this little book, all we can do is give an overview of the brain and peripheral nervous system, and look rather sketchily at such aspects as segmentation, cerebellar function, analysis of sensory input, or the different kinds of links between nerve and other cells.

As in all vertebrates, the fish nervous system is made up on the one hand of the brain and spinal cord (the central nervous system or CNS) with the motor and sensory nerves linking the CNS with effector organs and receptors; and, on the other, of the autonomic nervous system controlling involuntary visceral functions. Workers on the nervous system controlling the gut like to count it as a separate system, the enteric nervous system, although it is really just (a most important) division of the autonomic.

The brain is conventionally divided into fore-, mid-, and hindbrain (or prosencephalon, mesencephalon, and rhombencephalon), and since most texts deal with the vertebrate brain in this way, following this approach makes it easier to compare the fish brain with those of other groups (Figure 11.1). A difficulty in the tripartite approach is that this obscures the real interdependence of the different regions and, ideally, it would be better to consider the fish brain in terms of systems rather than regions. Yet work in the past decades has shown that the tripartite division of the brain is indeed partly underlain by ancient developmental mechanisms inherited from prevertebrates (next section). We have compromised here between an account of the regions and a survey of various systems.

In some special cases, for example the Mauthner neuron (Figure 11.25), the visual system, and the electroreceptive system (p. 374), a good deal is known of the way in which the anatomical arrangements function, but, especially in the brain, much more is known of anatomy and histology than of the part that particular anatomical arrangements play in the life of the fish.

Like all nervous systems, the fish nervous system is very largely based on the electrical activity of its nerve cells or neurons, which depend upon their

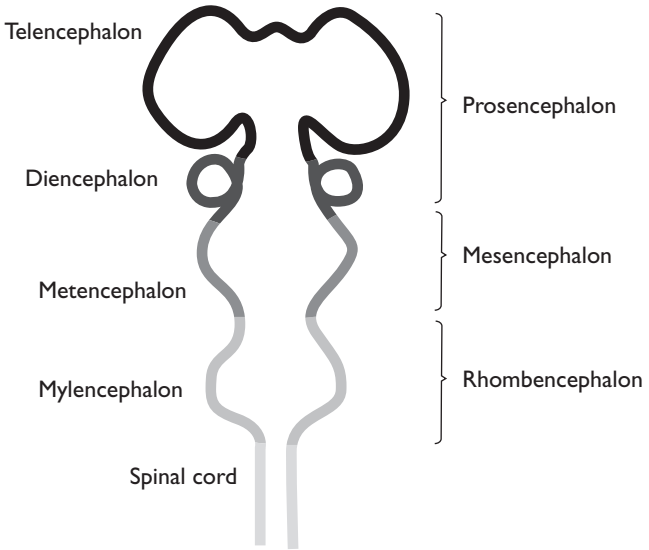

Figure 11.1 Brain regions in an embryonic vertebrate.

own intrinsic membrane properties, and the changes in these properties brought about by chemical and electrical synapses with other cells. The two kinds of synapses have interestingly different properties. Electrical synapses (gap junctions) between neurons permit very rapid signal transmission and precise timing, and also biochemical as well as electrical coupling (see Hormuzdi *et al.*, 2004). They are often bi-directional, in contrast to chemical synapses. The great number of ion exchanges across membranes that this involves is largely responsible for the high metabolic cost of the central nervous system.

11.1 Glia

We are accustomed to think of the CNS in terms of neurons synapsing with each other, and virtually all functional studies of the vertebrate CNS have concentrated on the properties and connections of neurons. But, it is important to bear in mind that neurons and their connections are not the whole story. Glial cells of various kinds (which also sheath the peripheral nerve fibers) make up perhaps half the volume of the CNS and over 50% of the cells within it.

Glial cells were at first regarded as simply "insulating," and "packing" material for neurons, which was convenient for students, since they could be more or less neglected however intriguing they looked in the superb metallic impregnation preparations of Golgi, Retzius, Ramon y Cajal, and Lorente de No. No one who has ever looked at a successful gold-sublimate slide of astrocytes could ever forget it. But understanding of their functions and the ways in which they interact with neurons has been revolutionized in the past decade, and it is important that this is understood. Indeed, at least some of the central glial types may be regarded as functional units with neurons (Fellin and Carmignoto, 2004; Beierlein and Regehr, 2005; Figure 11.2). Several recent studies have shown

electrical coupling between neurons and glial cells (see, e.g., Pakhotin and Verkhratsky, 2004), which may be related to transfer of second messengers rather than electrical transmission. The relation of glial cells with cerebral blood vessels is of great importance in regulating neuron environment (e.g. Koehler *et al.*, 2006), and may have important temporal effects where neuronal synapses and transmitter release and uptake are closely organized, as on the Purkinje cells of the cerebellum (p. 368).

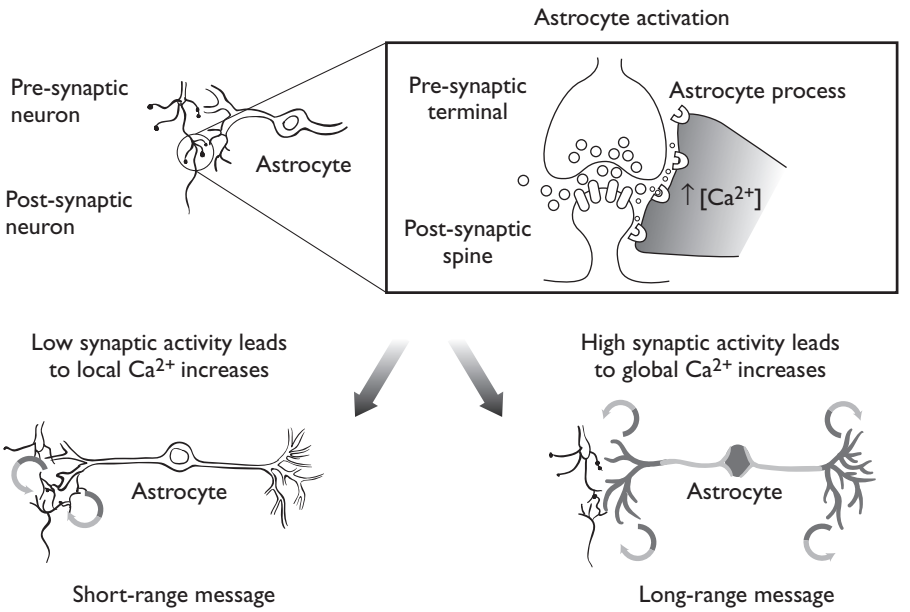

Figure 11.2 Neuronal-astrocyte interactions. From Fellin and Carmignoto (2004).

11.2 Origins

The longitudinal subdivision of the brain into fore-, mid-, and hindbrain is based on subdivisions in development seen as slight constrictions of the neural tube, the posterior being called rhombomeres or neuromeres (Figure 11.1). The three most anterior divisions are larger than the others and give rise to the definitive telencephalon, diencephalons, and mesencephalon. Behind these, seven further rhombomeres subdivide the major part of the rhombencephalon, leading to the spinal cord itself. Each rhombomere has an individual identity, as seen by the gene expression patterns of specific regulatory homeobox genes early in development. For instance, in zebra fish, which has been much used in these studies with remarkable and fascinating results (see, e.g., Maves and Kimmel, 2005), the zinc-finger gene Krox-20, which functions as a transcriptional regulator, is expressed very early in development in two patches which later develop as the hindbrain rhombomeres 3 and 5. Each rhombomere contains neurons with rhombomere-specific axon navigation behavior and neural crest cells with specific migration routes. In view of the

similarity of brain gene expression in all vertebrates, it is evident that the molecular basis of patterning in the developing brain of the convenient zebra fish has been conserved in all fish. When did it arise?

Whatever view we take of the relationships of protochordates and craniate chordates, (recollecting from Chapter 1 that rather than our respectably ancient amphioxus sister group, we may have to acknowledge ascidian tunicates as our sister group) the only protochordate central nervous systems at all comparable to those of fishes are those of the ascidian tadpole, and amphioxus. These look rather unlike the fish CNS, but as Shimeld and Holland (2000) pointed out, gene expression studies reveal that the embryos of vertebrates, amphioxus, and ascidia each have a distinct rostral domain of the neural tube, marked by *Otx2* expression, and a posterior domain marked by *Gbx2* expression, the boundary between the two being the midbrain/hindbrain boundary (MHB; Figure 11.3). Further scrutiny of the evolution of the MHB by Castro *et al*. (2006) has shown a single *Gbx2* gene in amphioxus, but none in tunicates, and we are left in the dark whether or not tunicates ever possessed an MHB, and even if they did, did it have organizing properties as in vertebrates? Perhaps the role of the MHB as an organizer appeared later in phylogeny, since the MHB in amphioxus apparently lacks organizing properties. The tri-partite division of the CNS in chordate neural tubes manifested by the expression of *Otx*, Pax 2/5/8, and Hox gene domains (Figure 11.3), corresponds to the fore-/midbrain, isthmo-cerebellar/midbrain/hindbrain-boundary, and the hindbrain/spinal cord of vertebrates. So fish inherited the genes controlling CNS structure from protochordates, as protochordates presumably did from earlier bilateria (see Reichert and Simeone, 2000 and Wilson and Hovart, 2004).

11.3 Spinal Cord

In gnathostome fish, as in other vertebrates, the CNS is formed by the inrolling and fusion of neural folds, and this leads to a more or less circular section spinal cord, with a central cruciform gray area surrounded by the fiber tracts of the white (Figure 11.4). In lampreys and hagfish the spinal cord is initially circular, but becomes flattened and ribbon-like during ontogeny.

White and gray are ancient anatomist's terms which refer to the naked-eye appearance of fresh sectioned cord (originally from animals and cadavers of the condemned). The central zones contain neuron cell bodies that are grayish by virtue of their lipofuscin pigment content, contrasting with the white fiber zones. When you look at fixed and stained sections of spinal cords, most will probably be treated to stain electively the myelin sheathes of nerve fibers (e.g. by Weigert's hematoxylin), and in this case the white matter will be dark grayish-blue and the gray matter paler and yellowish. Neither lampreys nor hagfish have myelinated axons in the spinal cord, so in Weigert preparations this appears uniformly pale. A small central canal contains the curious Reissner's fiber, continually secreted by an ependymal sub-commissural organ in the roof of the third ventricle. Studies in mammals have shown that Reissner's fiber consists of glycoproteins including sialic acid, and apparently regulates the cerebrospinal fluid (CSF) concentration of biogenic amines (such as noradrenalin) in the cerebrospinal fluid.

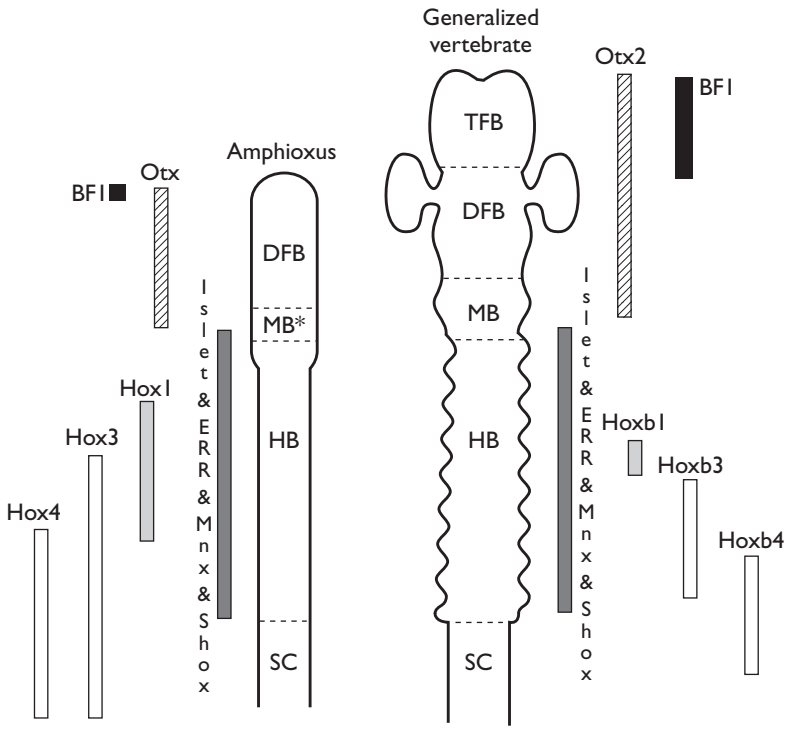

Figure 11.3 Comparison of anterior CNS regions in amphioxus and generalized vertebrate. Beside each, some key neural marker orthologous gene expression domains marked similarly. From Shimeld and Holland (2005).

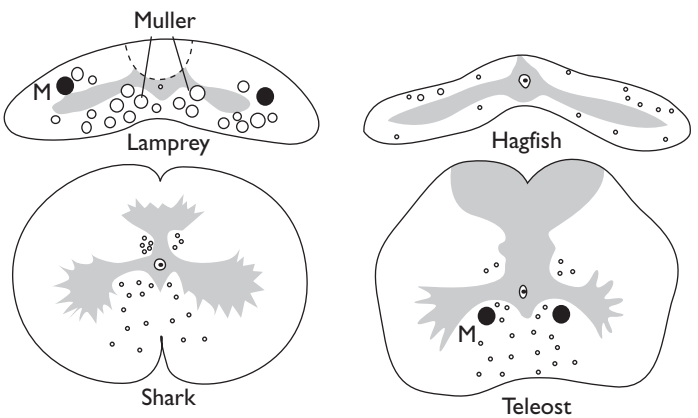

Figure 11.4 Cross-sections of the spinal cord in different fishes. Note large Mauthner axons (M) in lamprey and teleost, and the equally large Muller axons in the lamprey.

The early functional organization of the spinal cord, is best known in amphibian embryos (Figure 11.5) and is similar in lampreys, zebrafish, and the Australian lungfish *Neoceratodus* (Figure 11.6), the fish groups where it has been partially worked out. With these relatively simple segmental neuron patterns, the embryos are capable of oscillatory swimming and later, as adults, dogfish will swim as spinal fish (i.e. after the brain has been destroyed). So too will lampreys, and teleosts, but they may need some encouragement from various drugs to do so. Roberts (2000) has modeled the way in which these simple segmental patterns interact to generate swimming movements in embryonic amphibians, and it seems economical to suppose that they operate similarly in fish. In later just-hatching amphibians, where there are greater numbers of neurons, and the cord is more complex (Figure 11.7), the interactions of the cord neurons are likely to be essentially similarly organized to those in the earlier embryos.

One possible approach to the functional roles of spinal cord neurons is to examine the ontogeny of body movements together with the development of neurons in the spinal cord. Thus, for example, in the developing embryos of the Australian lungfish *Neoceratodus* (Figure 11.6) the first movements are myogenic (i.e. generated by the muscles themselves) before they are reached by the axons of motoneurons. At a slightly later stage the embryo bends away from touch on one side, and, later still, although it first bends away, this movement is followed by movements toward the stimulus, until finally, just before hatching, these flexions continue as a series of swimming movements. The first bending toward the stimulated side of the embryo correlates with processes of the transient Rohon–Beard column of dorsal sensory cells reaching the skin, and with the appearance of dorsal interneurons. Sustained swimming correlates with the later appearance of dorso-lateral interneurons with

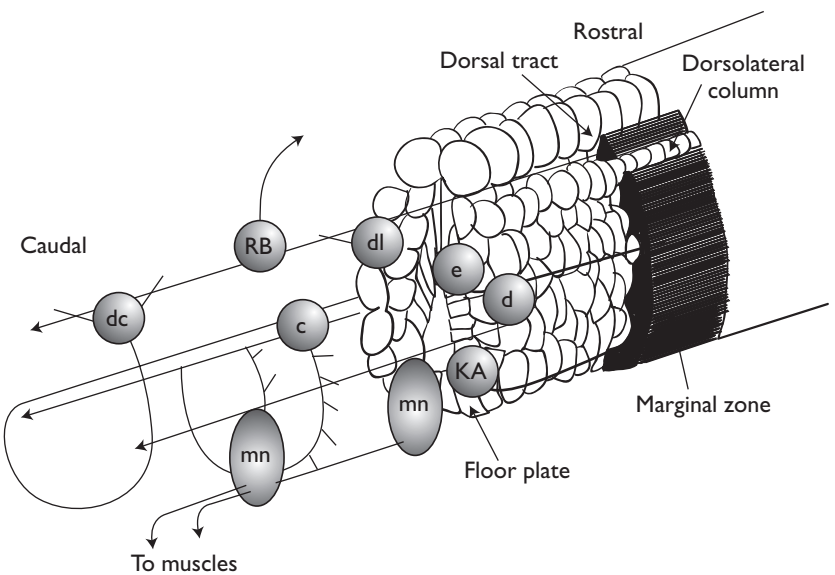

Figure 11.5 Amphibian (*Xenopus*) spinal cord at time of hatching.
RB: Rohon–Beard cells, mn: motoneurons. From Roberts (2000).

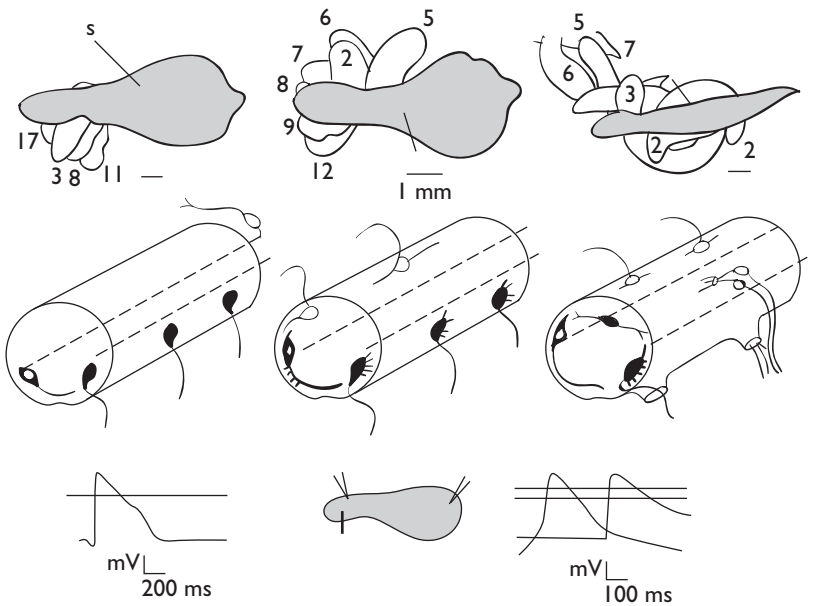

Figure 11.6 Early movements and spinal cord structure in the embryonic Australian lungfish, *Neoceratodus*. Upper: the gradual development of movements in response to stimulation by a sharp hair (s). In the earliest embryos to show responses (left) the response is head movement to the side away from the stimulus. At a later stage (middle) an initial movement away is followed by a movement towards the stimulated side. Last, at a later stage still (right), touch evokes a rapid swim. In each, the outline of the initial position of the embryo is shaded, and the numbers next to the other outlines are milliseconds after stimulation. Middle: spinal cord neurons at stages corresponding to the movement patterns shown above them. Sensory Rohon–Beard cells, clear; motor neurons, dark; internuncial neurons, nuclei shown clear. Bottom: intracellular records of action potentials evoked by touch in the skin of the embryo; right, action potential evoked by stimulation near one recording site travels along the skin to the second electrode (as shown in middle). After Whiting *et al.* (1992) and Bone *et al.* (1989).

bi-lateral dendritic fields, and with further development of ventral interneurons. Less is known at present of the circuitry in *Neoceratodus*, or other fish, than in amphibian embryos (Figure 11.7), but as McDearmid and Drapeau, (2006) have shown, further studies on zebra fish will redress this.

Although embryonic and larval fish have a remarkably large number of neurons in the Rohon–Beard system, all are merely transient and disappear at metamorphosis: they are replaced by the sensory neurons of the dorsal root ganglia. We will see (p. 355) similar massive neuron death in the motoneuron columns of the spinal cord.

The adult spinal cord is divided into functional regions, seen schematically in Figure 11.8, the dorsal neurons and fiber tracts of the somatic and visceral sensory divisions lie above the viscero- and somatomotor divisions which consist of the ventro-lateral neuron masses in the gray (Figure 11.8) and the large ventral ascending and descending tracts of the ventral white. In most fish,

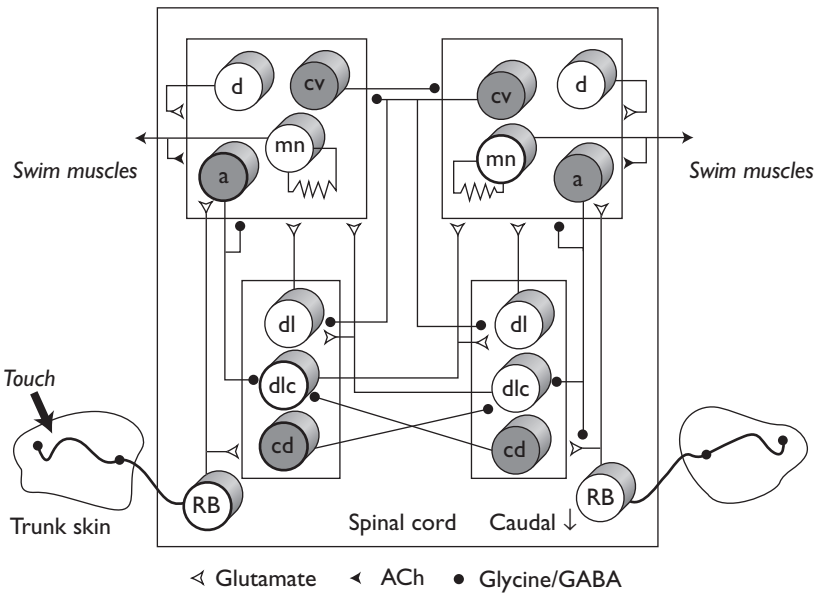

Figure 11.7 Speculative spinal circuit accounting for contralateral flexion followed by swimming, in response to touch on left side (at arrow). The circles represent longitudinal columns of neurons along cord. Shaded interneurons are inhibitory; so are dark circles representing synapses. Excitatory synapses represented by triangles. Zig-zag line represents electrical connections between motoneurons. RB: Rohon–Beard cells; dl: dorsolateral interneurons; dlc: dorsolateral commissural neurons; mn: motoneuron; cv and cd: ventral and dorsal commissural interneurons. From Roberts (2000).

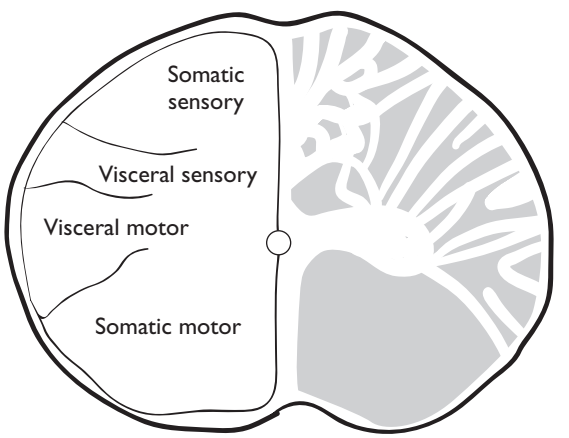

Figure 11.8 Diagram showing functional organization of spinal cord in the spurdog (*Squalus acanthias*). Right: distribution of myelinated fibers (stippled); left: the sensory and motor column of the spinal cord.

except sharks and rays, large paired Mauthner axons (see p. 373) are conspicuous as they descend in the ventral white, giving off collaterals to somatic motor neurons.

The organization of the fish spinal cord is well-conserved throughout the vertebrates as manifested by the distribution of neuropeptides in the dorsal horn of mammals and elasmobranchs. (Figure 11.9). The original design (possibly adumbrated in the ascidian tadpole, Brown *et al.* (2005)) was based on the control of oscillatory swimming and needed little modification for quadripedal locomotion. Indeed, the young African lungfish *Protopterus* walks along the floor of its tank using its extended thread-like pectoral and pelvic fins as legs, just like any terrestrial quadruped.

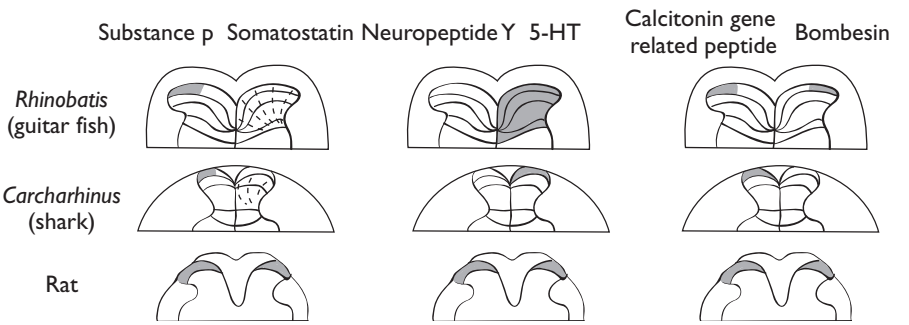

Figure 11.9 Comparison of the distribution of 5-HT (serotonin) and various neuropeptides shown by immunocytochemistry in the dorsal horn of the spinal cord in two elasmobranchs and in the rat. After Cameron *et al.* (1990).

Spinal nerves

Except in lampreys (where dorsal and ventral spinal nerves do not unite) the dorsal and ventral roots emerge separately from the cord and then join to form the mixed sensory and motor spinal nerves. The ventral roots contain mainly the axons of the spinal somatomotor neurons passing to the myotomal and fin musculature, and in addition visceromotor autonomic fibers. In the stingray (*Dasyatis*), there is monosynaptic input to the motoneurons from proprioceptors among the wing muscles, just as spindles have monosynaptic input to mammalian motoneurons (see p. 289). Monosynaptic proprioceptive input is absent in sharks, where there are only slower acting proprioceptors. In adult dogfish (*Scyliorhinus*) there are some 350 fibers in the mid-abdominal ventral roots, while in the much smaller zebrafish, around 100. These axon numbers in the adult are the remnant of a veritable holocaust or cull of spinal motoneurons during development, by apoptosis. For example, Sakamoto *et al.* (1999) found during angelfish development, the ventral roots had some 210 axons, whereas, later, only just around half this. There are also culls of other embryonic neuron types (Eisen *et al.*, 1990). The ventral roots pass out of the cord between the myotomes, so that the motor axons in each root supply two adjacent myotomes. There is also some overlap due to motor axons passing along the cord rather than emerging from the ventral root nearest to their cell bodies, and, in teleosts, axons may emerge in a ventral root and then cross

several myotomes before innervating muscle fibers. The axons in the sensory dorsal roots are from the sensory neurons in the dorsal root ganglia, whose dendrites go to the periphery.

Spinal swimming

Sharks are not immediately paralyzed if the brain is destroyed, as are other fish, and provided they are set up appropriately (with a flow of water over the gills) spinal dogfish continue a stereotyped slow swimming pattern for many hours (see Figure 3.9). Tail-beat frequency is around 0.6 Hz, a little below that of slowly cruising intact animals, but this slow spinal swimming can be modified in amplitude and frequency by various stimuli. Spinal dogfish were at one time much used for studying such questions as the role of sensory input in modifying the swimming pattern, or the existence of a spinal central pattern generator driving rhythmic swimming. Unfortunately, pharmacological "dissections" of spinal cord pathways in the dogfish using specific agonists and antagonists to different transmitters (for example glycine or glutamic acid) are not at all easy.

So later, spinal lampreys became the chosen preparation, mainly because the spinal cord is robust and very easily accessible, as are the (conveniently separate) spinal roots. Spinal lampreys do not swim spontaneously, but rhythmic motor activity from spinal cords in experimental baths can be evoked by adding small amounts of L-DOPA or D-glutamate to the bath (see Figure 11.10). The patterned motor activity of such "fictive" swimming may be recorded by suction electrodes placed on the ventral roots, showing the same phase lags between segments as in normal swimming. Similar phase lags (but out of phase with the myotomal muscles) are shown by the fin motoneurons (Mentel *et al.*, 2006). Glutamate inhibitors such as D-aspartate, abolish fictive swimming and glycine is likely concerned in linking phasic neuron activity on each side of the cord. Most recently, spinal zebra fish have been used (e.g. Masino and Fetcho, 2005). Zebra fish have the great advantage of having been extensively used for genetic studies. They show fictive swimming after addition of N-methyl-D-aspartate (NMDA), and have been used to study central pattern generators in normal and mutant embryos.

A striking difference between the spinal cord of adult fishes and those of terrestrial vertebrates is the absence of large numbers of proprioceptive fibers from muscle spindles (which seem absent from any fish, p. 289). Another difference from the better-known spinal cords of higher vertebrates, is that in fish there are very many more dendro-dendritic connections. For instance, the motoneurons and interneurons of dogfish or lamprey cords have relatively few synaptic inputs on their cell bodies (somata), instead connections are made on their enormous dendritic fields (Figure 11.8). Why this should be so is unclear.

11.4 Cranial Nerves

The cranial nerves I-XII were first named by human anatomists, so their names do not necessarily fit too well with fish. The facial (VII), for example, certainly innervates the snout, but also the spiracle, remote from the "face" of a fish. Only the vagus (X) aptly named for its wandering, wanders also in fish. The classical layout of the cranial nerves in a shark is seen in Figure 11.11A; their segmental

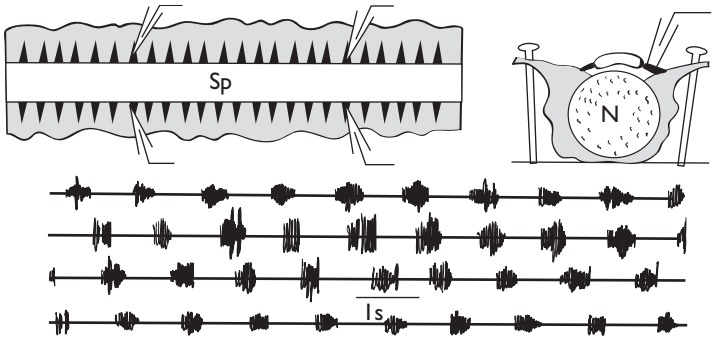

Figure 11.10 Rhythmic motor activity in isolated spinal cord. Above: experimental arrangement of lamprey spinal cord (Sp) isolated to record from ventral roots (black) with suction electrodes. N: notochord. Below: simultaneous records from four suction electrodes on different right (upper pair) and left (lower pair) ventral roots showing rhythmic motor output in preparation paralyzed with curare and bathed in a solution containing 0.6 mM D-glutamate. After Cohen and Wallén (1980).

affinity and functional components are given in Table 11.1, while Figure 11.11B shows their connections schematically. Both show how they accord with the dorsal sensory:ventral motor layout we have seen in the spinal cord. Dorsal and ventral cranial nerves remain separate (as in amphioxus and lampreys, possibly the primitive condition). Remembering the origin of the optic nerve (outpouching from the brain), it evidently does not form part of the segmental series. Both the nervus terminalis (0) that enters the brain just behind the olfactory nerve (I), and the olfactory nerve itself are also not obviously part of the segmental series of cranial nerves. The olfactory nerve is unique, because its cells of origin lie at the periphery, in the olfactory mucosa. Where the olfactory nerves are long, in a long snout, as in the gar *Lepisosteus*, this has yielded physiologists an excellent preparation of long uniform nerve fibers for experiments on the heat production of the nerve impulse.

Head segmentation

After some false starts by previous theorizers (including Goethe), the segmental relations of the cranial muscles, nerves, and gill arches were worked out by the Cambridge embryologist F. M. Balfour, who studied elasmobranch embryos at Dohrn's Stazione Zoologica in Naples, and then died in a climbing accident in the Alps when he was only 31. His conclusion that the head was merely a modified portion of the trunk and made up of serially homologous segments (Balfour, 1878) is shown in Goodrich's well-known diagram (Figure 11.11A), and in Table 11.1. This scheme was generally agreed by morphologists, and appears in classic texts such as the French *Traité de Zoologie*, or J. Z. Young's (1981) *Life of the Vertebrates*.

The alert reader will naturally enquire how this long-established scheme of head segmentation fits with the successive rhombomeres and the expression patterns of gene domains seen in Figure 11.3. The answer looks like being the curate's reply to the bishop's query about his breakfast egg: good in parts. Far from being a long-dead issue, head segmentation has recently generated lively debate. Despite over a century of universal acceptance, recent embryological

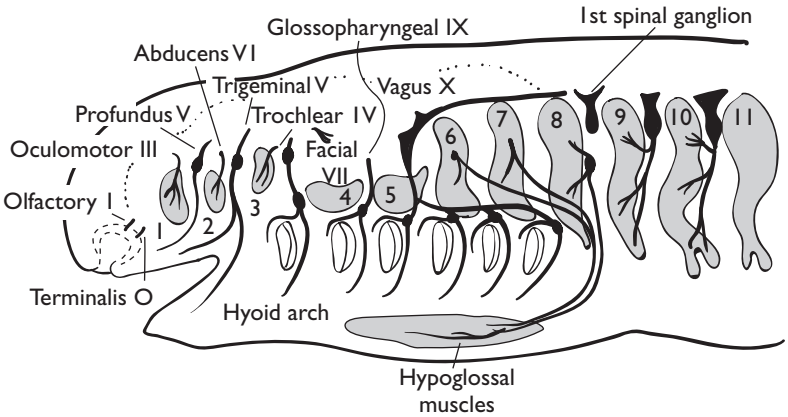

Figure 11.11A Cranial nerves of the embryo dogfish. Modified from Goodrich (1930).

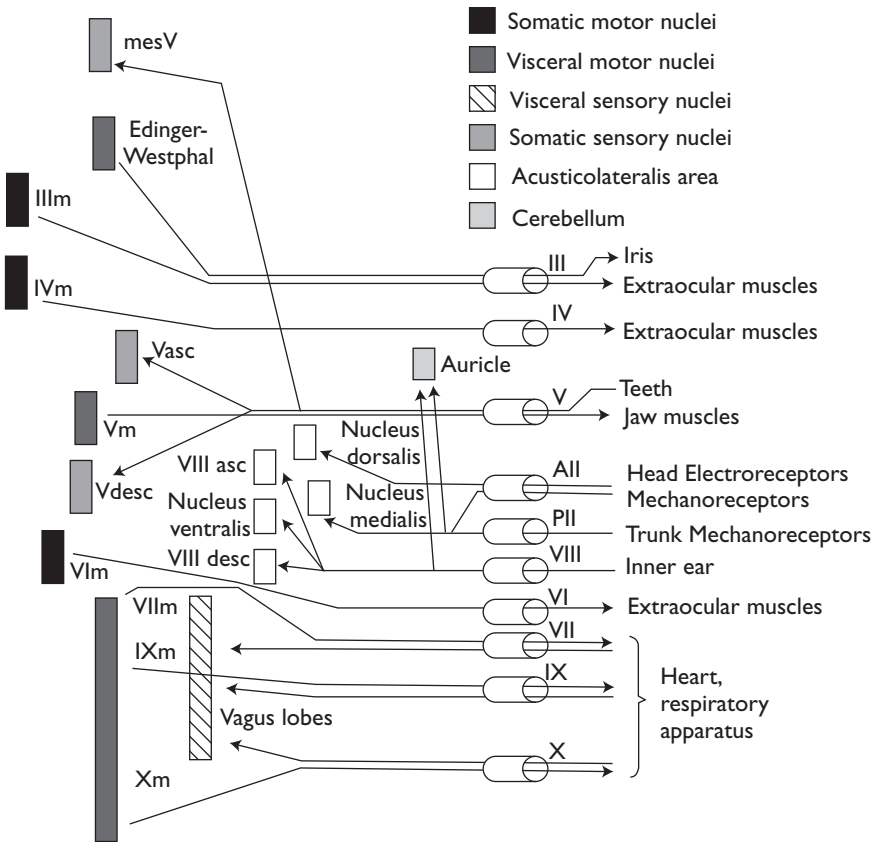

Figure 11.11B Schematic showing connections of nerves III–X, and anterior and posterior lateral line nerves (AII and PII): m: motor nucleus; asc and dsc: ascending and descending nuclei; mesV: mesencephalic nucleus of trigeminal. More ventral nuclei and tracts are mainly on left of the figure. From Hofmann (1999).

Table 11.1 The segmentation and functional components of the cranial nerves in fishes

Segment	Dorsal root	Ventral root	Components of dorsal root nerves
Premandibular	V (trigeminal) Deep ophthalmic	III (oculomotor)	General cutaneous
Mandibular	V (trigeminal) Superf. ophthalm., Max. and mandib.	IV (trochlear)	General cutaneous + visceromotor
Hyoid	VII (Facial) VIII (auditory)	VI (abducens)	Lateralis (somatic sensory) + visceral motor + visceral sensory lateralis (somatic sensory)
1st branchial	IX (glossopharyngeal)	Absent	Somatic sensory + visceral sensory + lateralis (somatic sensory)
2nd branchial	X (vagus) XII (hypoglossal) + XI (accessory)		Lateralis (somatic sensory) + somatic sensory + visceral motor

and gene expression studies have raised some doubts, considered in an interesting review by Olsson *et al.* (2005). The segmental repetition of the somites in the trunk, patterns other structures in the trunk. But in the head it is the gill pouch endoderm (which is segmented independently) that patterns the neural crest cells which stream to the existing pharyngeal arches, regulated by the *Dlx* gene family, much as the *Hox* genes govern antero-posterior boundaries. These are then significant in patterning the muscle, placodes and connective tissues of the head. The finding that both upper and lower jaw are derived from a single mandibular condensation perhaps does not support the older segmental theory of the head. A further problem linked to the segmental theory is posed by the premandibular region. Whatever view is taken of head segmentation, the agnathan ancestor of gnathostome fishes is agreed to have had a terminal mouth followed by a series of gill pouches, supplied by a series of cranial nerves, as in Figure 11.11A. The Norwegian palaeontologist Stensiö (1927, see also 1958) patiently ground a remarkable series of serial sections through the head shields of cephalaspids (Figure 11.12) which showed extraordinary details of natural casts of the brain and cranial nerves, and the outlines of the gill pouches. The illustrations in his papers deserve to be examined (as a truly astonishing feat of reconstruction from very beautiful fossils). However, the interpretation of some of the structures figured remains to some extent debatable. For example, Stensiö suggested that the lateral fields and their canals were electric organs and the nerves leading to them, but they may perhaps rather be part of an auditory system as Watson (1954) suggested. There is also some argument about the labeling of the nerves in Figure 11.12, for the numbering of the mandibular segments has yet to be finally agreed (see Whiting, 1977). However, this in no way detracts from Stensiö's astonishing achievement in his detailed "dissections" of ancient fishes.

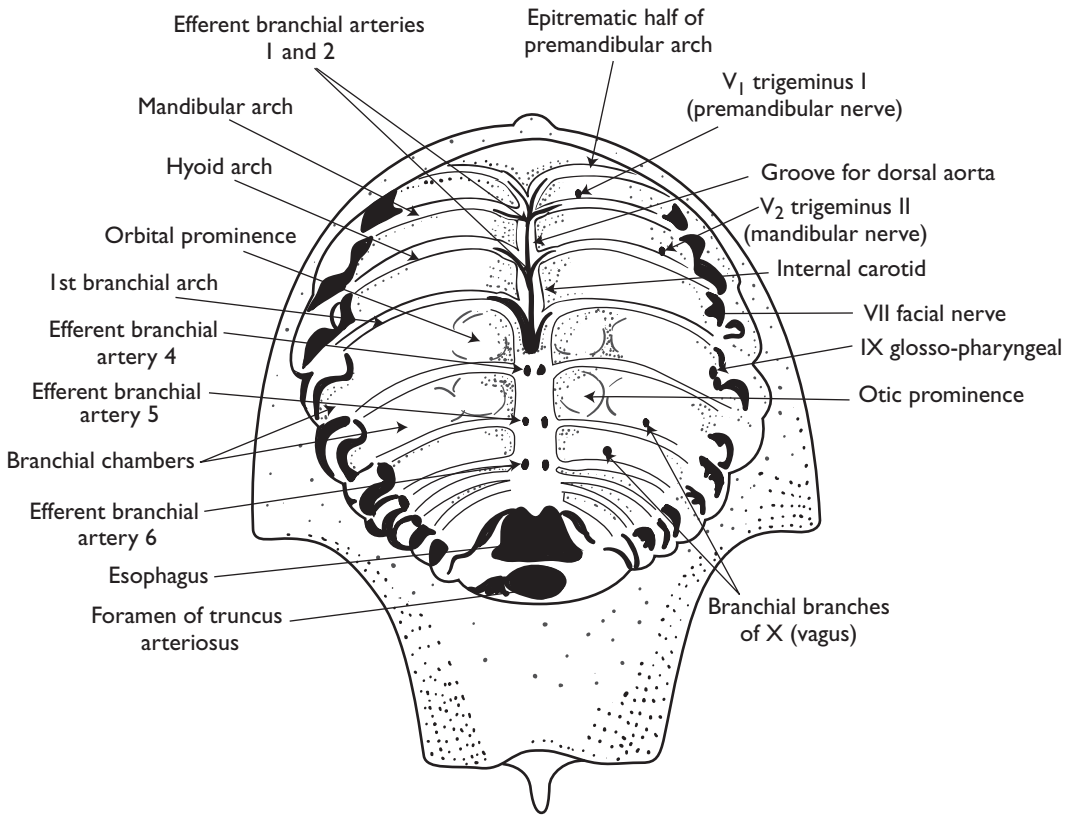

Figure 11.12 Cephalaspid branchial chamber showing segmental series of gill chambers. Some of the lettering and numbering of the different arches has been questioned (see text). From Stensiö (1958).

11.5 The Brain

To some extent, the brain can be regarded as an enlarged anterior region of the spinal cord, with hypertrophied centers associated with the development of the special sense organs. In its basic plan, the brain is similar in all fish, though there are very considerable differences between different fish groups in the relative development of the three different main regions (Figure 11.1). Obviously enough, even in fish of the same group, the size of the different regions depends on the importance of special senses, and the requirements of different lifestyles. Obviously enough, the optic lobes are enlarged where vision is important, but perhaps the most striking example of the relation of a brain region to a special sense is provided by the vastly enlarged cerebellum of electrolocating mormyrids.

Brain size

The relative sizes of different brain regions differs in different fish (Figure 11.13) as we should probably guess, according to their mode of life and taxonomic position (Figure 11.14). The absolute size of the brain in any species changes during its ontogeny (Figure 11.15), but, although the relative size of

different brain regions differs, the ratio between total *adult* brain weight and body weight is similar for all fish groups (except elasmobranchs and mormyrids), and much the same as that for amphibians and reptiles. It is only in comparison with birds and mammals, which have much higher brain:body weight ratios, that most fish can be considered as animals of little brain. Mormyrids are a most striking exception. The brain of the mormyrid *Gnathonemus petersi* weighs 3.1% of the body weight, exceeding our own (2.3%)! This is mainly because of the enormous valvulae of the cerebellum, where a later stage in processing of electroreceptor input takes place. As well as being very large, mormyrid brains consume 60% of the oxygen the fish extracts from the water, in comparison our brain only uses 20%! Elasmobranchs also have, in some cases, ratios approaching avian and mammalian values – as much as 400% greater than most other fishes – we do not really know why. Nilsson *et al*. (2000) suggested it was related to enzyme activity (see next page). The largest brain:body weight ratios are seen in the manta (*Mobula*) and in stingrays and eagle rays (*Myliobatids*), followed some way behind by active galeomorph sharks such as *Lamna* or *Carcharhinus*. Obviously enough, various rather different factors influence brain size, from neuron number, the sizes of their dendritic fields, and the complexity of the circuitry linking them, to the processing arrangements required for a huge amount of sensory input. Mantas are very maneuverable for their size, and have much enlarged cerebella, perhaps this may be due to the need to process information from the abundant proprioceptors regulating their flapping flight.

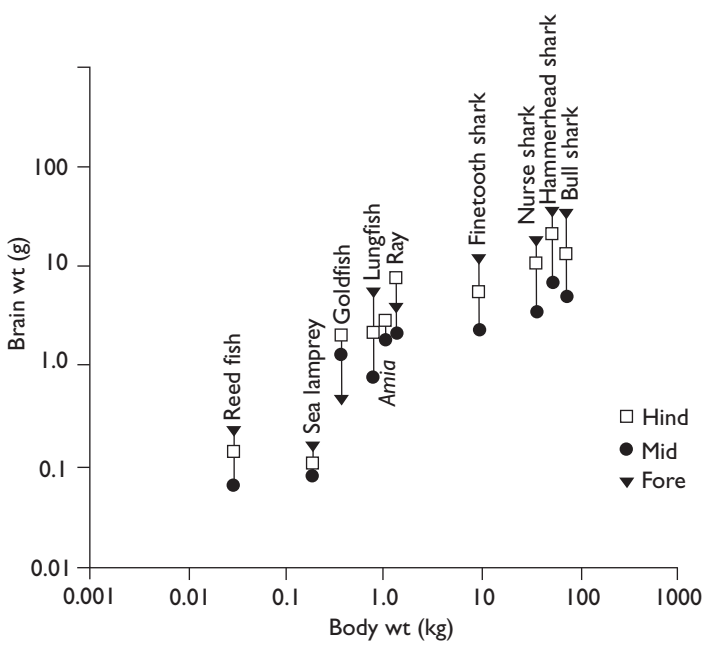

Figure 11.13 Relative sizes of different brain regions in fishes. Squares, hindbrain; triangles, forebrain; circles, midbrain. Note relatively small brain of lamprey. After Ebbeson and Northcutt (1976).

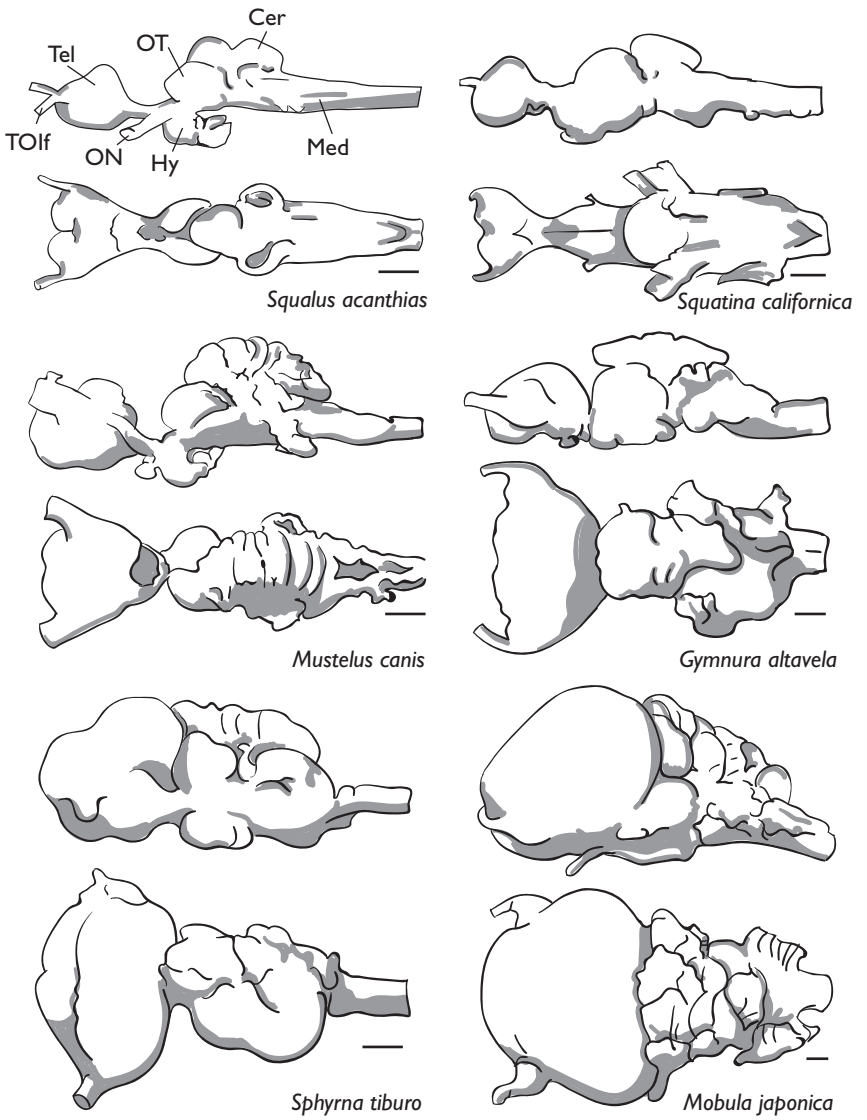

Figure 11.14 Lateral and dorsal views of brains of sharks and rays with different habits. Note that the sluggish bottom dwelling *Squatina* has a much smaller cerebellum than the active free-ranging *Mustelus*, and that the cerebellum in the two pelagic rays *Gymnura* and *Mobula* is large. After Hofmann (1999).

A quite different view was proposed by Nilsson *et al.* (2000) who found that the activity of the ion-pumping enzyme Na^+ /K^+-ATPase was relatively much less in elasmobranchs than in teleosts, so that the large elasmobranch brain was less expensive energetically to run. Curiously, elasmobranch brains share with mammals other features as well as size. One such is the elaborate system of mesencephalic dopaminergic neurons apparently lacking in actinopterygians, but again, the significance of this is unclear. Northcutt (2002) gives an

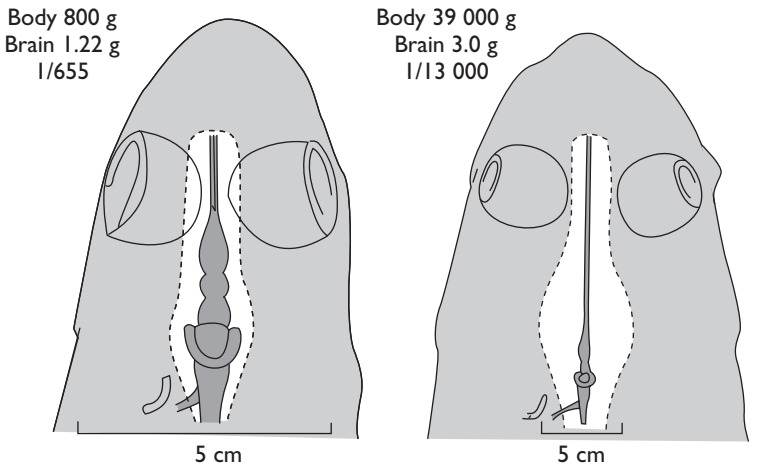

Figure 11.15 Notable change in relative brain size from oviducal embryo to adult of *Latimeria*. Head seen in dorsal view, note vast size of adult cranial cavity. After Anthony and Robineau (1976).

interesting review of brain evolution, including brain size, while Ekstrøm and Ohlin (1995) discuss the role of the GABA-immunoreactive axon scaffolding of path-finding fibers in teleost brain development.

Brain temperature

In most fish, the brain, like the rest of the body, is at the same temperature as the ambient water. In swordfish (*Xiphias*) and marlins (*Makaira*), however, it is at least 3–4°C warmer. Carey (1982) collected data by telemetry from an intracranial probe in *Xiphias*, and observed temperatures as much as 10°C–14°C above cold ambient water. The brain is warmed by a special heat-generating liver-like tissue associated with the eye muscles, and the eyes are also warmed (p. 325). The porbeagle *Lamna* also has a warm brain but in this case a vein passes intracranially and transfers heat from the myotomal muscles (Wolf *et al.*, 1988) as well as a rete associated with the eye muscles (Figure 11.16A). So far no temperature measurements have been made, but a complex rete-like system of vessels overlying the cerebellum and under the brain of manta rays (Figure 11.16B), suggests that they may have a warm braincase, warmed from the wing cruising musculature.

11.6 Elasmobranch Brain Regions and their Connections

The shark brain will be described as an example of the design of a fish brain, but the reader should beware of supposing that this is because it is chosen as a model of a "primitive" fish brain, as used to be imagined. However, because of the cartilaginous cranium, the curious reader will find that, if available, shark brains and cranial nerves are very much easier to dissect than those of teleosts. Figure 11.17 shows sagittal sections of the brains of the small sharks *Squalus* and *Scyliorhinus*.

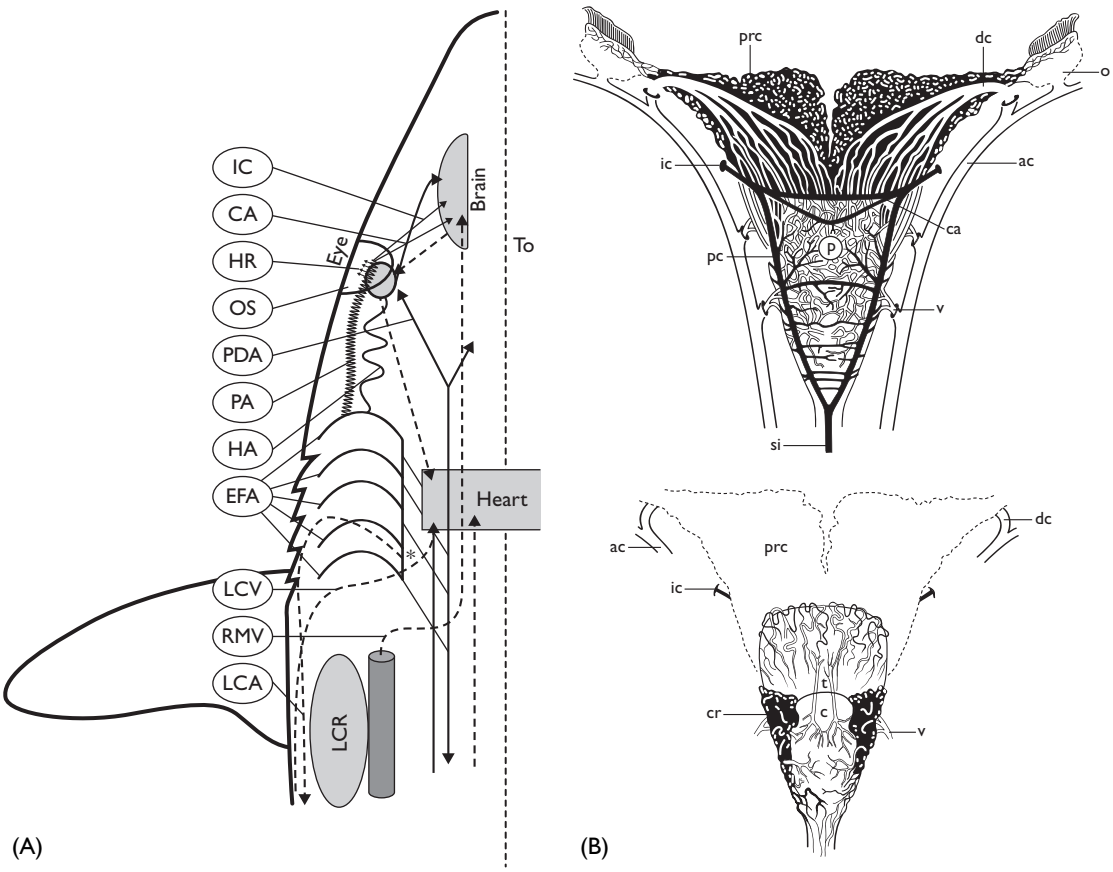

Figure 11.16A Brain and eye warmed in lamnid shark. CH: cerebral artery; EFA: Efferent branchial arteries; DA: Dorsal aorta; IC: internal carotid; HA: Hyoidean artery; HR: Hyoidean rete; LCA: Lateral cutaneous artery; LCV: Lateral cutaneous vein; OS: Orbital sinus; PA: Pseudobranchial artery; PDA: Paired dorsal aorta; PCV: posterior cardinal vein; LCR: Lateral cutaneous rete; RMV: red muscle vein. From Wolf *et al.* (1988).

Figure 11.16B Cranial retia of *Manta birostris* brain. Arteries black, veins white. Ventral view above, dorsal more caudal view below showing cerebellar rete. ac: anterior cardinal sinus; c: cerebellum; ca: anterior communicating artery; cr: caudal rete; dc: dorsal collector vein; ic: internal carotid artery; o: olfactory sinus; P: plexus; pc:deep cerebral artery; prc: precerebral rete; si: unpaired spinal artery; t: telencephalon; v: cerebral veins. From Alexander (1996).

Telencephalon

At the front of the brain all elasmobranchs have large olfactory organs and olfactory bulbs formed by forward bulging out of the forebrain and connected to it by stalks of varying lengths. In some species the olfactory bulbs are particularly large, for example in smooth hounds (*Mustelus canis*); spurdog (*Squalus acanthias*) or the hammerhead (*Sphyrna*), reflecting the importance of olfaction in their feeding. Olfactory fibers enter the bulbs, where they gather in glomeruli connected via interstitial cells to the olfactory tract running to the

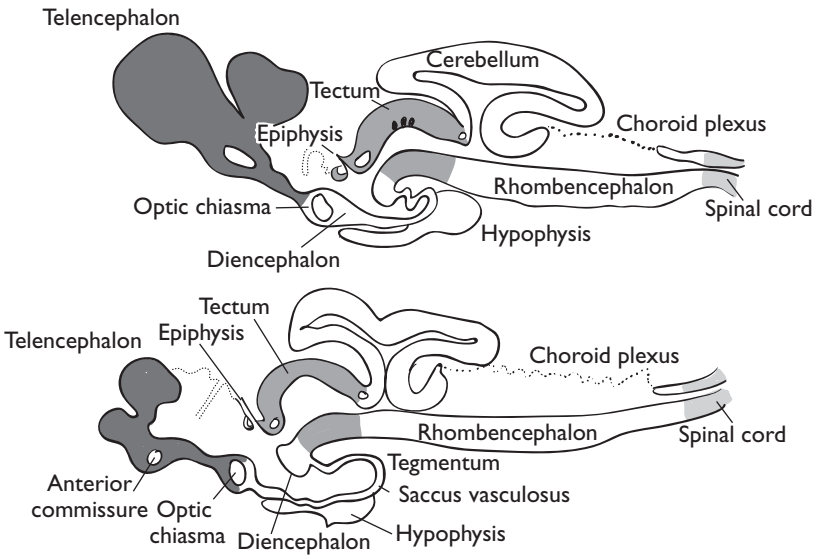

Figure 11.17 Sagittal sections of the brains of *Scyliorhinus* (above) and *Squalus*. Dark shading: telencephalon; medium shading: mesencephalon; light shading: spinal cord. The dots in the dorsal mesencephalon next to the ventricle represent the position of mesencephalic Vth neurons seen in Figure 11.18. After Smeets *et al.* (1991).

forebrain. Olfactory input to the glomeruli seems at least in part to be mapped according to the position of the receptors in the olfactory epithelium (such as the retinotopic maps in the visual system or the somatotopic maps in weakly electric fish). Output to the telencephalon from the olfactory bulbs is also partly localized, lateral regions of the epithelium being linked to the lateral parts of the tectum. The large telencephalon, however, is not merely an olfactory center, for major parts are concerned with vision and probably other sensory modalities also: perhaps only 10% of the telencephalon is concerned with olfactory activity. Indeed, in associating different sensory inputs with different motor output programs, it resembles the mammalian limbic system, as for example in rays, when electroreceptive input from the hindbrain is relayed via the mesencephalon to the diencephalon, and thence to the upper pallial layer of the telencephalon.

Diencephalon

The diencephalon is divided into the dorsal epithalamus (pineal or epiphysis, and the habenular ganglia) and the thalamus and hypothalamus ventrally. There is good evidence that the pineal is in dogfish a sensitive photoreceptor, and some evidence that it is involved in the paling that dogfish undergo in darkness, though less is known of melatonin in elasmobranchiomorphs than in lampreys or teleosts. The thalamus receives direct retinal and secondary visual input from the tectum, descending telencephalic fibers, and ascending fibers from cerebellum and spinal cord. Thalamic output passes to these regions, and to the mesencephalic tectum and the motor systems of the brain stem.

Hypothalamic input comes from the telencephalon, as do taste (gustatory) fibers from the medulla, while thalamic fibers descend to the reticular formation of the hindbrain. A curious feature is the saccus vasculosus at the caudal end of the hypothalamus. This is a folded pigmented sac with special coronet cells contacting the ventricular fluid. It may be involved in the secretion and absorption of the fluid. At the opposite end of the hypothalamus, the large neurosecretory cells of the supraoptic nucleus send granular secretory material down their axons, to the underlying neural lobe of the pituitary (p. 269).

Diencephalic functions are not yet fully understood, but it seems that in elasmobranchs, as in mammals, the hypothalamus (in association with the telencephalic limbic centers) regulates feeding, escape, attack, and sexual behaviors, as well as the homeostatic control of such bodily functions as color change.

Mesencephalon

As in all fish, the elasmobranch mesencephalon is larger, roofed by the layered tectum. This often referred to as the optic tectum, but since it receives inputs from other sensory systems, it seems best to refer to it simply as the tectum. Like the telencephalon, it is evidently an associative and integrative center. Almost all optic fibers decussate in the midbrain floor before rising to end in the tectum, where, by analogy with other fish groups, there is at least one retinotopic map of the visual field, manifested by the orderly well-patterned neuron layers. Some tectal neurons send efferent axons to the retina, experiments in teleosts suggesting that these are of two kinds, one probably neurosecretory, but just how they operate us unclear. The tegmental floor of the mesencephalon (merging with the rhombencephalon) contains the large multipolar neurons of the reticular system. In the mid-line, next to the ventricle, there is the mesencephalic nucleus of nerve V, whose function is well understood. The neurons in this nucleus have large rounded cell bodies, and are hence amenable to intracellular recording (Roberts and Witkovsky, 1975). Their axons pass to high threshold touch receptors on the teeth and around the mouth. When the teeth are tapped, the neurons fire a short burst of action potentials (Figure 11.18) to the motoneurons of V that innervate the jaw-closing muscle, and the jaws reflexly snap shut. As we might have guessed these motoneurons have synaptic input from other brain regions, so snapping at prey is not only a consequence of touching the teeth.

Cerebellum

The cerebellum in elasmobranchs has the same well-ordered and rather complex circuitry as in higher vertebrates (Figure 11.19), with an interesting difference. As in teleosts, climbing fibers from cells of origin in the olive nucleus of the brainstem end only around the bases of the very beautiful Purkinje cells, rather than climbing around Purkinje dendrites in the molecular layer. Purkinje cells carry the German name of their discoverer, the distinguished Czech physiologist and histologist Purkynë, a friend of Goethe's, who also gave his name to the fast-conducting fibers of the mammalian heart, and founded the world's first physiology department in 1839.

Both the elasmobranch and teleost cerebellum apparently function in the same way as that in higher forms despite the non-climbing "climbing" fibers.

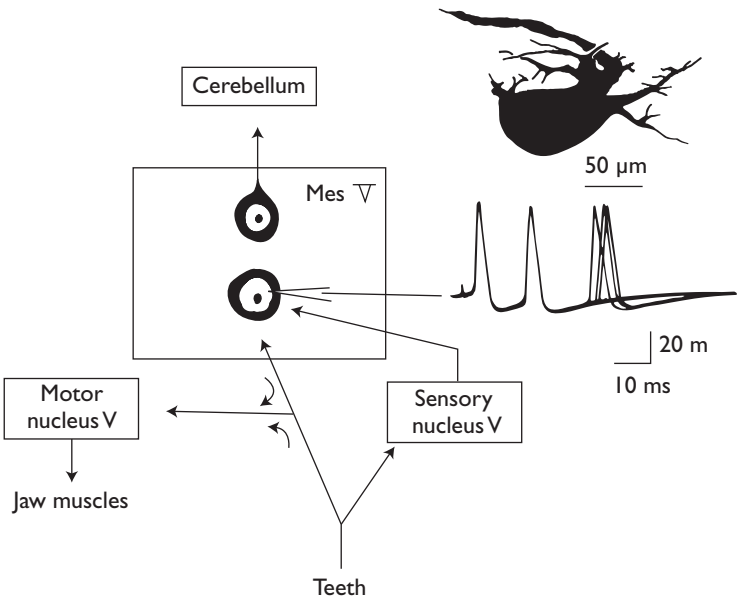

Figure 11.18 Some features of the mesencephalic Vth neurons involved in the jaw-closing reflex. On the right above, a neuron visualized by the Golgi method, and below it, a record of a series of action potentials evoked by tapping the teeth. After Roberts and Witkovsky (1975).

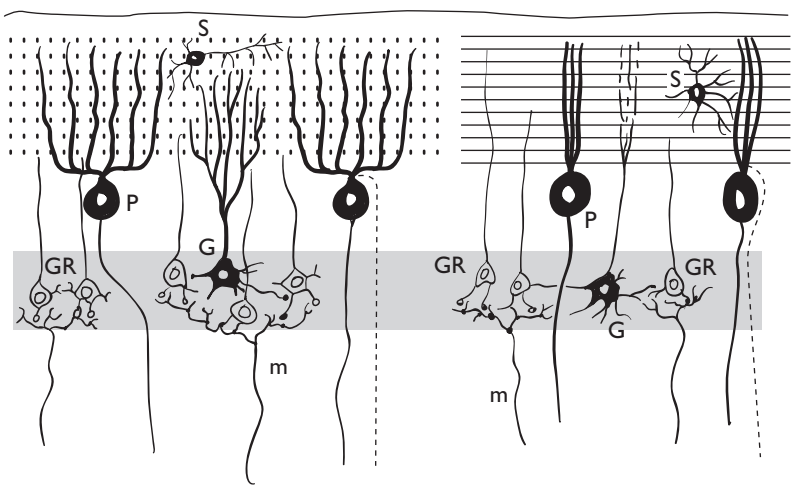

Figure 11.19 The major cell types of the cerebellum. Left and right of the diagram show sections in planes at right angles to each other; note the remarkable regularity of the parallel fibers and the planar arrangement of the Purkinje dendritic trees. Inhibitory: black. Climbing fibers do not climb in fish but remain around the somata of the Purkinje cells, otherwise the pattern is like that of other vertebrates. Purkinje cells (P) provide the only output system. Mossy fiber input (m) synapses with the abundant granule cells (GR) whence the parallel fibers of the outer layer arise, modulated by the Golgi (G) and stellate (S) cells. Partly after Eccles *et al*. (1967).

The consequent separation of somatic and dendritic synapses is usefully preadapted for neurophysiological experiment, but provides something of a puzzle if in higher vertebrates climbing fiber links with Purkinje dendrites are involved in modifying Purkinje/climbing fiber synapses. In electrolocating teleosts, Purkinje axons do not leave the cerebellum, but end on huge eurydendroid cells, the sole excitatory output. Mossy fiber input drives granule cells, whose axons are the parallel fibers, exciting Purkinje cells and stellate cells. In higher vertebrates there are specialized types of granule cells, the unipolar brush cells, which enter into complicated synaptic glomeruli with mossy fibers, but these have yet to be discerned in fishes. Golgi and stellate cells are interneurons, regulating Purkinje cell activity via GABAergic synapses, while climbing fibers have glutaminergic synapses on Purkinje cells. Other afferent connections ending in the granular layer come from a variety of motor nuclei (e.g. oculo-motor and trigeminal), and from the spinal cord, and some motoneurons have collaterals passing to the cerebellum, thus sending to it efferent copies of motor commands.

Here, then, is a complicated and specialized circuit whose function still proves rather puzzling, although in higher vertebrates it is obviously somehow concerned with the control of movements, since cerebellar removal produces profound motor disturbances, as well as cognitive and emotional problems. What does it do in fish? Experiments on the dogfish *Scyliorhinus* by Paul and Roberts (1979), who studied the effects on fin movements and spinal swimming after removing various parts of the brain (Figure 11.20) produced very interesting results. After removal of the forebrain (decerebrate fish), about one-third of the Purkinje cells discharged rhythmically in phase with body movements. Since this activity continued in curarized fish where the muscles were paralyzed, it was presumably driven by spinal cord generator circuits. The role of the cerebellum here seems to be to monitor (perhaps via efferent

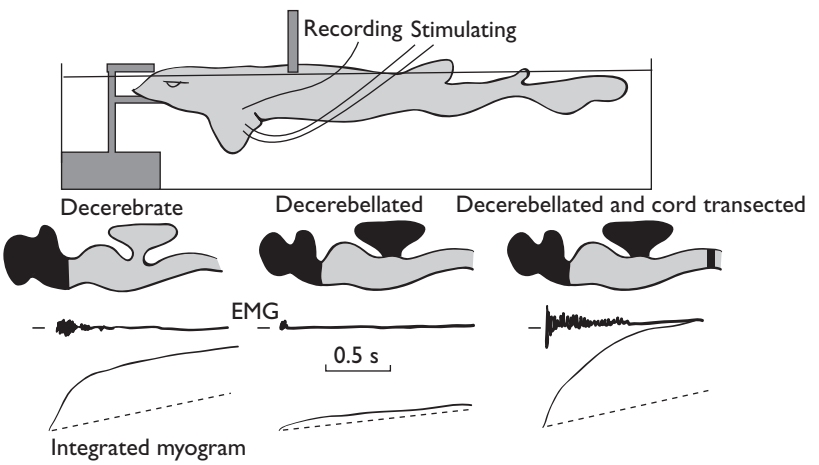

Figure 11.20 Role of cerebellum in regulating movements. Upper: experimental arrangement; middle: parts of brain removed (black); lower: muscle responses. EMG: electromyogram; below this, the integrated myogram. Note dotted line shows response of integrator in absence of EMG. After Paul and Roberts (1979).

copies of the spinal motoneuron discharges), the state of spinal cord circuits during movement. The experiments on reflex fin movements gave a clear idea of a different role for the cerebellum, the control of motor patterns arising elsewhere in the CNS. Dogfish raise their pectoral fins reflexly if they are touched on the upper surface (check this if you have access to live small sharks). A rapid upward movement is followed by a longer-lasting tonic phase. Cerebellar ablation does not abolish this reflex movement, but modifies it by increasing the response threshold, and reducing the tonic phase (Figure 11.20). Only a few Purkinje cells in the posterior region of the cerebellum increase their firing rate when the fin is moved (after a short delay showing that this discharge did not initiate the reflex but was appropriate to modify it). These Purkinje cells monitor spinal motor circuits, and inhibit a set of cerebellar nuclear neurons which are themselves normally inhibitory to brainstem descending pathways for the same spinal cord circuits. Transection of the cord removes this inhibitory action, allowing the fin reflex to be expressed fully, while at the same time (via other sets of neurons in cerebellar nuclei) descending inhibition is increased to other motor systems to prevent their unwanted expression.

So the cerebellum is thought to control how much of a particular motor pattern arising elsewhere in the nervous system is to be expressed. There is an elementary somatotopic map so that different cerebellar regions deal with different motor systems. In the middle of the auricles, for example, Purkinje cells give complex responses when the corresponding granule cells are excited by stimulation of nerve VIII. These are probably concerned with regulating movements controlled by vestibular responses.

We should expect from this, that the size and elaboration of the cerebellar cortex would be related to the complexity of the movement patterns in different fish. Certainly, this seems correct, for example *Mustelus* is an active free-swimming shark and has a much more elaborate cerebellum than the sluggish bottom-dwelling *Scyliorhinus*, while the very active short fin mako (*Isurus*) has the most elaborately-folded cerebellum (Figure 11.21). It is also large in the pelagic batoids such as butterfly rays (*Himantura*) and mantas (*Mobula*), possibly related to the large numbers of proprioceptors involved in their delicate wing movements.

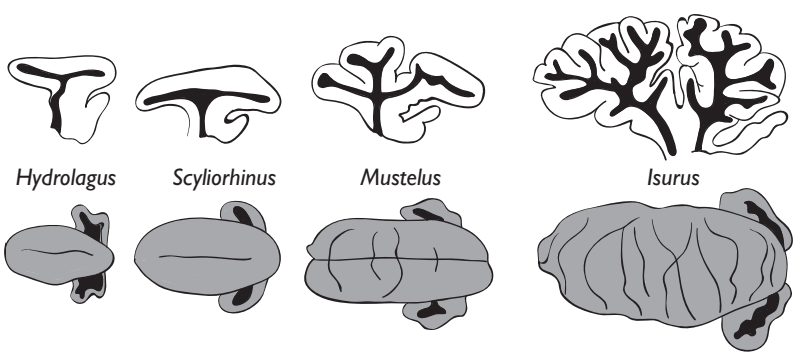

Figure 11.21 Cerebellar development in different elasmobranchs.
After Smeets *et al.* (1991).

Another quite different view of cerebellar function arose because the cerebellum shares strong anatomical similarities with the cerebellar-like nuclei of the hindbrain, which are clearly sensory and process electrosensory and lateral line information, possibly suggesting a similar cerebellar sensory processing function (Montgomery *et al*., 2002). Of course, the suggested functions for the cerebellum need not be mutually exclusive. Rodriguez *et al*. (2005) have shown in goldfish that while cerebellar lesions do not produce any obvious motor deficits, they severely impair spatial cognition and such conditioned responses as heart rate changes (Figure 11.22). The possibility that the cerebellum may contain sensory maps seems very likely.

New (2001) has reviewed possible functions of the elasmobranch cerebellum, emphasizing the suitability of small sharks and rays for further experimental studies.

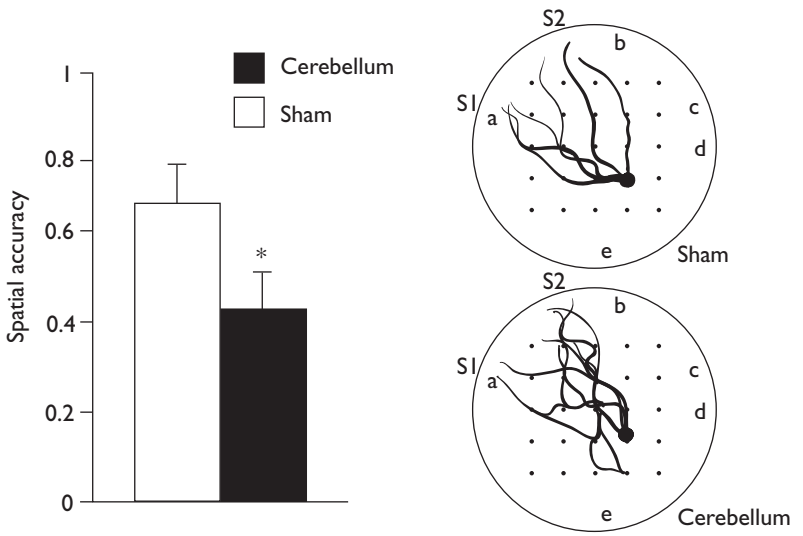

Figure 11.22 After cerebellar ablation goldfish are significantly less accurate at finding their way around in their tank, and are less successful at finding bait in a baited feeder. From Rodríguez *et al*. (2006).

Medulla oblongata, brainstem, rhombencephalon

The brainstem contains the nuclei from which arise all the cranial nerves and into which their sensory input passes, excepting the olfactory and the nervus terminalis. The walls are thickened by the primary nuclei of the acustico-lateralis sensory systems, and the basal central core contains the reticular formation. This is most striking in elasmobranchs, since it contains very large neurons with extensive dendritic arborizations. Many of the cells of the reticular formation are driven synaptically by ascending fibers from the spinal cord, others by cutaneous afferents from the head and pharynx, while the descending reticular axons influence the intrinsic spinal cord segmental locomotor circuits. Cranial outputs from other motor systems of the rhombencephalon control eye, jaw, and respiratory movements. In mammals, the reticular formation receives and processes

information from other parts of the brain, taking part in the control of locomotion, eye movements and sensori-motor activity, but there has been little experimental work on its functions in elasmobranchs.

11.7 Brains of Other Fishes

The remainder of this chapter points out some obvious differences from elasmobranch brain morphology seen in other fishes, and then considers some of the work (mainly on teleosts) where known pathways are involved in specific behaviors.

Telencephalon

The most striking difference between elasmobranchs (and bony fish and sturgeons) is in the telencephalon. Here, the brain roof is simply a thin sheet of ependymal cells, and lateral ventricles are absent, so that in section (Figure 11.23) it is quite unlike the elasmobranch forebrain. This condition of "eversion" has long made comparison with other vertebrates difficult, but more recently tracing of connections and of vascular supply have shown that despite differences in adult gross morphology and embryological development, the functional organization of the "everted" telencephalon is similar to that of more conventional brains (Butler, 2000). An interesting recent view (Striedter and Northcutt, 2006) suggests that eversion was a consequence of spatial constraints in the small embryos of early ray-finned fish, which had adopted the reproductive strategy of many small young versus a few larger offspring. The telencephalon has no space to expand in the normal vertebrate manner, but instead spreads into the spaces dorsal and caudal to the developing nasal epithelia, so becoming "everted" during ontogeny.

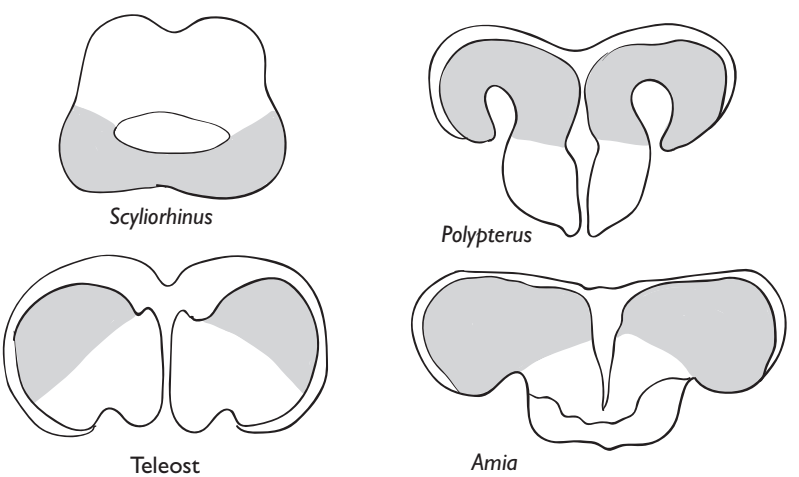

Figure 11.23 Transverse sections of the telencephalon: in the elasmobranch *Scyliorhinus* (top left) compared with various bony fishes showing the thin roof in the latter. Part of the pallium stippled. After Vanegas (1981).

Mauthner cells

Another notable difference between the elasmobranch brain and that of other fish groups is that the important pair of Mauthner neurons is only found in young stages (Bone, 1977). These very large and conspicuous neurons lie in the medulla at the level of the VIIIth nerve, and drive the rapid C-start escape reaction. Their axons descend the cord as the largest fibers within it, after crossing the mid-line in the Mauthner chiasma, and are linked by collaterals to spinal motoneurons (Figure 11.24). A large lateral dendrite passes towards the entry of VIII, covered with synapses from VIII fibers (Figure 11.25). Other excitatory

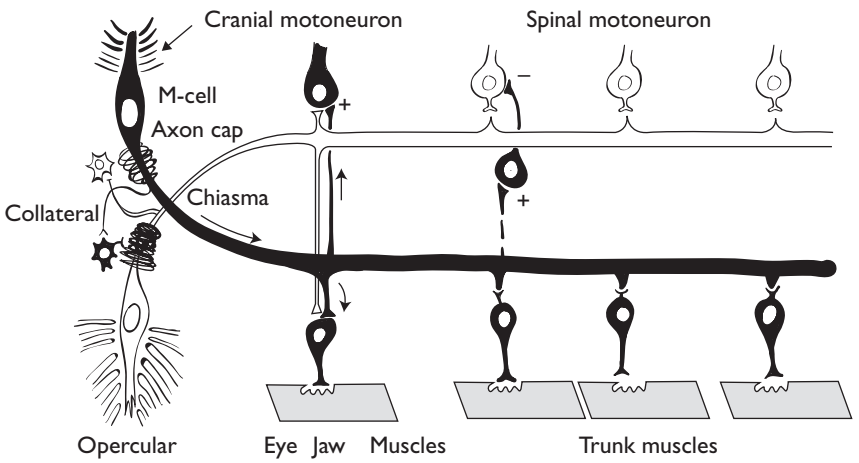

Figure 11.24 The Mauthner system. Schematic diagram showing Mauthner cells, their axons crossing at the Mauthner chiasma before descending the cord and connecting with contralateral motoneurons.

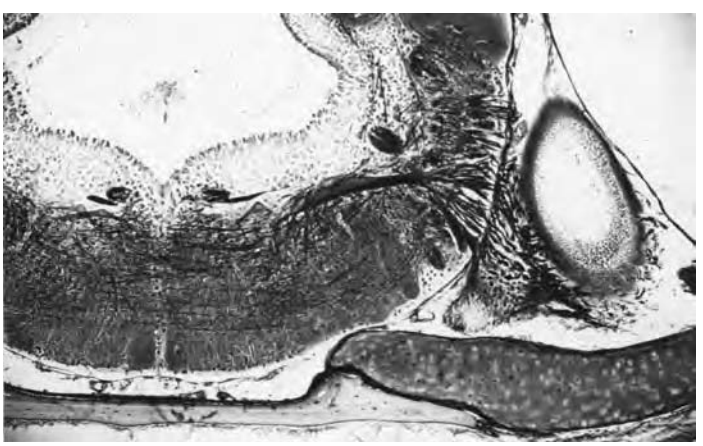

Figure 11.25 Mauthner cell of the lungfish (*Protopterus*) seen in thick reduced silver wax section at level of VIII. Note large fiber synaptic input to lateral M-dendrite from ganglion cells of VIII (GC) and M axons passing towards chiasma. The macula of the inner ear on right is innervated from VIII.

input comes from the main nucleus of V, and from cerebello-tegmental and tecto-bulbar pathways. Inhibitory input comes from the contralateral Mauthner cell and smaller neurons associated with an axon cap. The decussation at the chiasma means that when the Mauthner cell on one side is stimulated to fire, the C-start moves the head (the most vulnerable part) away from the stimulus. Figure 11.26 shows the operation of the system. In some teleosts, such as syngnathids, eels, and angler fish, a C-start escape response is inappropriate, and the Mauthner cells have disappeared.

The Mauthner system is a fine example of a "hardwired" central system driving a fundamental rapid escape reaction appearing early in ontogeny. Hardwired systems are much more common in invertebrates, perhaps because stereotyped "invariant" responses are less suited to the more complex vertebrate CNS. Interestingly, some rapid escape reactions can be evoked *without* Mauthner activity, just as (and equally puzzling) rapid escape jettings can be evoked in squid without involving the rapidly conducting huge diameter giant axons.

Striking evidence of segmentation is provided by work on Mauthner and allied reticular neurons of zebra fish, where Liu and Fetcho (1999) have shown (by laser ablations) that the Mauthner cell is one of three repeated reticulospinal neurons in successive hindbrain segments which are concerned with rather different escape responses from predators. If the Mauthner cell alone is

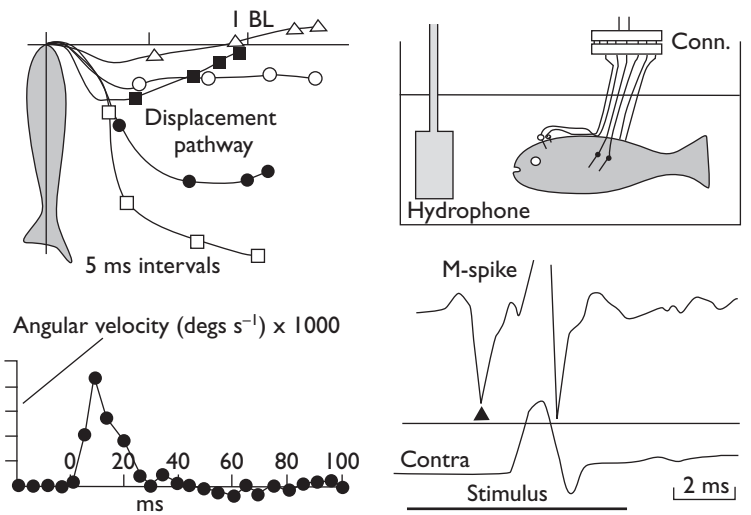

Figure 11.26 Responses of the Mauthner system. Left: displacement pathway of goldfish head during five Mauthner responses (points indicate 5 ms intervals); angular velocity during response. Right: experimental arrangement and extracellular records obtained from hindbrain near M-cell (the M-cell spike evoked by a sound stimulus is followed by a large movement artefact), contralateral muscles contract, but as expected no response from ipsilateral muscles (middle line). After Zottoli (1978); Eaton and Bombardieri (1978).

eliminated, changes from tail-directed stimuli are changed, if all three, there are no rapid responses to head or tail stimuli. It seems that there are segmentally arranged functional groups of hindbrain neurons (Nakayama and Oda, 2004), just as in each spinal cord segment, there are three pairs of primary motoneurons (Eisen *et al.*, 1990).

The cerebellum in electrolocating teleosts

Many fish of different groups (see p. 303) have modified part of the acusticolateralis receptor system for electroreception. In such electroreceptive fish, a special dorsal electroreceptor nucleus, separate from the acustico-lateralis nuclei, projects directly to the cerebellum. Remarkably, two groups of freshwater electroreceptive fish have independently evolved weak electric organs (see p. 307), with which they gain a sophisticated view of their surroundings, and communicate with each other. The ways in which the brain processes electroreceptive input in such fish is complex, involving specialized circuits for processing signals arriving with microsecond time differences, delay circuits, layers of sensory maps, and the generation of negative images of "expected" sensory input. Once again, these fascinating topics can only be glanced at briefly, but we can at see why weakly electric fish have contributed so much to the understanding of how specialized circuits function.

Circuitry of cerebellum-like sensory structures

Mormyrids, gymnotids, and elasmobranchs share the generation and subtraction of expected electrical input from the responses of tuberous electroreceptors, allowing them to detect novel features. The expected input is a negative image of the past electroreceptive input, with correct phase, polarity (i.e. opposite polarity to the sensory input), and amplitude. This image is produced as a result of plasticity at parallel fiber synapses with the ganglionic Purkinje-like cells. Bell (2001) has shown how in mormyrids the circuitry of the electrosensory lateral line lobe (ELL) where this processing takes place, is very similar to that of the cerebellum proper, and Meek *et al.* (1999) point out that the ELL is derived from the cerebellar crest cells and fibers. The latter authors show (Figure 11.27) part of what is known of the circuitry in the ELL. In the cerebellum, the espaliered dendritic trees of the Purkinje cells differ from those of the ELL, because they are more strictly regular and all arranged across the longitudinal axis, at right angles to those in the ELL.

Rather similar cerebellar-like circuitry is seen in the teleost optic tectum (Figure 11.28) where large pyramidal cells send dendritic trees upward into the marginal layer of parallel fibers. In all cases, the arrangement of these cerebellar-like circuits, with sensory input via both rapid and delayed lines, seems dedicated to the analysis of small time-differences.

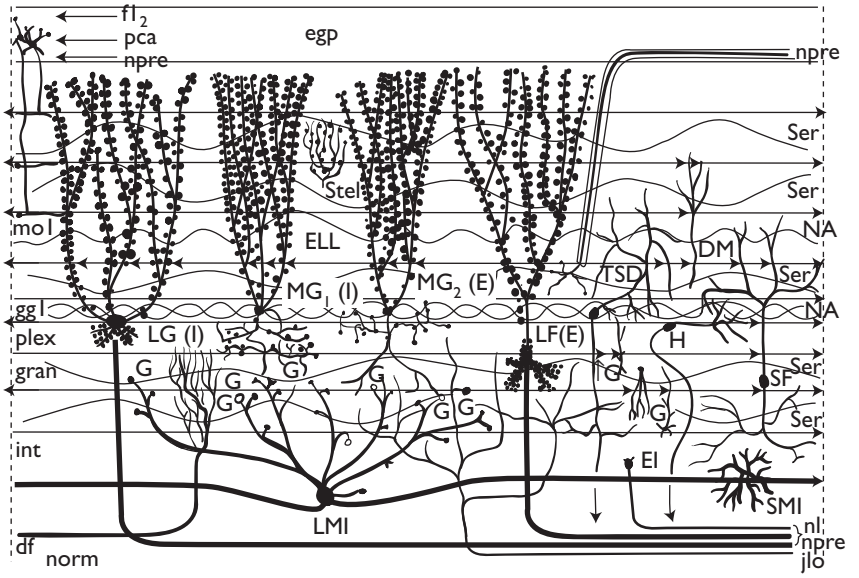

Figure 11.27 Neuron types and circuitry of mormyrid electrosensory lateralis lobe (ELL). This diagram reviews the results of both electrophysiological and histological studies, and shows a complex arrangement, containing as it does (*inter alia*) time-delay circuits, input timing circuits and subtraction of actual from expected input. The original diagram in color distinguishing different transmitters is available (free) on the web at the *J. Exp. Biol* site www.biologists.com. Dendritic trees and axonal arborizations are drawn semi-schematically. Axonal arborizations are connected with horizontal lines to indicate their main layer of termination and their (possible) targets. Presynaptic terminals are indicated by small dots and unequivocally demonstrated synaptic connections are indicated by small dots contacting postsynaptic dendrites or cell bodies. Presumed but not (yet) definitely demonstrated synaptic contacts are indicated by blunt arrows, while sharp arrows indicate that structures continue beyond the frame of the drawing. df, deep fiber layer; DM, cell of the deep molecular layer; E, excited by primary afferent input; egp, eminentia granularis posterior; EI, efferent cell of the intermediate layer; fl2, second funicular nucleus; G, granular cell; ggl, ganglionic layer; gran, granular layer; H, horizontal cell; I, inhibited by primary afferent input; int, intermediate (cell and fiber) layer; jlo, juxtalobar nucleus; LF, large fusiform cell; LG, large ganglionic cell; LMI, large multipolar intermediate layer cell; MG, medium-sized ganglionic cell; MG1, first subtype of MG cell, with basal dendrites in the plexiform layer; MG2, second subtype of MG cell, with basal dendrites in the granular layer; mol, molecular layer; morm, mormyromast primary afferent; NA, noradrenaline; nl, nucleus lateralis (of the torus semicircularis); npre, nucleus preeminentialis; pca, paratrigeminal command-associated nucleus; plex, plexiform layer; Ser, serotonin; SF, small fusiform cell; SMI, small multipolar intermediate layer cell; Stel, stellate cell; TSD, cell with a thick smooth dendrite. From Meek *et al.* (1999).

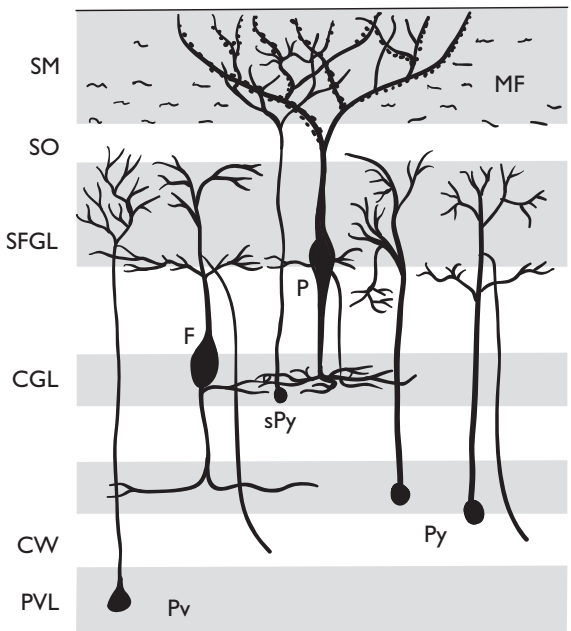

Figure 11.28 Some cell types of the teleost optic tectum, as seen after impregnation by the Golgi technique. Note similarity to those of the cerebellum and ELL of the previous figure and Figure 11.19. MF: marginal fibers; SO: marginal layer; SFGL: superficial fibrous and gray layer; CGL: central gray layer; CW: central white layer; PVL: periventricular layer; pv: periventricular cells PY: pyriform layer; sPY: small cells of pyriform layer (= climbing fiber cells); P: Purkinje cells. After Vanegas (1981).

11.8 The Autonomic Nervous System

Autonomic nerve fibers innervate smooth (involuntary) muscle, for instance in spleen, gut, and urinary tract, around blood vessels and the heart, and glandular chromaffin tissue (p. 274). In teleosts, but not in elasmobranchs, they innervate the pigment cells of the skin. Thus autonomic fibers pass to almost every part of the body, regulating visceral functions to maintain homeostasis. In every case, these efferent pathways from central viscero-motor neurons do not pass directly to the target organ, instead synapsing first with a peripheral ganglion cell which then innervates the organ, as seen in Figure 11.29. So the fibers from the CNS are called pre-ganglionic fibers; those from the ganglion cells to the target organ, post-ganglionic fibers. Early workers on the fish autonomic naturally adopted the mammalian divisions of the system into the *sympathetic* with thoracic and lumbar connections to the spinal cord, the *parasympathetic* with pathways in the cranial and sacral nerves, and the enteric intrinsic neurons of the gut plexuses. But it seems more sensible in fishes (where the distinction between sacral and lumbar for instance, is much less clear than in mammals) to simply divide the autonomic system into cranial, spinal, and enteric systems. All three are best known in elasmobranchs, thanks to the work of J. Z. Young, who studied dogfish and rays at

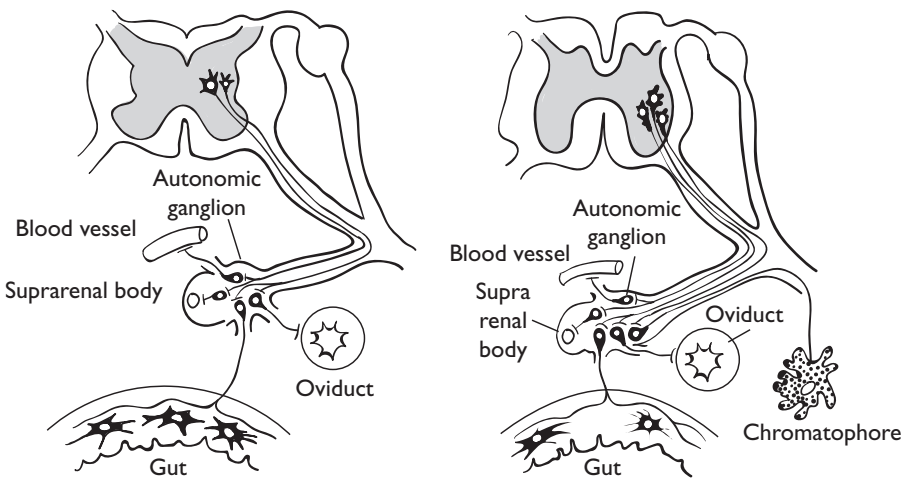

Figure 11.29 Comparison of autonomic pathways in an elasmobranch (left) and teleost. Note recurrent axons supplying teleost skin chromatophores. After Nilsson and Holmgren (1988).

Naples in 1931 (Young, 1933), and returned to the topic at Plymouth some years later (Young, 1980)! In the first paper, Young described the morphology of the autonomic; in the second the effects of a range of neuropeptides and other possible neurotransmitters on the enteric nervous system.

The arrangement of the autonomic in the dogfish is shown in Figure 11.30. Cranial autonomic fibers pass out in nerve III to the ciliary ganglion close to it, whence post-ganglionic fibers innervate the iris, and optic blood vessels. Textbooks repeat the idea that the retractor lentis is also innervated but at least in dogfish this seems incorrect (see p. 314). The iris sphincter is light-sensitive and contracts in response to light (unlike teleosts), the iris autonomic innervation controls the radial muscles which expand the pupil. Other

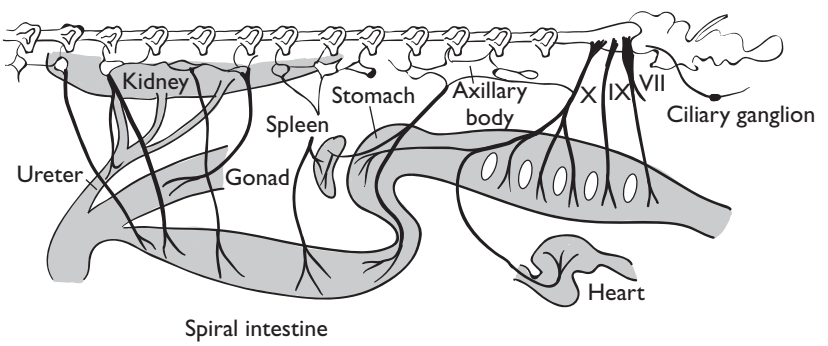

Figure 11.30 Schematic diagram of the autonomic system in *Scyliorhinus*. After Nilsson and Holmgren (1988).

cranial autonomic fibers emerge from nerves VII, IX, and X; innervating the smooth muscle of the pharynx and gut, and in X (the vagus) the heart.

Spinal autonomic fibers run to segmental paravertebral ganglia associated with masses of chromaffin cells (p. 274) containing catecholamines, mainly noradrenaline. The longitudinal links between the ganglia are irregular and there are no distinct "sympathetic" chains such as are seen in teleosts and higher vertebrates. Post-ganglionic fibers passing to the gut in cranial and spinal outflows modulate and control the intrinsic activity of the enteric nervous system. An astonishing variety of neuropeptide transmitters from different families are found here, including (among many others) the tachykinins, the vasoactive intestinal peptide (VIP) family, and the neuropeptide Y (NPY) family (Holmgren and Jensen, 2001). This list could be extended almost to fill the page, so diverse are these signaling molecules of the neurons in the autonomic system. Other neuropeptides not belonging to any family, such as galanin, are important in the neurons of the enteric plexus, and unconventional neurotransmitters such as adenosine triphosphate (ATP) are also involved. Work by Young and his colleagues has shown that gut contractions are modified by vagal stimulus frequency.

In teleosts, and other bony fishes (Figure 11.29, right and Figure 11.31) there are two notable differences from the elasmobranch set up seen on the left of Figure 11.29 and in Figure 11.30. First, the spinal autonomic ganglia are linked to the spinal nerves not only by the pre-ganglionic fibers from the cord, but also by branches carrying post-ganglionic fibers, which run to the skin to innervate melanophores. Second, from nerve III posteriorly, there is a chain of autonomic ganglia linked by connectives (Figure 11.31). The cranial autonomic ganglia are essentially part of the spinal division of the autonomic, since they receive pre-ganglionic fibers only from the trunk region, not from their own segments. This is the arrangement seen in terrestrial vertebrates. Pre-ganglionic fibers in nerve III go to the ciliary ganglion and make up the cranial autonomic system, which constricts the iris sphincter, while pupil expansion is controlled by spinal autonomic fibers passing forward in the ganglionic chain.

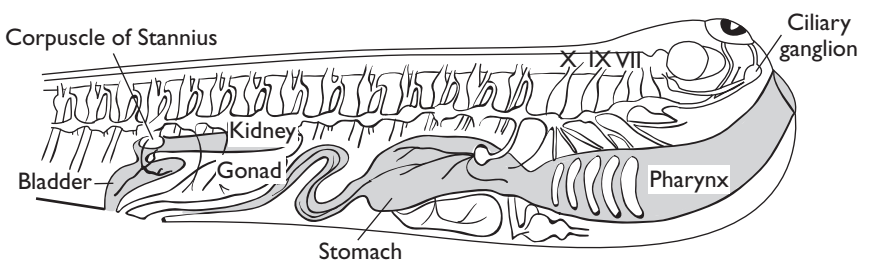

Figure 11.31 The autonomic system of the teleost *Uranoscopus*. After Young (1933).

Envoi

No other organ system is as fascinating and as complex as the nervous system, and in fish it is especially intriguing. Many features of the fish nervous system are model systems for studying those of higher vertebrates and ourselves, for instance the complex roles of the cerebellum, or the development of the spinal cord mechanisms for rhythmic behavior. This chapter has glanced at only a few such features, but all well deserve further reading.

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12 The Immune System

Fish, like all other organisms, are constantly exposed to pathogens (parasites and disease causing organisms) in their environment. This has led to the evolution of defensive immune systems consisting of a variety of molecules, cells, and tissues. Although these different systems operate in different ways in different species, the basic requirement of any immune system is to distinguish between cells belonging to one's own "self" and "non-self" cells. The organisms must then possess a means to eliminate or neutralize the "non-self" cells.

Two types of immune systems are distinguished: innate and acquired. Both systems include both cell-mediated and humoral responses. As their name implies, cell-mediated responses rely on direct involvement by cells that may engulf and destroy (phagocytize) or otherwise attack pathogens. Humoral immunity is often restricted to just those responses that involve antibodies, but in a broader sense the term can include those responses in which any defensive molecules are delivered via bodily fluids such as blood, interstitial fluids, or exocrine secretions. Innate immunity exists in virtually all plants and animals and so is believed to be the earliest form of immunity (Khalturian *et al.*, 2004). It is non-specific, that is, it acts against many different types of organisms and does not become more effective following repeated exposure to the same ones. Mechanisms of innate immunity include physical barriers that prevent entry of microorganisms (for example the skin or mucosal lining of the digestive tract), chemicals such as cytokines, agglutinins, precipitins (opsonins, primarily lectins), and interferon may be contained in secretions (mucus or digestive juices) or generalized responses such as inflammation, the labeling of antigens (opsonization) or phagocytosis by non-specific effector cells, such as macrophages and non-specific cytotoxic cells. The complement, and histamine systems of vertebrates are other examples of such non-specific mechanisms.

In contrast, acquired (or adaptive) immunity permits the immune system to respond to specific antigen molecules carried on pathogens despite no prior exposure. This acquired immunity (lacking in plants) then provides additional protection against re-exposure to the same antigen. In most fish, as in higher vertebrates, adaptive immunity systems include two types of lymphocytes: B-cells, which ultimately are responsible for the production

of immunoglobulins (antibodies, usually denoted by the abbreviation Ig) and a family of T-cells which include both effector and regulator cells which are involved in cell-mediated immunity. B-cells acquire their name from their origin in bone marrow, while T-cells, although initially produced in the marrow, finish their development in the thymus. Although fish lack bone marrow, and jawless fish lack a true thymus, these same functions still occur in equivalent tissues located elsewhere in the animal. The development and activity of B- and T-cells are under the control of genes and the proteins they code for, known as the Major Histocompatibility Complex (MHC). The actual structure of antibodies is controlled by immunoglobulin genes (abbreviated with uppercase letters: IG) and the structure of the functional receptors on the T-cell membranes is controlled by T-Cell Receptor (TCR) genes. The occurrence of these and other associated genes and proteins, such as the Recombination Activating Genes (RAG-1 and RAG-2) are, for the most part, limited to jawed vertebrates. Table 12.1 illustrates the occurrence of genes and molecular systems associated with immunity in invertebrates and vertebrates.

The evolution of the adaptive immune system, however, has not made innate immunity obsolete. The two systems can work sequentially with the innate system functioning as a first line of defense, fending off or keeping in check attacks by pathogens until an adaptive immune response can develop. Most fishes hatch or become free-living at a relatively undeveloped larval stage, but they must still be capable of dealing with pathogens in their environments. Innate defenses develop early in life, generally before hatching, while specific immunity may require several weeks or months after hatching before becoming effective. The two systems can also work together to reinforce or facilitate one another's activities as illustrated by the complement system which not only functions as part of the innate system, but also contributes to the development of an acquired immune response and so bridges the innate with the adaptive immune response.

Despite possessing developed acquired immunity, innate defenses, particularly phagocytosis, probably play the more important role in the lives of most fishes. For example, in elasmobranchs the induction period for the humoral immunity response can be so long as to be of questionable value (Hunt and Rowley, 1986). Even in teleosts with better developed specific immune responses, these are suppressed by cold temperatures and environmental toxins (chemicals) to a degree that they may not be useful. When this happens, phagocytic activity is increased in what appears to be a compensatory mechanism.

The origin of acquired immunity is a matter of considerable debate. Some studies have found evidence that hagfish (*Eptatretus*) were capable of rejecting allographs (i.e. "non-self" recognition), producing a humoral response, and possessing putative Ig molecules and T-like leucocytes, yet most other studies have just the opposite results. Cells resembling plasma cells, but still lacking certain defining criteria, have been found in *Myxine*. Finally, although genes and their products for regulating the early development of the immune system have been found in jawless fishes, genetic studies have failed so far to reveal genes analogous to the IG, TCR, and MHC genes that form the critical basis of the developing immune system in jawed vertebrates (Khalturian *et al.*, 2004; Rombout *et al.*, 2005).

Table 12.1 The origin of the vertebrate immune system and its relationships with molecular aspects of innate immunity mechanisms. X indicates the occurrence of the mechanism and/or associated genes. PPO: prophenoloxidase; Phag: phagocytosis; Toll/TLR: Toll or Toll-like receptors ; C′: complement; Cytok: cytokines; MHCIII. Major Histocompatability Complex-III; Ag pres LMP: antigen presenting low molecular mass polypeptides; MHC I-II: Major Histocompatability Complex I and II; V-C2: C2 Ig-like set of the variable (V) domain (C2 is believed to be the most primitive while the following two sets, I and C1, are increasingly more derived); V/I set: I set of the V domain; V-C1: C1 set of the V domain; TF Lympho: transcription factors of the lymphoid cell lineage. For further explanation of the role of each of these mechanisms the student should consult any general immunology textbook, such as Paul (2003). Adapted from Du Pasquier (2004)

	Innate immunity						Adaptive immunity						
	PPO	Phag.	TOLL/ TLR	C′	Cytok	MHCIII	Ag presenting LMP	MHC I-II	Tapasin	V-C2	V/I	V-C1	TF Lympho
Vertebrata gnathostomes		X	X	X	X	X	X	X	X	X	X	X	X
Agnatha cyclostomes		X	X	X	?	X	X			X	X		X
Protochordata	X	X	X	X	X	X	X			X	X		X
Echinodermata deuterostomes	X	X	X	X						X	X		X
Arthropoda protostomes	X	X	X	X						X	X		
Porifera metazoa	X	X	X	X							X		
Plants	X	X	X										

Gut-associated lymphoid tissue (GALT), containing lymphocytes and lymphoid cell aggregates, occurs in all jawed vertebrates, and similar tissues are found associated with the gut in jawless vertebrates as well. Matsunaga (1998) and Matsunaga and Rahman (1998) suggested that the adaptive immune system of vertebrates may have first evolved within the gastrointestinal tissues of primitive jawed fish. According to their jaw hypothesis, GALT evolved in gnathostomes to deal with the traumatic stresses caused by ingesting harder, and potentially more dangerous foods, thus leading to the evolution of adaptive immune systems and creating the remarkable difference between the immune systems of jawless and jawed. Highly organized arrangements of GALT are known in higher vertebrates (e.g. Peyer's patches in mammals), where they are considered a primary immune tissue, providing mucosal immunity. The early appearance of GALT in the development of most fish species reinforces this theory, as does the near absence of GALT tissues in the syringe-feeding seahorse or other species with restricted diets such as the suction feeding North American sturgeon *Scaphirhynchus platyrhynchus*. Unfortunately, in the absence of a living placoderm, or a time machine by which we could travel back 450 million years to find one, when and where acquired immune functions first appeared among fishes will remain a mystery.

Cartilaginous fish are the earliest vertebrate group (or at least the earliest that still survives) that possess an immune system comparable to that found in higher vertebrates.

As might be expected in the most diverse group of vertebrates, actinopterygian fishes exhibit considerable morphological diversity in their immune systems as well as specializations (synapomorphies) that distinguish them from both chondrichthyan and sarcopterygian fishes.

Sarcopterygian fishes including African lungfish (Ota *et al.*, 2003) and *Latimeria* (Betz *et al.*, 1994) possess functional Ig and MHC genes, but little else is known about their immune systems.

12.1 Why is Knowledge of the Fish Immune System Important?

Effects of disease on aquaculture and capture fisheries

Understanding the causes of disease as well as the mechanisms that prevent disease in aquatic organisms can be very valuable. Although traditionally little has been done to attempt to control diseases in natural populations, such as are exploited by most capture fisheries, the recent expansion of aquaculture has led to a need for increased knowledge of fish diseases. Bacterial and viral diseases create major economic and environmental problems in the industry. The use of antibiotics, often administered through food, remains an important mechanism for the control of bacterial diseases in aquatic organisms, however, to reduce the risk of development and spread of antibiotic resistant bacteria, more environmentally friendly methods of administration must be developed. The development of vaccines effective against viral diseases requires an understanding of the principles of adaptive immunity. As in the case of antibiotics, widespread vaccination of fish is not without controversy.

The immune system of fish is fascinating from a phylogenetic perspective

The existence of an acquired immune response appears to be a vertebrate (or gnathostome) characteristic (synapomorphy) made possible by extensive gene duplication just prior to the emergence of the first vertebrates and later the first gnathostomes. Consequently, the study of variations within that system sheds light on vertebrate (or gnathostome) phylogeny. There are only two extant groups of jawless fish – lampreys and hagfish – and accordingly they represent the most ancestral vertebrates. Both possess, to differing degrees, the genes and other fundamental building blocks of the vertebrate immune system which can provide information on the relationships of these two groups with one another and with other groups of fishes, as well as insights into the evolution of the immune system in all vertebrates. The recent discovery of a unique and alternate form of adaptive immunity in the lamprey and hagfish (Pancer *et al.*, 2004) probably represents one of the most significant discoveries in recent biology. At the other end of the fish spectrum, teleosts, like all jawed vertebrates, possess polymorphic class I and class II MHC molecules; however, unlike in other jawed vertebrates, these are not linked. This unlinked characteristic would then be a derived character for teleosts (whether it occurs in other groups of actinopterygian fishes is not known at present). Figure 12.1 presents an illustration of the principal groups of chordates and a summary of fundamental molecular mechanisms possessed by them.

Study of the immune system of fish can yield valuable insights into the human system

Despite these variations, the vertebrate immune system overwhelmingly demonstrates far-reaching consistencies, meaning that the relatively simple systems of fish are valuable as models for more complex immune systems such as that of humans.

The use of organismal health in assessing ecosystem health also relies on knowledge about and use of immune system response

The immune system of fish typically responds to contaminants at concentrations much lower than those which typically elicit responses commonly used in ecological hazard and risk assessments (Anderson, 1996; Barton, 2002).

12.2 Anatomy of the Fish Immune System

The immune system of fishes is simple and less well differentiated than that of mammals, yet the systems of teleosts and elasmobranchs can evoke responses that are comparable to those of mammals showing that functionality is achieved even in the absence of anatomical sophistication. In many regards, lymphoidal tissues are defined in a circular fashion, they are tissues that contain lymphoidal cells such as white blood cells (leucocytes) and fixed cells that perform the functions of immune responses, blood cell production (hemopoiesis), and removal of damaged or worn-out blood cells and cellular debris. Lymphoidal tissues function primarily at molecular level, their products are distributed via circulatory (and in higher vertebrates lymphatic) pathways and

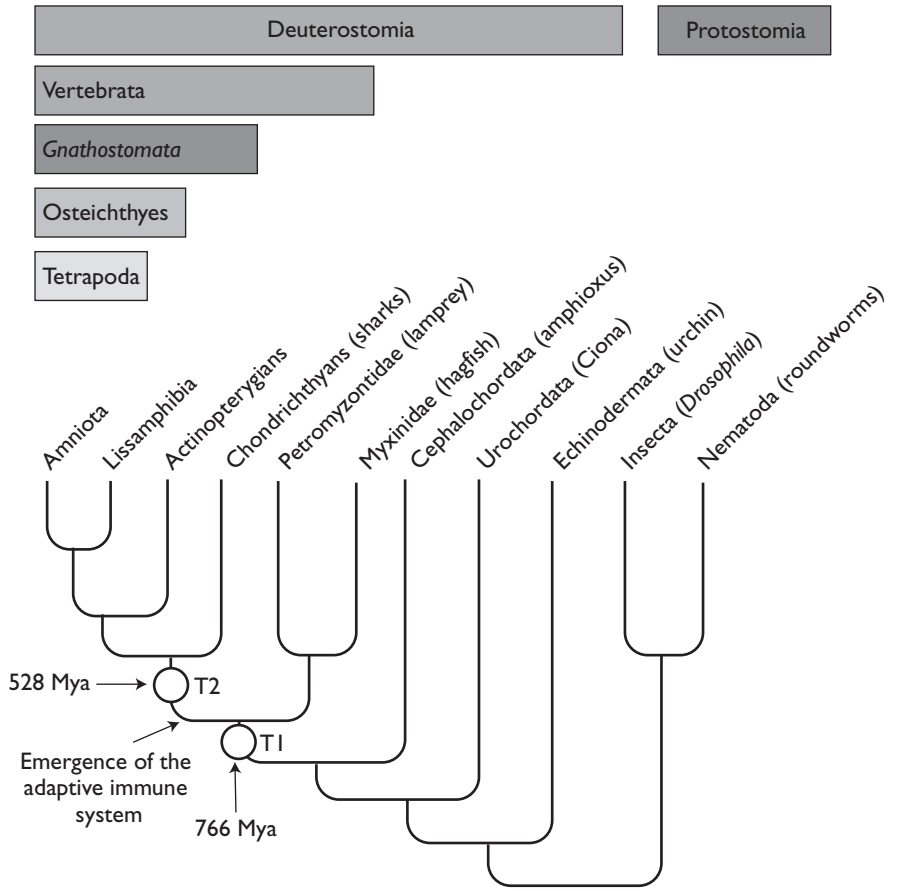


Figure 12.1 Occurrence of major histocompatibility complex components among metazoans. The arrows indicate bloc duplication events (labeled T1 and T2) in the proto-MHC and the emergence of the adaptive immune system, including the MHC. From Danchin *et al.* (2004).

so, analogous to the endocrine system, there is no need for structural complexity of the organs.

The occurrence and development of lymphoidal organs, tissues, and cells in different groups of fishes is summarized in Table 12.2 and illustrated in Figure 12.2.

Epithelial tissues and mucus

The mucus producing skin of fish represents an almost unique first line of defense against foreign invaders. Mucus contains immunoglobulin (IgM), complement proteins, lectins, pentraxins, and lysozyme. While early studies suggest that this immunoglobulin was non-specific, specific antibodies have been reported in mucus of *Ictalurus punctatus* (Lobb, 1987; Zilberg and Klesius, 1997); *Oncorhynchus mykiss* (as *Salmo gairdneri*, St. Louis-Cormier *et al.*, 1984), and other species (Buchmann, 1999; Magnadottir, 2006).

Table 12.2 Occurrence of lymphoidal cells, organs and tissues in different groups of fish

	Agnatha		Chondrichthys		Osteichthyes		
	Cyclostomes		Holocephalans	Elasmobranchs	Actinopterygii		Crossopterygii
	Hagfish	Lampreys			Chondrosteans and holosteans	Teleosts	
Thymus	No	No	Yes	Yes	Yes	Yes	Yes
Spleen	No	No	Yes	Yes	Yes	Yes	Yes
Bone marrow	No	No	No	No	No	No	No
Bone marrow analogs containing lymphoid tissues	Larval opistonephros Central mass Fat column or supraneural body	Typhlostole or "primative spleen" exists in larval forms	Orbital tissues Cranium	Leydig's organ Epigonal organ Meninges	Cranium (meninges) Choroid plexus Heart	Kidney	No
Lymph nodes	No	No	No	No – cavernous bodies of gill arches may have similar role	No	No – secondary circulatory system may have similar role	No
GALT	No[1]	No[1]	Yes	Yes	Yes	Yes	Yes
Lymphocytes	No	Yes	Yes	Yes	Yes	Yes	Yes
Plasma cells	No	Yes	Yes	Yes	Yes	Yes	Yes
Macrophages	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Granulocytes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

[1] But concentrations of lymphoid-like tissues are found in and near the GI tract.

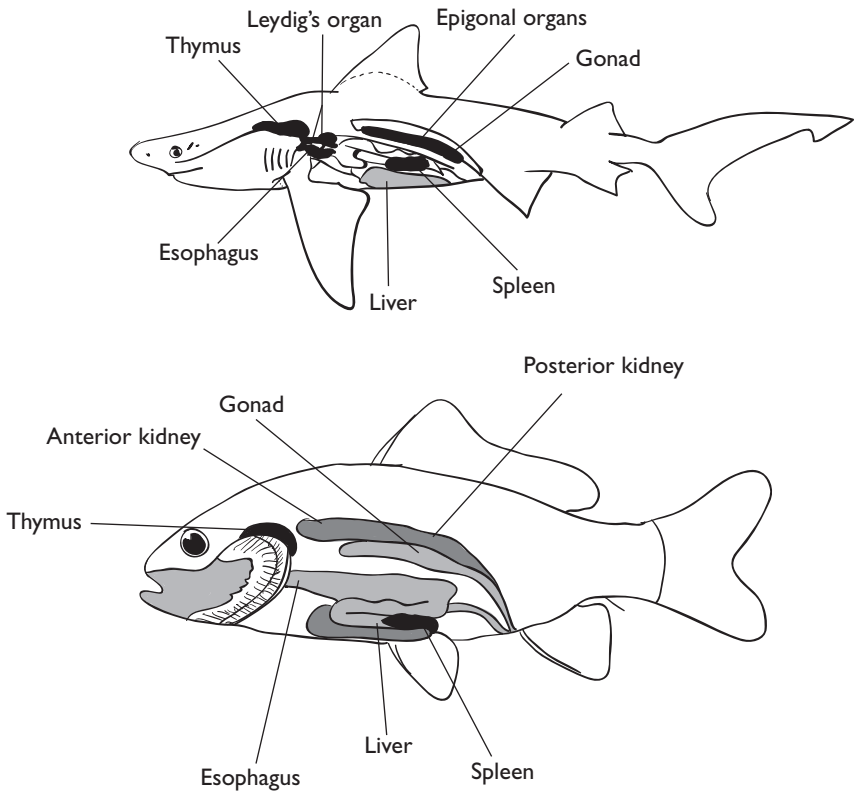


Figure 12.2 Anatomy/outline of shark and bony fish.

The internal mucosal surface area is about 400 times that of the external surface area as well as being the principal site of gas and nutrient exchange, making it a major route for pathogen entry. Mucosal associated lymphoid tissues (MALT) are present throughout much of the internal mucosal surfaces as a generalized defensive mechanism and are viewed as the probable source from which the thymus evolved as a specialized area (Bowden *et al.*, 2005).

Elasmobranchs and salmoniform fish possess abdominal pores through which coelomic fluid may exit the body. These pores also function as part of the reproductive system in some fishes. Following an abdominal infection (or injection of artificial substances into the cavity) strings of macrophage-laden mucus are excreted indicating the linings of the coelom must have an immune system function as well.

Gut associated lymphoid tissues (GALTs)

All vertebrates, including agnathans, (Uzell *et al.*, 2003) contain lymphoid cells in the lamina propria and intestinal epithelium, although well organized lymphoid aggregates first occur in Chondrichthyes. Considerable species-specific variation in size and development of these occurs in teleosts (Figure 12.3) but such tissues still appear to have a basic similarity of structure and function. GALT is so well developed in the posterior gut walls of most teleosts

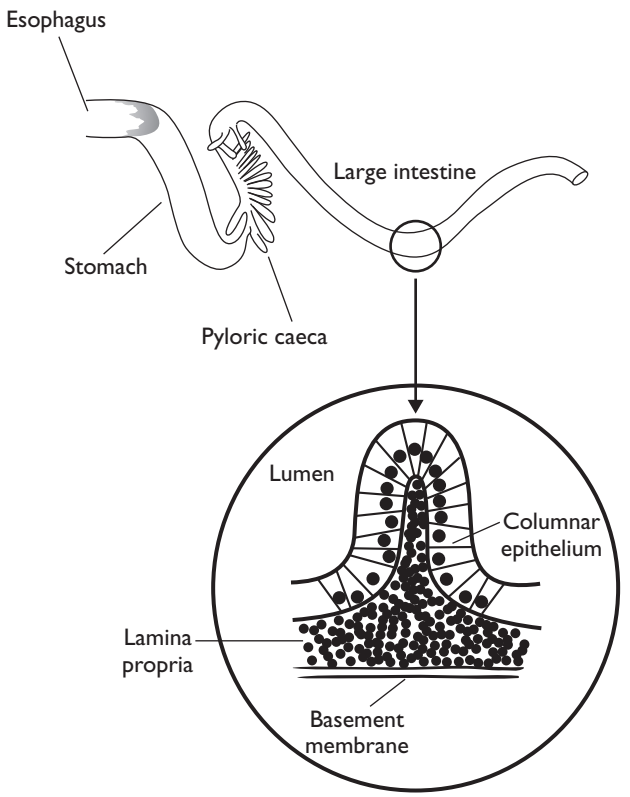


Figure 12.3 GALT tissue in the salmon gut in which the large intestine is responsible for the uptake and transport of antigens. The enlargement shows the lumen where antigens are transported to large intraepithelial macrophages in the columnar epithelium. Macrophages process and present antigens to other lymphoid cells. Although Peyer's patch-like structures (as found in mammals) are not seen in salmon, many lymphoid-like cells are present, scattered throughout the large intestine and presumably function analogously. From McLean and Donaldson, 1990.

that this section is sometimes distinguished as a "second gut" whose primary function is immunological, not digestive. (Press and Eversen, 1999; Rombout *et al.*, 2005).

Bone marrow analogs

Fish do not possess bone marrow, however, a variety of other tissues functions as sites for both blood cell production (hemopoiesis) and lymphoidal immune functions. A summary of these tissues is presented in Table 12.3.

Elasmobranchs have two lymphomyeloid organs, Leydig's organ in the lining of the esophagus (Mattisson and Fänge, 1982) and the epigonal organ in close association with the gonads (Fänge and Mattisson, 1981; Honma and Tamura, 1984). All elasmobranchs have one or both organs (Honma and Tamura, 1984) however Fänge (1984) suggests there is a reciprocal relationship in their development, for example *Prionace* and nurse sharks have well developed epigonal organs but lack Leydig's organ.

Table 12.3 Bone marrow equivalents found in different groups of gnathostome fishes

Group	Site of erythopoietic/lymphopoietica
Chondrichthyes	
Holocephali	Orbit and cranium, possibly spiral valve, cardiac epithelium in larvae
Elasmobranchi	Epigonal organs, Leydig's organ, meninges
Osteichthyes	
Actinopterygii	
Chondrosteans	Cranium, heart, kidney and gonads, spiral valve
Holosteans	Cranium, heart, kidney and gonads
Teleostei	Cranium, heart, kidney and gonads
Brachiopterygii	
Polypteriformes	Olfactory sac, meninges, kidneys
Sarcopterygii	
Dipnoi	Spiral valve, kidneys, gonads
Crossopterygii	Unknown, possibly visceral mass

Holocephalans lack epigonal and Leydig's organs. Instead, granulocytes are produced in an organ lining the orbit, whereas lymphocyte production occurs in spleen, orbital tissue, and the large thymus that lies beneath the palate (Fänge and Sundell, 1969; Hine and Wain, 1988).

Lymphoidal tissues are also found in the meninges of elasmobranchs, and some primitive bony fishes. The cranial cavities of sturgeon, bichir, and some elasmobranchs contain a saddle-shaped, lobular, tissue mass overlaying the medulla oblongata and rostral spinal cord (Fänge, 1986). Lobes contain sinuses filled with blood/plasma, and contain both white and red blood cells in different stages of development (the latter sometimes giving the tissue a pinkish appearance) indicating that they are probably produced there.

Fish do not possess lymph nodes either, however, their secondary circulatory system offers many similarities to the lymphatic systems of higher vertebrates (Ishimatsu *et al.*, 1995). The cavernous bodies, columns of specialized tissues found in the basal third of the gill septa between afferent filamentar arteries and afferent lamellar arteries of elasmobranchs, contain vascular sinuses lined with macrophages. These anatomically resemble mammalian lymphatic capillaries, except that the interlamellar vessels are directly fed by arteriovenous-like anastomoses. However, they develop from mesenchymal endothelium, and so, despite having a similar function (phagocytosis, cleaning up debris, etc.), are not embryological equivalents of the mammalian lymphatic system (Olson, 2002).

12.3 Major Organs of the Lymphoid System

The thymus, kidney, and spleen are the major lymphoid organs of teleosts (Manning, 1994) and the thymus and spleen also fulfill similar roles in elasmobranchs (Luer *et al.*, 2004). Hemopoietic activity first occurs in the pronephric or head kidney; however, the thymus is the first tissue to develop a true lymphoidal character. The spleen appears to be the last organ to develop lymphocytes in freshwater fishes (although it may be an early site of red cell production), but interestingly enough develops earlier than the thymus in marine species. This dichotomy may be an artifact due to the selection of only a few species for study, since among freshwater fishes only cyprinids and salmonids have been studied. However, marine fishes studied constitute a much broader phyletic spectrum including serranids, sparids, scorpaenids, pleuronectids, and gadids (Zapata *et al.*, 2006).

The thymus

The fish thymus develops from the third gill pouch, dorsal to the gills, on either side and just beneath the surface of the opercular lining, and, unlike in higher vertebrates, retains a connection with the pharyngeal endoderm. The thymus shrinks after adulthood is attained and there is a replacement of secretory with fatty tissues. This process, known as involution, is common in higher vertebrates but less common in fishes where the thymus may persist throughout life in some species (e.g. *Lophius*). The thymus contains mostly lymphocytes, although macrophages may also be common.

The kidney

The kidney is an organ important in both immunity and hemopoiesis. It is the site of blood cell differentiation as well as the development of early immune responses in fish embryos. With maturity, the anterior portion (head kidney) has no renal functions, instead it contains lymphomyloid tissues that produce lymphocytes (specifically B-cells) and adrenal-like endocrine tissues.

A great deal of variation is seen in the development of the kidney among different major groups of fishes, but, in all cases, parts of it are involved in immune system function. Kidney development begins as a pronephros, a few primitive kidney tubules attached to a single nephric duct. This type of kidney is found in all larval fishes and is retained by hagfish (and a few others) throughout life. In most other fish, the pronephric kidney is replaced by a mesonephric (also called opistonephric) kidney in late larval, juvenile, and adult organisms. Although chiefly an excretory organ, the structure of the pronephros requires that blood flows through it only very slowly, permitting it to function not only as a site for early hemopoiesis, but also as a site for other functions, such as cleansing and endocrine secretion, to occur. In myxinoids, hemopoietic tissues occur in/around the nephrostome of the pronephros. In lampreys the larval pronephros and opisthonephros function as lymphoidal organs, however these roles are largely taken over by other organs after regression of the larval opistonephros (see Table 12.4).

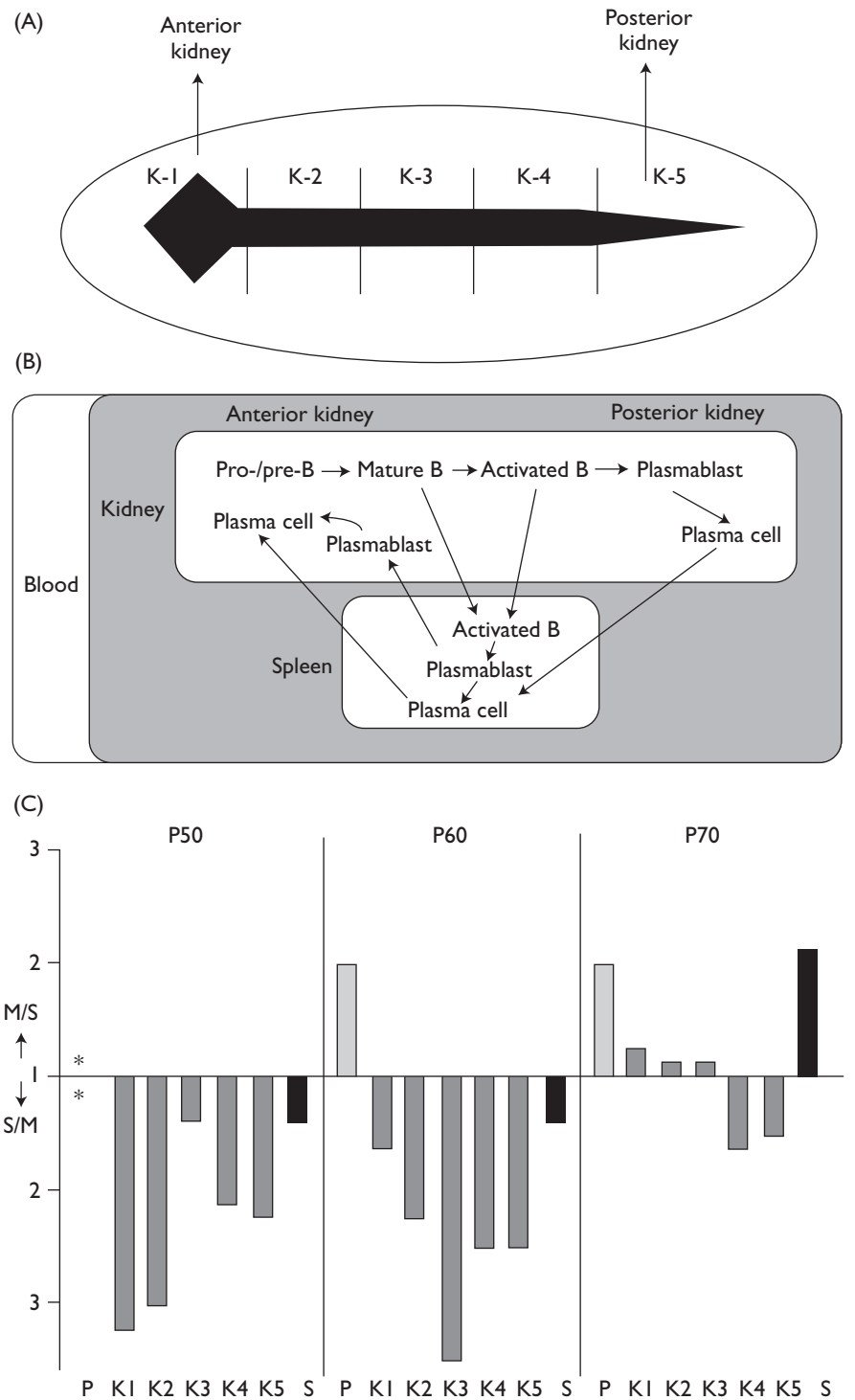
The kidneys of cartilaginous fish have been shown to contain lymphohemopoietic tissue only during embryonic development. These functions are apparently taken over by tissues such as the Leydig's organ and epigonal tissue

Table 12.4 Development of lympho/hemopoietic tissues in lampreys

Life history/developmental stage	Lymphopoietic organs
Ammocoetes (stages I–III)	Typhlosole, nephric fold, larval opistonephros, adipose tissues (stages I & II only)
Metamorphosing (IV)	Typhlosole, supraneural body
Macrophthalmus (V)	Supraneural body
Parasitic adult (VI)	Supraneural body, adipose tissue, adult opistonephros

in adult elasmobranchs (Zapata *et al.*, 1996). Considerable variation is also seen in bony fishes. In the primitive paddlefish (*Polyodon*) the structure of the kidney does not change suddenly, but the anterior portion contains mostly hemopoietic and lymphoidal cells representing a remnant of the larval pronephros (Georgi and Beedle, 1978). In most teleosts the pronephros functions as an excretory organ only in larval stages. In juveniles and adults, the tubules of the anterior region degenerate, and the "head kidney" becomes a hematopoietic, lymphoid, and endocrine organ. The mid- and posterior kidneys contain both renal and immune tissues. B-cells originating in the anterior kidney mature and are activated as they pass posteriorly, finally resulting in plasmablasts and functional plasma cells. B-cells at all stages of maturity may be released and occur in the blood or spleen as illustrated in Figure 12.4 (Zwollo *et al.*, 2005).

Figure 12.4 Kidney hemopoiesis. A) Schematic drawing of the trout kidney to indicate the location of segments K1–K5. The kidney was divided into five sections, K1–K5, based on anatomical location. K1 corresponds to the most anterior site of the kidney, residing below the most anterior seven vertebrae (anterior kidney). Each progressive section consists of the kidney below the next seven posterior vertebrae, with K5 terminating the posterior kidney. B) Model outlining trout kidney function during B cell development and activation and immunological relationships with spleen and blood. In this model, B cell stages and developmental capacity were determined experimentally. Migratory routes are hypothetical. B cell precursors develop in the anterior side of the kidney, whereas mature B cells migrate to the posterior kidney or the blood. Posterior kidney and spleen are both sites for B cell activation. Plasmablasts formed in posterior kidney or spleen differentiate into plasma cells. Plasma cells migrate to the anterior kidney where they may become long-lived plasma cells. C) Results from RT-PCR on membrane and secreted forms of heavy chain Ig. Amplified DNA fragments were separated by agarose gel, and relative intensities were quantified using NIH Image analysis software. *Top three panels*, M_S: ratios of membrane to secreted values (M/S) for cell subsets with M_S. *Bottom three panels*, S/M: ratios of secreted to membrane values (S/M) for samples with S<M. *n*=3. [light hatching], PBLs (P); [medium hatching], kidney (K-1 through K-5); [dark hatching]s, spleen (S). P-number 50, 60 and 70, refers to cell density as determined by a Percol separation. In general the higher the number the fewer activated cells in the sample. Adapted from Zwollo *et al.* (2005, Figures 1, 3, and 6, pp. 6609, 6612, and 6614).


(A)
Anterior kidney
Posterior kidney
K-1
K-2
K-3
K-4
K-5
(B)
Anterior kidney
Posterior kidney
Kidney
Pro-/pre-B → Mature B → Activated B → Plasmablast
Plasma cell
Plasmablast
Plasma cell
Blood
Activated B
Spleen
Plasmablast
Plasma cell
(C)
P50
P60
P70
3
2
M/S
1
S/M
2
3
*
*
P K1 K2 K3 K4 K5 S P K1 K2 K3 K4 K5 S P K1 K2 K3 K4 K5 S

The spleen

When present, the spleen contains lymphocytes and macrophages and is involved in immune reactivity as well as being the most important hemopoietic organ in fish. Hagfish lack a defined organ but *Myxine* and *Eptatretus* possess islands of hemopoietic tissue around the portal veins (Fänge, 1986) and spleen-like tissues occur in the intestinal spiral valve of lampreys (Hart *et al.*, 1986). In elasmobranchs the spleen is an elongate strip of tissue attached to the mesenteries between the stomach and duodenum, and is supplied with arterial blood which drains into the portal vein. In teleosts, the size, shape, and extent of development varies. Generally, it is more compact than that in sharks, consisting of masses of red and white pulp (respectively sites of RBC and WBC production) surrounded by numerous small capillaries.

12.4 Cells, and Molecules

Cells

The cells of the immune system can be either fixed or migratory. Fixed macrophages and dentritic cells are found in the skin and mucosal membranes as well as the various lymphoidal organs. Migratory cells, also known as leucocytes or white blood cells, occur both in the blood (both primary and secondary circulatory systems) and in interstitial tissue fluid. Although the total number of leucocytes varies with stress, diseases, and environmental conditions, fish generally have greater white cell counts than mammals; Mulcahy (1970) notes 79 800 to 137 000 cells per cubic mm in pike.

Fish have at least seven types of leucocytes (Figure 12.5): three or more eosinophilic granulocytes, one heterophilic/neutrophilic granulocyte, a rarely reported basophilic granulocyte, lymphocytes (the most common variety, comprising roughly 20% to over 99% of the total white cell count) monocytes and thrombocytes. There is a great deal of confusion in the literature as to the nomenclature of these cells, especially the granulocytes. Nomenclature of fish leucocytes is based on similarities with those found in mammals, and, while in some cases there are clear parallels between fish and mammal leucocytes, most authors agree that there is no absolute correspondence. However, they continue to utilize the same names (Fänge, 1992; Rombout *et al.*, 2005).

The principal cells in involved in innate immunity are phagocytic granulocytes (neutrophils) and macrophages as well as non-specific cytotoxic cells (NCCs) or natural killer cells (NKC), while acquired immunity depends on the activities of different types of lymphocytes.

Granulocytes

Granulocytes are distinguished on histochemical grounds as eosinophils, neutrophils, and basophils, the last of which are only rarely reported in fishes.

Neutrophils (often categorized as heterophils) are the most common variety of granulocyte. They are part of the mobile defense mechanism that is transported to sites of infection or injury. They migrate through capillary walls, and engulf and destroy invading bacteria. Dogfish neutrophils and those of some teleosts too, have been shown to possess a bacteria-destroying peroxidase system similar to that found in higher vertebrates. Sturgeons (and other primitive

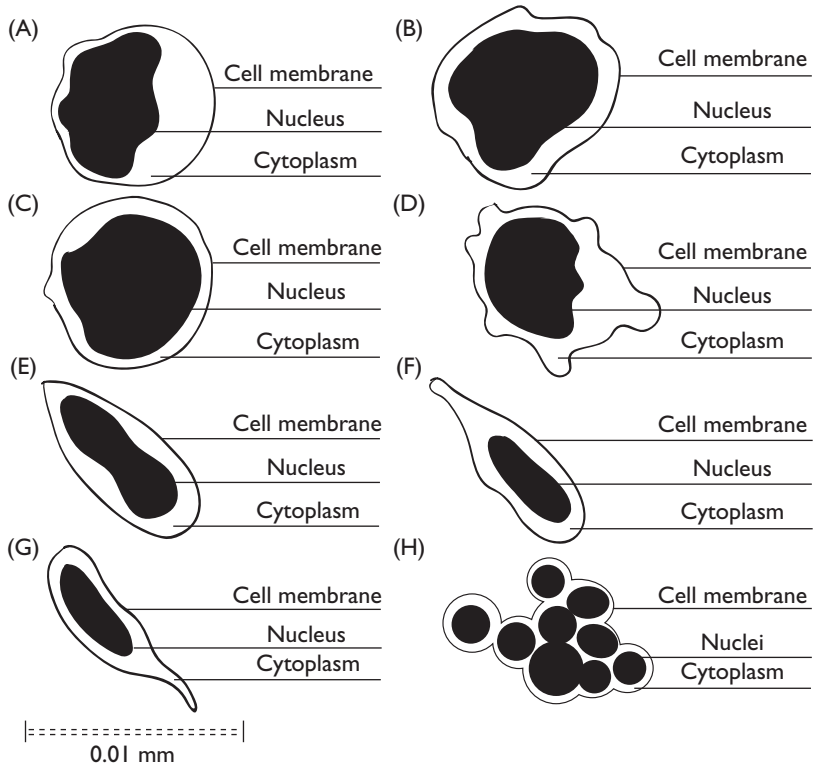


Figure 12.5 Representative fish leucocytes: (A) granulocyte; (B) lymphocyte; (C) immature lymphocyte; (D) monocyte; (E) oval thrombocyte; (F) spindle thrombocyte; (G) spiked thrombocyte; (H) cluster of fragmented thrombocytes. All drawings to same scale.

bony fish) possess an analogous system in their eosinophils. Eosinophils have limited phagocytic capacity, nevertheless they can attach to the surface of and destroy large parasites by releasing cytotoxic enzymes into the parasite. (Reite and Evensen, 2006). In higher vertebrates, basophils release histamines, a chemical that is part of the inflammatory response, the scarcity of basophilic-staining cells in fish probably is reflected in the apparent scarcity of these chemicals as well.

Monocytes

Monocytes are large circulating cells that develop into macrophages at the site of an infection.

Macrophages

Macrophages are fixed cells that develop from monocytes that migrate to the spleen, liver, and kidney where they form interlacing networks of lymphoidal tissue. Macrophages are also found in the walls of the atrium, the intestinal walls and mesenteries. Besides having a role in the removal of pathogens and cellular debris by phagocytosis, macrophages also help regulate the activity of T-cells.

Non-specific cytotoxic cells/natural killer cells

Non-specific cytotoxic cells are a variety of lymphocyte that function primarily as part of the innate immune system. Again, there is some debate as to the analogy between fish NCC cells and mammalian natural killer cells (NKCs), hence the preferred use of the term non-specific cytotoxic cells for fish. Some fish seem to possess only NCCs, others have both NCCs and cells that resemble mammalian NKCs. In either case, they function much in the same way, by attacking the body's own cells; either cells infected by a virus or cancerous cells. The NCC/NKCs mode of destruction is not phagocytosis but an attack on the membrane of the target cell, in a manner similar to the mode of action of cytotoxic T-cells, which causes that cell to lyse (break open) but which unlike the T-cell action does not require a particular antigen to be present.

Lymphocytes

With the exception of Agnathans, extant fish, like higher vertebrates, possess three basic types of lymphocytes. Natural killer cells, discussed above, B-cells which are responsible for humoral immune responses through the production of immunoglobulins (antibodies) to fight extracellular infections (bacteria, fungi, etc.) and T-cells that are responsible for cell-mediated immune responses and include both effector and regulatory cells. Cytotoxic T-cells ($CD8^+$) attack and destroy host cells, which have become infected by viruses or other intracellular pathogens. Distinct from the action of natural killer cells, cytotoxic T-lymphocytes require specific antigen presentation to recognize target cells. Helper T-cells ($CD4^+$) activate both B-cells and cytotoxic T-cells for the attack, while suppressor T-cells await the signal to change, slow, or end the assault.

Sharks possess lymphocytes homologous to mammalian B- and T-cells, although B-cells may not be identical with the mammalian B-cell (Anderson *et al.*, 2004), B-cells in bony fishes, for example, have been shown to possess phagocytic capabilities (as shown in Figure 12.6) that are absent in cells of higher vertebrates (Li *et al.*, 2006).

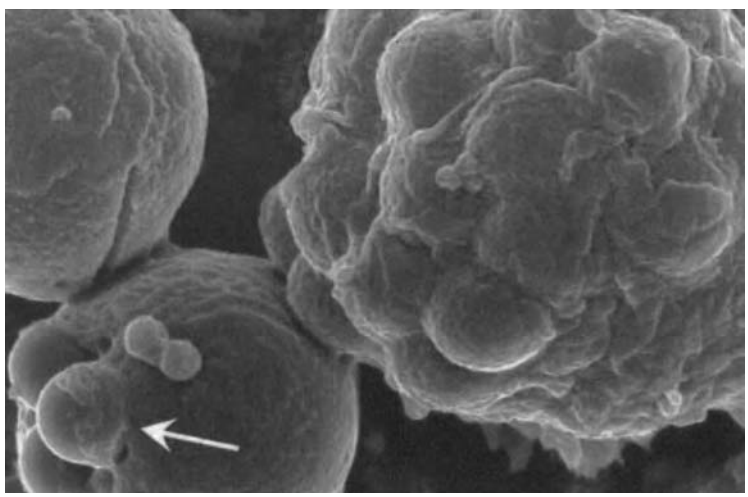


Figure 12.6 B lymphocyte phagocytosis. Lymphocyte-like cell (white arrow) in process of ingesting three 1 μm latex beads and larger neutrophil-like cell containing several ingested beads. From Li *et al.* (2006, Figure 4b, p. 1119).

The occurrence of lymphocytes in lampreys (Shintani *et al.*, 2000) has quite recently been explained by the discovery of their unique (among vertebrates as well as all animals) system of rearranging antigen receptors.

Thrombocytes

Fish thrombocytes come in a variety of shapes; usually with either an oval or spindle-shaped nuclei and possess limited phagocytic capacity. They also play a role in blood clotting similar to that in higher vertebrates. In mammals thrombocytes rarely occur in general circulation, but are the precursors of blood platelets which are the cellular basis of the clotting mechanism.

Plasma cells

Plasma cells develop from activated B-cells and are the actual sites of antibody production. They appear to be normally restricted to the spleen, the head kidney, and other lymphoidal tissues, and are not normally found in circulation.

Rodlet cells

Enigmatic cells, first described as Sporozoan parasites, which may have an immune function. These are possibly an immature cell type that later develops into an eosinophilic granulocyte (Manera and Dezfuli, 2004; Reite and Evensen, 2006).

Molecules

Both the innate and acquired immune systems include humoral components. These consist of molecules that are present in the blood, interstitial fluid, and exocrine secretions. Molecules which function in the innate system include: lytic enzymes such as lysozyme which is found in the mucus, lymphoid tissues, and serum of most fish species; lectins that bind to carbohydrates on the surface of pathogenic organisms to result in opsonization, phagocytosis or activation of complement; iron binding transferrins, that have an important role in the nutrition of the organism as well a defensive role by denying necessary iron to bacteria, and complement, the latter also bridging the innate and acquired immune responses. Cathepsins may have a bactericidal role in the skin and intestine of fish. Molecules particular to the acquired immune system include immunoglobulins (antibodies) and antigen receptor molecules found on T- and B-cells, however, various signaling molecules such as cytokines and complement are also present.

Humoral factors have been found in hagfish and lampreys which appear to be similar to or identical to most of the non-specific factors found in higher vertebrates. Although the basic cohort of genes that define the vertebrate acquired immunity (RAG, Ig, TCR, and MHC) are absent, some agnathan genes may conveniently be viewed as analogs or functional precursors to genes involved in immunologically relevant activities of higher vertebrates, earlier reports of apparent immunoglobulins in jawless fish are probably based on the occurrence of a C-3-like complement molecule.

Despite extensive searching, acquired immune mechanisms represented by rearranging antigen receptors or MHC genes have not been found in these primitive fishes. On the basis of negative evidence it had generally been assumed that they do not possess an adaptive immune system, making them the only group of vertebrates without one.

Elasmobranchs possess a variety of nonspecific molecules, which act as their first line of defense. In fact, the first antimicrobial agent to be identified was squalamine, a steroid with broad-spectrum antibiotic activity found in the liver and intestine. It may serve as a systemic antimicrobial agent in elasmobranchs, and has been shown to act against bacteria such as *Escherichia coli*, strains of *Staphylococcus* and *Streptococcus*, and fungi. Chitinase, an enzyme usually associated with digesting chitin-shelled arthropods has also been found in lymphoid tissues and blood plasma where it presumably has a role contradicting chitin-clad parasites, such as myxosporidian spores and anchor worms. Lysozyme activity has also been detected in shark leucocyte lysates (Bird *et al.*, 2002).

Bony fish possess a broad repertoire of molecular defenses ranging from bactericidal and bacteriostatic compounds in their mucus, skin, and other epithelial membranes to a well-developed system of signaling proteins and antibodies.

Nitric oxide

Nitric oxide is one of the most potent defense mechanisms utilized in innate immunity. The binding of bacteria to macrophages results in the production and release of nitric oxide (NO) in nurse sharks and several teleost species (and presumably all other fishes as well) which is toxic and can kill microorganisms in the vicinity of the macrophage.

Histamines

Histamines play a powerful role in initiating inflammation in most vertebrates, however, there are conflicting reports as to the presence of histamine in fish. Instead, fish thrombocytes and eosinophils release serotonin which appears to have the same vasodilatory and other inflammatory effects. Histamines, and their effects, in fish may be normally limited to brain and nervous tissues.

Scombroid fish poisoning and other food intoxications are generally associated with eating fish, especially spoiled fish, which have high levels of histamines to which humans react by presenting an elevated fever and gastrointestinal disorders. The histamines in these cases are thought to be the products of bacterial decomposition of fish proteins and not naturally occurring compounds.

Cytokines

Cytokines are proteins (usually glycoproteins) of relatively low molecular mass (17 000–650 K daltons) and usually consist of a single chain. Cytokines are signaling chemicals secreted by various leucocytes to activate other cells, coordinate, and regulate all important biological processes. Homologs of well known mammalian cytokines including interleukin and interferon have been found within jawless and bony fish, and recently in cartilaginous fish as well (Bird *et al.*, 2002).

Complement

Complement is a system of serum proteins, named for their cooperation with other defense mechanisms, activated in a cascade sequence. It is part of the non-specific immune system that generally deals with bacterial infections. Complement activates macrophages, aiding their ability to find and digest

foreign cells and attracts neutrophils to the scene of an infection, which can then kill the bacteria by producing peroxide.

Fish appear to possess activation pathways similar to those in mammals, and the fish complement proteins identified thus far show many homologies to their mammalian counterparts (Holland and Lambris, 2002). The complement system of mammals includes three different activation pathways, known as the "classical pathway" because it was the first discovered, the "alternative," a somewhat shorter pathway believed to represent a more primitive system parts of which are found in agnathan fishes and deuterostome invertebrates, and the "lytic" or "lectin" pathway which is viewed as an evolutionarily late addition that ties the complement system into acquired immune system functions. Both cartilaginous and bony fishes possess all three complement activation pathways.

Toll-like receptors

Toll-like receptors (TLR) are components of innate immunity occurring in all vertebrate groups, except cartilaginous fishes where their existence has not been established. However, they should be expected here due to their occurrence in invertebrate groups (Purcell *et al.*, 2006; Roach *et al.*, 2005).

Toll-like receptors are proteins found in the membranes of dendritic cells, one of the earliest cell types that recognize microbes once they have breached physical barriers such as the skin or intestinal tract mucosa, and activate immune cell responses. They are believed to play a key role in the innate immune system, and form an evolutionary linkage between the innate-only systems of invertebrates and the acquired immunity found in vertebrates. Their name derives from their homology to a family of molecules first found in the fruit fly *Drosophila melanogaster*, known as Toll (which in German, means "amazing" or "cool") which regulate fly development as well as playing a role in the immune response.

Antibodies

The antibody-based immune system (AIS) is a key part of the most highly evolved system by which organisms protect themselves against pathogens and parasites. In higher vertebrates, rearranging DNA segments coupled with random mutations provide a basis for 10^{14} different antibody combinations. In contrast, the variable leukocyte receptors (VLRs) of lampreys and hagfishes are proteins containing varying numbers of leucine-rich segments, which bind to antigens in a manner similar to that of antibodies. Although based on only a single gene in lampreys, and two genes in hagfishes, VLRs have been shown to produce as much protein diversity as that found in higher vertebrates (Alder *et al.*, 2005) resulting in a functionally equivalent adaptive immune system, but one that lacks antibodies. Antibodies are a class of protein, also known as immunoglobulins (Ig) produced by B-cells in response to foreign substances (antigens). Unlike all other proteins in the body, the genes that control antibody production are not fixed, but consist of DNA sequences that can produce proteins with slightly variable structures permitting them to respond to an almost limitless number of antigens. This differentiation occurs prior to B-cell formation, so that every B-cell produces its own unique antibody without the need for exposure to any antigen.

Each antibody molecule is roughly "Y" shaped (Figure 12.7) and consists of two identical heavy and two identical light chains of amino acids each of which contains a constant and a variable region. Different isotypes of immunoglobulins (IgM, IgG, IgA, IgD, and IgE; Figure 12.8) have heavy chains consisting of different amino acid sequences in their constant regions. The variable region includes 110–130 amino acids of the light and heavy chains, and is responsible for binding to antigen. This part of the antibody shows variations in amino acids when the specificity of the antibody for antigen is changed.

Although antibodies are produced without prior antigen exposure, once an organism has been exposed it develops specialized B-cells, known as memory cells, which reside in a resting state and upon re-exposure to the same antigen allows the organism to quickly produce more of the necessary antibodies.

Lamprey, and hagfish. Despite much effort and early reports to the contrary, antibodies have not been found in the agnathans. Lampreys do, however, possess a highly expressed family of highly diverse proteins built up from leucine-rich repeats (LRR). These lymphocyte receptors seem to be generated by a novel rearrangement mechanism that generates an enormous amount of diversity; and like immunoglobulins and T-Cell Receptors, can be expressed clonally by the lamprey lymphocytes. These receptors may explain old data that suggested that lampreys indeed had adaptive immunity. The significance of this system is greater than just that it is different from what is found in higher vertebrates, it demonstrates that there can be diverse ways of coping with pathogens, a phenomenon that repeatedly is seen in different immune mechanisms of all types of fishes as well as other organisms.

Cartilaginous fish. These animals were once believed to have only IgM, but now have been shown to have at least two other isotypes: IgNAR and IgW. Sharks have two forms of IgM, a pentameric and monomeric, generally expressed in equal amounts, which makes them unique among vertebrates. Shark IgMs are also distinctive in having only two combining regions, one on each arm whereas mammals have six. IgNAR (NAR = "New Antigen Receptor," also called IgH) is distinctive in that it consists of only two heavy chains. A fourth immunoglobulin isotype, IgR, has been reported from rays (hence the "R" designation), however it appears very similar to, if not the same as, IgW from other elasmobranchs. They also appear to have some fundamental gene-based differences in antibody production. In bony fishes, as in mammals, antibody diversity is derived from a system in which RAG genes assemble antibodies by "cutting and pasting" segments of DNA. In contrast to this system, known as class switching, most shark antibody genes are encoded by regions of DNA that are arranged in the order of the final antibody product (Figure 12.9). A shark will need to possess many times more antibody encoding regions since most only encode for a particular antibody (there are still some in which class switching activity occurs), however these antibodies can be produced much more quickly than in teleosts and appear to be inheritable. Until recently there has been no evidence of a memory response within the elasmobranchs as well (reviewed in Flajnik, 1996; but see Dooley and Flajnik, 2005).

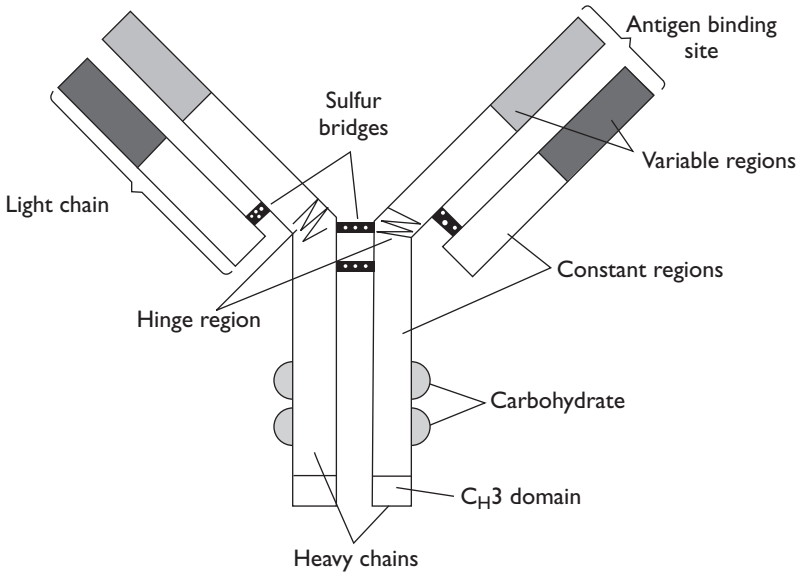


Figure 12.7 Y-shaped structure of an antibody molecule. Two antigen binding sites are present at the ends of either light chain arm. This will vary in amino acid sequence between different antibody molecules, while the remainder of the molecule (the constant regions) will be the same in all antibodies. Chains are held together by sulfur bridges as indicated.

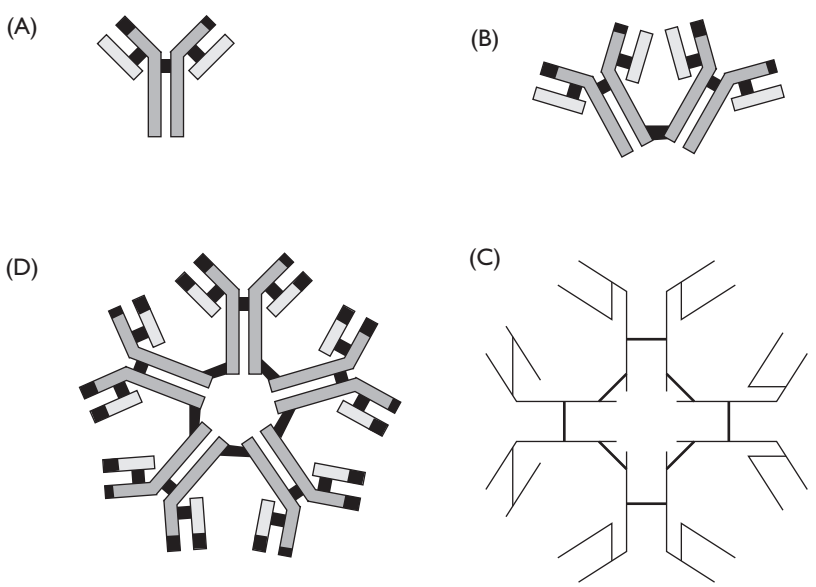


Figure 12.8 Various Ig (antibody) molecules found in fishes illustrating: (A) unimeric, (B) dimeric, (C) tetrameric, and (D) pentameric configurations.

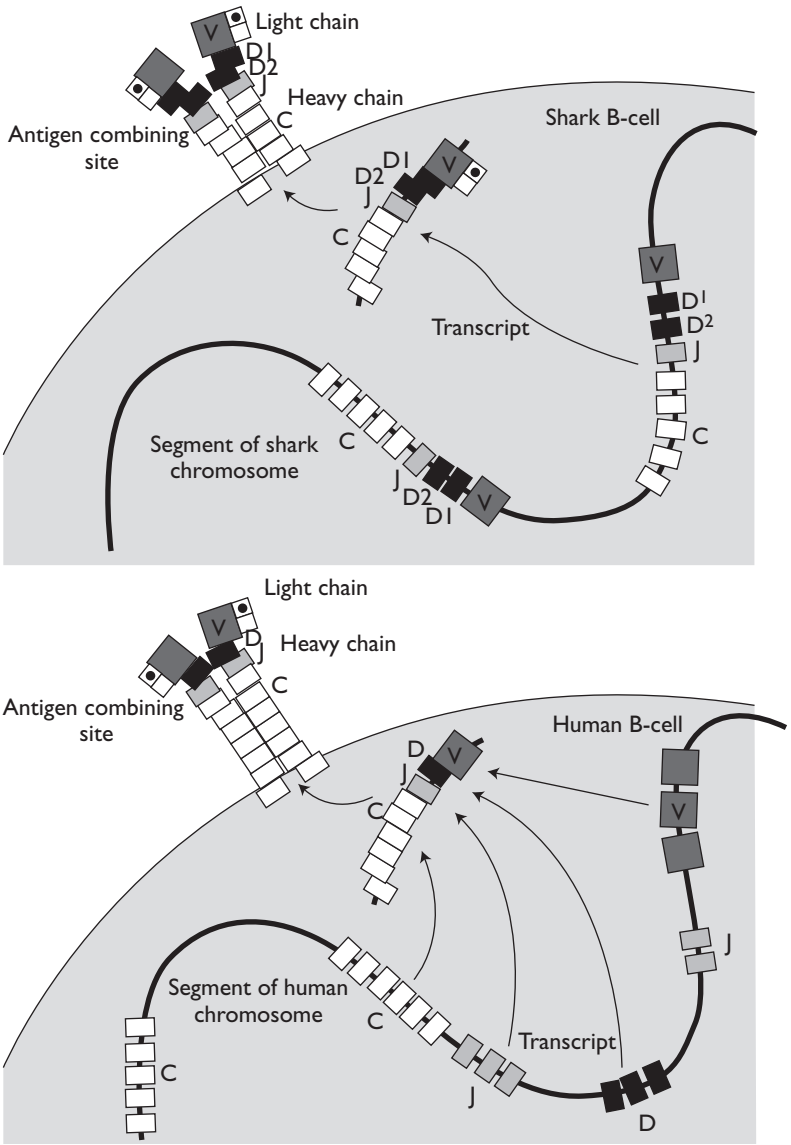


Figure 12.9 Comparison of the shark and mammalian antibody gene system. Note differences in the arrangement of gene segments on the chromosome that recombine to produce antigen receptors. A simplified version of formation of the heavy chain molecule that makes up part of the receptor is shown, which is an IgM antibody common to both sharks and mammals. In mammals the gene segments that come together are scattered along a relatively long length of one chromosome, whereas in sharks the gene segments are aligned next to one another on any one of several chromosomes. From Rao (2005, Figure 1.2, p. 10).

Actinopterygian fish. Like all jawed vertebrates, they have IgM, which is found only in a tetrameric form. Also, a form that seems related to mammalian IgD has been found in several teleost species as well as one or more additional isotypes (IgT, for teleosts, and IgZ for zebra fish). This diversity of isotypes

shows that these, and probably all, bony fishes have a greater capacity for functional diversity in their immune systems than has been imagined in the past. The bony fishes also appear to have memory, and within some species of this class there is evidence of maternal transfer of antibodies (Bly *et al.*, 1986).

Sarcopterygian fish. Lungfishes posses IgM and IgW, previously believed to be specific to cartilaginous fish as well as a low molecular weight Ig known as IgN. The small size of IgN indicates that it too probably only contains two C-domains.

Envoi

The study of the immune system of fishes presents some of the most interesting and challenging opportunities for comparative biologists and fish epidemiologists. As a distinctive characteristic of vertebrates, yet derived from genes and molecular systems present in many invertebrates, their immune system, or more correctly systems, since it is increasingly obvious that these do not represent a common feature, but rather evolutionary variations on a common theme, demonstrate the close relationship as well as diversity of vertebrate groups. Of greater practical value, the study of the immune system of fishes can lead to the development of vaccines and medications necessary for keeping farmed and wild fish populations healthy.

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13 Behavior and Cognition

13.1 Introduction

Behavior

Behavior refers to the observable or measurable actions or reactions of an organism in response to a stimulus originating from its environment. Or, more simply put, it is "anything an animal does" (Keenleyside, 1979). The stimulus may be some environmental change such as day-length or temperature, or it can be the activity of another organism, often a potential predator, prey or mate. In most cases this stimulus acts as a *releaser*, initiating a series of events within the endocrine or nervous system which culminate in an external, observable, response. Cognition is defined as the mental process of knowing, including aspects such as awareness, perception, reasoning, and judgment. The study of cognition in animals has developed out of comparative psychology, and has also been strongly influenced by ethology and behavioral ecology. Much of what used to be considered "animal intelligence" is now regarded as cognition.

Cognition

The capacity for brain function is commonly assumed to be related to brain size, complexity, and sophistication. Hence, fishes, because of their relatively small (except in the case of sharks) and simple brains, have not been regarded as possessing much capacity for memory, learning, or other cognitive functions. But many fish live in complex social systems that require a cognitive capacity much greater than previously assumed (Laland *et al.*, 2003). While there is much compelling evidence that fishes "teach" or consciously pass on information or knowledge to others, they have been shown to learn by observing and mimicking the actions of other, more experienced (and hence knowledgeable) individuals. Fish learn to recognize others as individuals, not only members of their own species (Binoy and Thomas, 2006), but also members of other species (Stummer *et al.*, 2004). They learn from others becoming more efficient at finding mates and food or avoiding predators. They can spatially encode, and remember, complex environments, allowing them to navigate obstacles and return to precise locations despite the absence of immediate sensory inputs (Burt de Perera, 2004).

Fishes from the earliest times on have had basically the same brain pattern. Despite its anatomical simplicity, *Myllokunmingia* shows a characteristic craniate pattern with optic and nasal capsules, and sclerotic cartilages (Northcutt, 2002). From this simple beginning the brains of modern fishes with their capacity for learning and cognition must have developed (Figure 13.1). As explained in Chapter 11, the cerebral hemispheres of actinopterygian fishes develop in a radically different pattern from that found in other vertebrates, everting and expanding laterally rather than envaginating inwardly, a scheme possibly necessitated by the evolution of small size especially in larvae while maintaining a large optic capacity necessary for finding food.

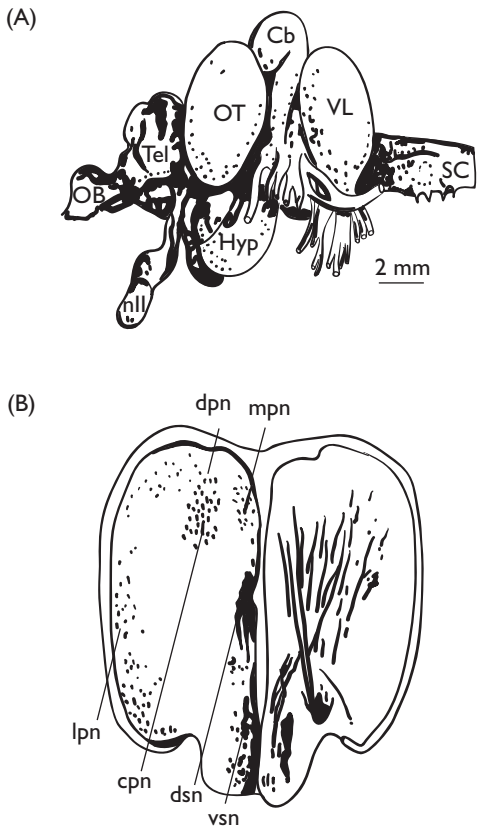

Figure 13.1 Schematic diagram of the brain of a general teleost fish. (A) Lateral view of the goldfish brain showing the key large-scale structures. Hindbrain, including cerebellum (Cb), vagal lobe (VL), and spinal cord (SC); midbrain, including the hypothalamic lobe (Hyp), optic tectum (OT); and forebrain, consisting of the olfactory bulb (OB), optic nerve (nII) and telencephalon (Tel). Adapted from Broglio *et al.* 2003. (B) Transverse section through telencephalon of a typical teleost: cpn = central pallial nucleus, dpn = dorsal pallial nucleus, lpn = lateral pallial nucleus, mpn = medial pallial nucleus, dsn = dorsal subpallial nucleus, vsp = ventral subpallial nucleus (adapted from Nieuwenhus 1967). The different pallial and subpallial nuclei receive sensory inputs from the primary sensory areas as well as the thalamus and other specialized relay nuclei and have been shown to be involved in the processing of various sensory modalities and are thought to play a role in spatial mapping and memory.

The cerebellum of teleost fish is involved in learning as well as motor responses. Damage impairs classical conditioning but does not impair observable reflex-based motor responses such as swimming or avoiding obstacles. Conditioning is reflected by increased levels of cytochrome oxidase (COX) activity in the cerebellum. Damage to the cerebellum also effects spatial learning – in comparison to controls or sham-operated fish cerebellar damaged goldfish show stereotypical and inefficient exploratory behavior when placed in an obstacle course (Rodríguez *et al.*, 2005).

13.2 Behavior as a Discipline

Although the various responses of an organism may be described or studied in the context of the different anatomical and physiological systems or the ecology of a species, for example the endocrine system or feeding behavior, there is merit in consideration of behavior as a separate discipline (Goodson, 2005). A behavioral observation is like a photograph of the fish at a particular point in time. It integrates a broad spectrum of anatomical features and physiological mechanisms into a single observable, sometimes quantifiable, response about which we may draw conclusions as to its importance or role in the survival of an individual. Behavioral adaptations can take their place along with morphological and physiological adaptations, increasing the fitness of individuals and through natural selection the evolution of species (Taborsky, 2001; Gilmour *et al.*, 2005).

How is behavior studied?

In the past, behavioral observations were often limited to the interpretation of observations (Aristotle wrote about schooling behavior in fishes) or the oftentimes crude quantification of responses. While these techniques are still used, often with valuable results, technology has afforded many improvements. Digitized images can be analyzed to show precise patterns of motion and times of responses. Telemetry delivers data recorded at field sites to distant laboratories for analysis and archiving. Acoustic tags, Passive Integrated Transponder (PIT) tags detected by magnetic readers, satellite tracking, and telemetry all permit not only precise tracking of individuals, but sometimes also the recording of physiological and ecological conditions while the fish are freely swimming (Hawkins and Usrquhart, 1983). Increasingly sophisticated and sensitive physiological instrumentation can be used to measure responses both in the laboratory and the field. Long-term recordings can record the presence, absence, or types of activities without the need for the investigator's presence.

Categorization of investigators

Traditionally the students of fish behavior have been divided into two camps: ethologists and behavioral ecologists. These differences probably are more a reflection of the investigators' background and training than of the methodologies or even ultimate goals of either. Ethology is somewhat a blend of ecology and psychology and traditional ethologists analyze behavior in terms of instinct and learning, breaking down complex behavior "patterns" into components, and investigating the neural and sensory basis of behavior. Many of today's ethologists are less concerned with learning and more concerned with the

development of non-learned or innate behaviors. Ethologists may also take an evolutionary perspective, examining the development of behavior between different phyletic lineages or among organisms of different complexity.

Behavioral ecologists study behavior in the context of ecology, looking at behavior in the wider context of the lifestyle or life history. They investigate metabolic aspects of behavior, optimal foraging, predator–prey relations, and schooling, migration, and spawning as related to the seasons and life history. Behavioral ecology also stresses that behaviors are adaptations, subject to natural selection and so serve to increase the fitness of individual organisms. Frequently there are practical applications in such work if the fish species is exploited. The study of behavior can allow for the prediction of where fish concentrations occur at different times of the year or even the design of fishing gear to permit greater capture efficiencies (Bardach *et al.*, 1980). Recent efforts have also been devoted to teaching hatchery-reared fishes intended for release or restocking in nature how to better survive. As 95% of hatchery fish succumb to predation or starvation within weeks of their release, higher survival rates achieved by properly conditioning and training (Brown and Laland, 2001) should be beneficial to both the fish and the fishery.

Categorization of behavior

Because behavior is controlled by the nervous as well as endocrine systems the complexity of the behavior of an organism is often thought to be related to the complexity of its nervous system. In the past, ethologists have attempted to categorize behavior into inborn or innate (instinctive) and acquired (learned) categories with the latter, more complex behaviors, associated with more complex nervous systems. Innate behaviors are inherited and instinctive. They are believed to be programmed by genes and are often highly stereotyped (similar each time in many individuals). They occur without the need for training or even prior experience. Ethologists recognize four categories of innate behaviors (Figure 13.2):

1. Kinesis: the change in the speed of random movement in response to environmental stimulus, for example when subjected to a rapid change in salinity some estuarine species may increase their rate of turning. This behavior, in theory, should result in the fish moving back into a more favorable, stable salinity regime.
2. Taxis: a directed movement toward or away from a stimulus; positive and negative taxes. Most fish exhibit a positive rheotaxis – orienting into the current flow. Examples of both positive and negative phototaxis (attraction to or repulsion from light) are common among different species. Such responses are important in maintaining orientation. Most fishes have a normal posture or primary orientation which is almost certainly innate; the longitudinal axis is usually kept horizontal and the dorsal surface uppermost. But there are notable exceptions such as the upside-down catfish, (*Synodontis nigriventris*) whose images have been found in ancient Egyptian art. As might be expected, this species derives its common name from its habit of hanging out on the underside of rocks, leaves, caves, and driftwood. Several species of gobies such as the upside-down goby (*Bryaninops nexus*)

use their fused pelvic fins as a sucker device with which they are able to cling to objects vertically or even upside-down. *Chilodus punctatus*, the headstander of tropical aquaria, adopts an oblique posture while swimming and a few species such as eel leptocephali, sand eels, and seahorses may have a normal vertical posture. Other times abnormal postures are adopted to obtain food. Some cichlids may lie on their sides on the bottom to sham death, making a meal of smaller fish that venture too near.

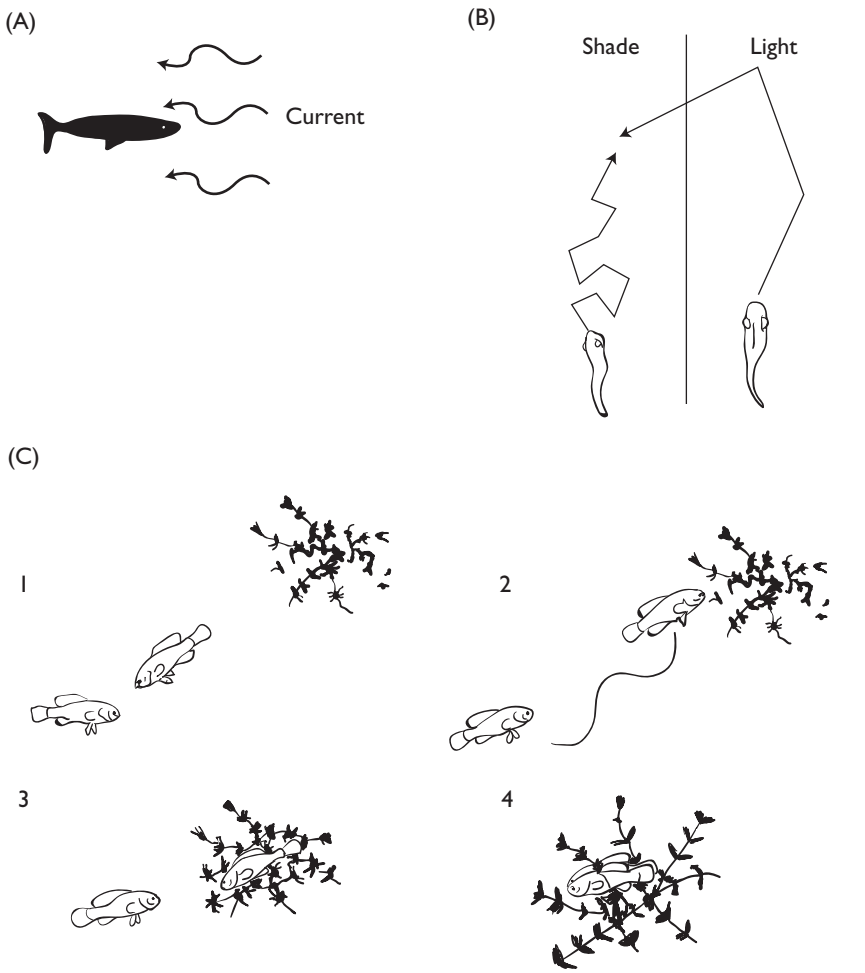

Figure 13.2 Different levels of behavioral complexity demonstrated by fishes. (A) Taxis – attraction or repulsion from stimulus, here the fish orients into the current (rheotaxis). (B) Kinesis – stimulus affects rate and frequency of turning. Fish in the shaded area (left) swim slowly and turn more frequently, hence remaining in the shade; fish in sunlight (right) swims faster and turns less often, ultimately entering the shade. (C) Fixed action patterns (FAP) illustrated by the courtship behavior of pygmy sunfishes (*Elassoma*). Each step of which is dependent on the occurrence of the prior step: 1. Male identifies a potential mate; 2. Male performs "wiggle dance" leading female toward vegetation; 3. Female approaches; and 4. Enters vegetation, where spawning occurs. Adapted from Miller (1964).

Although fish may make oblique movements upward or downward, they return to their primary horizontal orientation which is continuously monitored by the eyes and by proprioceptors in the inner ear. Superimposed on the primary orientation is a secondary orientation which determines where a fish remains within its habitat, for example whether it is near the surface, in midwater or near the bottom, pointing up-current or down-current, and so on. As explained by Fraenkel and Gunn (1961), fish move at a fixed angle to a light source, called a *light compass reaction*. A modification of the light compass reaction is the ability to move along a fixed compass bearing using the sun as a reference point (the *sun compass reaction*) and by compensating for the movement of the sun across the sky, the bearing can be maintained throughout the day. Sun compass orientation appears to take advantage of the polarization of sunlight by water, an effect that has shown to occur in clear waters as deep as 200 m meaning that this could be used by fishes anywhere in the photic zone. We do not know to what extent these forms of secondary orientation are innate or to what extent they are learned.

One of the most common responses to light, demonstrated by many species, is the dorsal light reaction, a form of primary orientation in which the fish orients with its dorsal surface toward the light which is normally coming from above. Von Holst (cited in Fraenkel and Gunn, 1961) showed that the wrasse (*Crenilabrus rostratus*) oriented at right angles to light and tilted if the light tilted (Figure 13.3). They would, however, only tilt to a limited extent. If the inner ear was removed surgically on both sides, the fish remained normal to the light and swam upside-down if the light came from below, so showing the interplay between the eye and the inner ear. Similar results may be obtained by subjecting fish to temperatures just above their cold-lethal level when they may lose their ability to maintain equilibrium before they lose the ability to swim, resulting in schools in which some individuals swim normally, some on their sides, and some upside down.

3. Reflexes: the movement of a body part in response to stimulus. Reflexes are similar to simple taxes in that they are stereotyped and innate but generally they involve only parts of the body, for example nystagmus (back-and-forth movement) of the eye to a moving light source. One of the best known reflexes in fish is the startle response or C-start, as described in Chapter 3 an extreme flexion of the body in response to a potentially harmful stimulus, usually away from the side that the stimulus is applied (p. 80). The resulting escape movement is fast and brief but sufficient to take the fish out of the attack path of a predator or away from the nematocysts of a predatory medusa. Similar movements seem to be spontaneously generated even before hatching and may assist the larvae in escaping from the chorion. C-starts are usually associated with giant Mauthner neurons or reticulospinal cells (p. 372).
4. Fixed Action Pattern (FAP): a "stereotyped and often complex series of movements, or other responses to a specific stimulus (the releaser)." Such behavior is often complex, being made up of a number of acts within a hierarchy in which each particular act depends on a preceding act and influences a succeeding act. As a case study, one of the best known examples of reproductive behavior, that of the three-spined stickleback, *Gasterosteus*

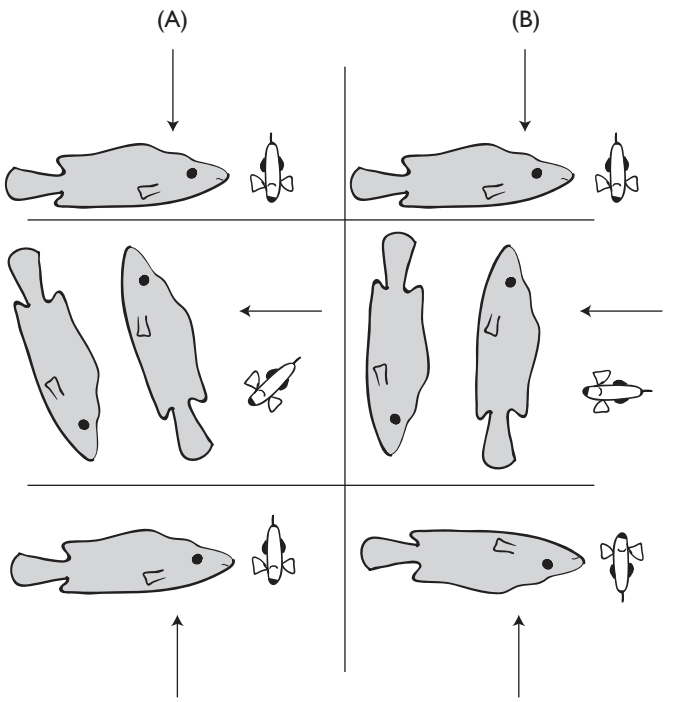

Figure 13.3 Dorsal light reaction of the wrasse (*Crenilabrus rostratus*) when the light comes from above, the side or below, as shown by the arrows. (A) Intact labyrinth; (B) labyrinths removed. Fish are shown in lateral view and from the front. Without labyrinths the orientation is entirely controlled by the dorsal light reaction. With the labyrinths intact, the fish becomes somewhat tilted when the light is from the side but does not swim upside down when illuminated from below. Redrawn from Fraenkel and Gunn (1961) after von Holst (1935).

aculeatus (Figure 13.4) so well described by Nikko Tinbergen (1951) (who might be described as the father of fish ethology and who won the Nobel Prize for Medicine with Konrad Lorenz and Karl von Frisch in 1973). At the beginning of the breeding season the males isolate themselves from the schools and select territories. The eyes develop blue color, the back becomes greenish and the belly red. The males protect their territory by a ritual form of aggressive behavior but rarely fight intruding males. The male then builds a nest of algae in a shallow pit. When this is complete the male goes through a characteristic dance if a female approaches. A receptive female turns towards the male and adopts a semi-upright posture and is then led down to the nest by the male. At the nest the male thrusts its snout into the entrance, the female penetrates the nest, her head on one side, her tail on the other. The male prods the female which begins to spawn. The female then leaves the nest; the male enters and fertilizes the eggs and subsequently chases the female away. The eggs are then protected by the male until they hatch. Each step of this type of hierarchical behaviour cannot start without the previous step occurring first.

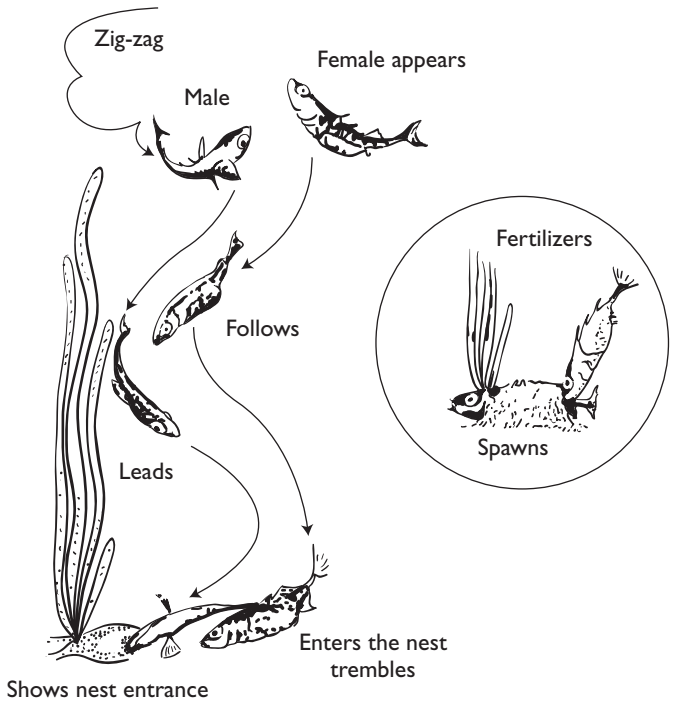

Figure 13.4 Courtship and spawning behavior of the stickleback. Redrawn from Tinbergen (1951).

Learning

Learning implies the transfer of information from other organisms such as parents, siblings, prey or predators, and the incorporation of this experience into new or modified behavior. Many early investigators felt that fish, because of their "simple" nervous systems were incapable of communicating learning to other individuals, but it is now established that fish can learn, not only from trial-and-error experiences, but by observing and then mimicking or following the lead of other fish. Learned behavior can be assigned to five categories:

1. Imprinting. Imprinting is a stimulus that must be learned, rather than being innate. The classic example is the imprinting of olfactory clues from the surrounding water upon newly hatched salmon that will ultimately allow these fish to return to the same stream in which they were spawned despite many years absence, during which they may have swum thousands of miles. Imprinting typically occurs during a critical period.
2. Habituation. Habituation is another form of learning by which organisms become adapted to their environment. In this case the organism learns to ignore a stimulus that does not reward or harm them. Male Siamese fighting fish (*Betta splendens*) will generally behave antagonistically toward other males, or toward anything brightly colored. If, however, the colored object does not reciprocate aggressively, the behavior in the initial male will be extinguished and further encounters with bright colors ignored, until an appropriately aggressive stimulus occurs. Habituation can also result in the

evolution of characteristics among organisms that derive benefit from being ignored. For example, a fish may learn to ignore organisms with a particular color pattern or outline if these generally are not predators. This certainly opens the door for predators that have evolved resemblances that mimic these harmless forms as some predators have done by mimicking cleaner species. As long as they remain much less common than the harmless organisms they will continue to be ignored by their prey species.

3. Conditioning. Conditioning involves training, usually in a laboratory setting, in order to use the behavior of an organism as a signal. There are two types of conditioning:
   A. Classical conditioning. Classical conditioning experiments have trained fish to respond in observable ways (for instance by changes in respiratory rate or electric discharge) to environmental changes that otherwise would not be quantifiable, or even noticeable.
   B. Operant conditioning. In operant or instrumental conditioning the fish are trained to perform new and more complex tasks. For example, they might be trained to choose between two sources of stimulation, movement to one resulting in a reward of food, movement to the other the absence of reward (Figure 13.5). Differences between the sources can be increased or reduced to test discriminative ability.

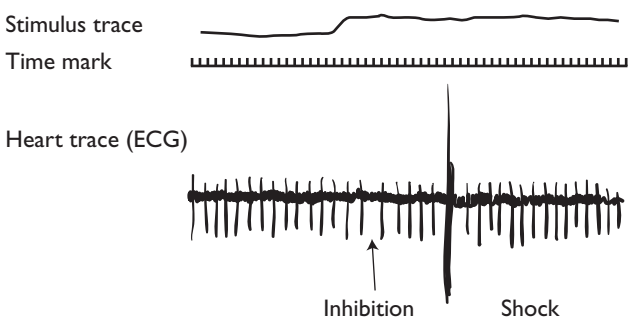

Figure 13.5 Electrocardiograph of a fish such as cod (lower line) with the heart beating about once per second. The middle line shows time marks and the upper line shows when the stimulus is applied. Note the loss of a heart beat after the stimulus but before the shock is applied (picked up on the ECG electrodes), so demonstrating that the fish has perceived the stimulus.

4. Trial and error learning. Unlike laboratory conditioning, trial and error learning is a form of conditioning that occurs in nature. At its simplest, it should be obvious that fishes which fail to find food, avoid predators, or locate suitable habitats may not survive; however, experimental results have demonstrated that fishes which survive an attack from a predator exhibit a "learning curve," becoming more adept at avoiding future attacks.
5. Insight or reasoning. Insight or reasoning occur when an animal applies past experiences to solve new problems without a period of trial and error. Although most "higher" cognitive functions have yet to be demonstrated in fishes, fish have been shown to be capable of communication of learned behaviors and of learning by observing others.

The notion that fish (and particularly goldfish) have a three-second memory seems to be well entrenched in popular literature and culture, and even appears in the introductions of many recent scientific papers on learning and behavior of fishes (Laland *et al.*, 2003). However, the origin of this statement in the scientific literature is obscure and, since it has repeatedly been refuted, it is probably best to consider it a myth and avoid it altogether in the future.

French (1942) demonstrated that memory retention in goldfish was temperature dependent. Initial learning proceeds faster at moderate or high temperatures, but even at the coldest test temperature (4°C) fish retained learned responses up to 8 weeks post-conditioning. Subsequent studies on goldfish and other species have shown that fish may retain memories for 3 or more months, but like other animals they tend to forget information that is not frequently used. Rats forget learned responses after 2–3 months, a somewhat analogous period of retention to that of many university undergrads.

Fish regularly "eavesdrop", watching others around them and apparently learning from them (Bshary *et al.*, 2002). Female guppies choose males that have been more frequently chosen by other females, male *Betta*s modulate their aggressiveness to other males based on what they have observed – when a male *Betta* is allowed to observe fights between other males, it will attack less aggressive males with greater intensity than males which have been observed to behave more aggressively. Fishes accompanied by experienced "demonstrators" are better at trawl avoidance than naive, unaccompanied fish.

Helfman and Schultz (1984) found that translocated French grunts (*Haemulon flavolineatum*) adopted twilight migratory paths used by resident fish between daytime schooling/feeding sites and nighttime resting sites. When the residents were first removed, translocated fish moved more randomly. When Warner (1988, 1989) transplanted bluehead wrass, these established "new" nesting sites even when previously used (supposedly "good" ones) were available due to removal of prior residents. Guppies learn to find hidden food from established (knowledgeable?) tank mates. Young rock bass choose the same food eaten by older fish. In absence of older fish their choices are broader; and some foods favored by the other group were completely avoided.

13.3 Schooling

It has been estimated that between 50 and 80% of all fish species school, the difference probably reflecting differences in how the term "school" is defined. The terms "school" and "shoal" are sometimes used synonymously, but it seems sensible to follow Pitcher (1993) in using "school" to describe a group of fish swimming at about the same speed in roughly parallel orientation and maintaining constant nearest-neighbor distance (NND; Figure 13.6). "Shoal" describes all social groups of fish including schools as well as aggregations of fish with random orientation and varying NND. One of the more unusual shoals is the pod or ball, where the NND is effectively zero (Figure 13.6) and one of the more deceptive parallel orientations is seen in the ranks of trout sheltering behind riffles in the bed of a stream (Figure 13.6), which is an example of a taxis with the distances between individuals probably being determined by hydrodynamic conditions and not a social group at all.

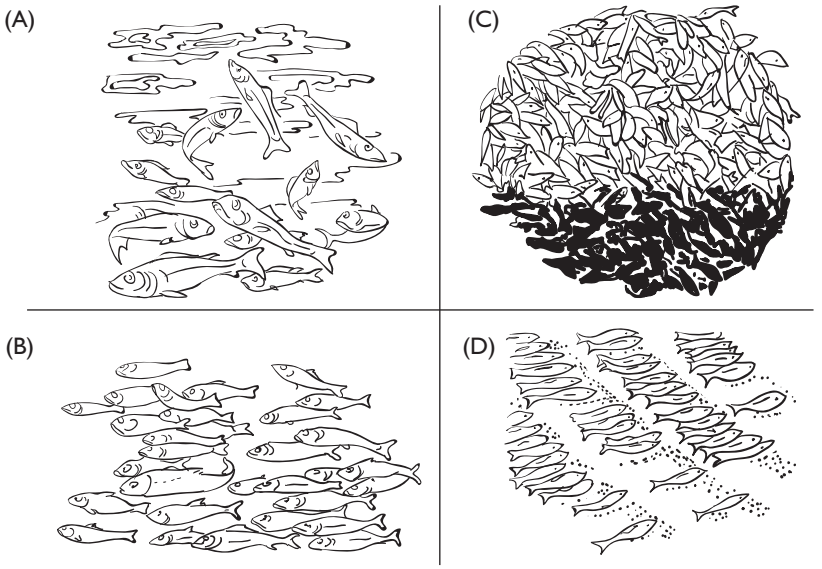

Figure 13.6 (A) School of herring breaking to feed below surface. (B) School of herring swimming in a tank. (C) "Pod" of *Sebastodes paucispinus*. (D) "Orderly files" of rainbow trout sheltering behind riffles on the bed of a stream. (A) and (B) redrawn from original underwater photographs; (C) and (D) redrawn from Breder (1959).

The NND determines the extent of packing and is usually of the order of one-half or one body length. Such schools break ranks during feeding and may then themselves be more vulnerable to predators (Figure 13.6). Shoals can comprise anything from a few individuals to many millions, possibly billions, for example in the clupeoids and *Cyclothone*. Large schools are often oblate spheroid in shape, said to minimize the detection envelope under water and so reduce predation.

Schools usually lose their integrity at night. The level of activity falls but it is doubtful whether fish ever sleep in the human sense. Some parrot fishes (scarids) of coral reefs rest immobile and secrete a protective mucous cocoon around themselves at night, and it hard to believe that they are not then asleep. Schools break up at dusk (p. 325) once the illumination has dropped below a visual threshold of 0.1 mc (0.05 uW cm^2) but they reform at dawn. Most schooling species, such as the clupeoids and scombroids, follow this pattern, although the mackerel has a very low visual threshold of 10^{-7} mc. Section of the lateral line nerve in saithe (*Pollachius virens*) causes the fish to swim closer; leading to a plausible theory that NND is determined by the interplay of attraction mediated by visual stimuli and repulsion by lateral-line stimuli. Although blind saithe were able to school in a specialized experimental situation, it is likely that both vision and the lateral line are normally used to maintain school structure and dynamics. The amazing thing about schooling herring, for example, is how quickly they move, apparently without colliding, in an impressive display of coordination. There must be a rapid and effective exchange of information to achieve this (see p. 301).

Species which school are often distinguished by "schooling marks," prominent spots typically on the shoulder (humeral region) or base of the tail, or longitudinal stripes that are easily seen and provide visual references to maintain distance and cohesiveness. Members of a school are also generally of about the same size as suggested by theories of hydrodynamic advantage (Pitcher *et al.*, 1985). Individuals that differ in size by about 30% simply do not fit in. Shoals of mixed sizes do occur, but these appear to be transient phenomena, probably to find food or protection. Sometimes this leads to opportunities for intraspecific schooling when one to many individuals will school with similar sized fishes of different species. Mixed schools of grunts (*Haemulon*), often juveniles, provide some of the best examples of intraspecific schooling.

Schooling is seen as an example of an emergent principle (Parrish *et al.*, 2002) – one which is possessed by a group, that is, the school of fish, but not by the individuals. But from an evolutionary perspective, emergent properties confer an advantage upon members of a group, because of their membership. Non-members do not receive such benefit. It has been suggested that schooling and other forms of shoaling play important roles in searching for food, predator avoidance, spawning, and energy conservation. Predatory fish may also gain an advantage through schooling. Since many prey species are themselves schooling species, the distribution of prey is quite patchy. A large number of predators can search for food over a wider area than an individual and, so long as there is a means of communication, chiefly through vision or audition, the discovery of prey by one will benefit the larger group (Figure 13.7). Shoaling may play a part in searching for food but its major role is protective. It is difficult for predators to select individual prey from a shoal (confusion effect) and predators usually feed on stragglers. Mackerel attack shoals of sprats in a haphazard way to split off individuals for subsequent consumption. Shoals of minnows re-form, or become denser, when a predator is sensed. This may be determined by a "sentry" effect in which the fish nearest the predator on the outside of the shoal first respond and information is rapidly passed across the school, almost certainly initiated by changes in locomotor behavior.

Despite their reputation as a fierce, pack-hunting predator, red-bellied piranha (*Pygocentrus natterei*) are also subject to predation from dolphins, birds, and larger fishes. Evidence has been presented that schooling is an antipredator mechanism while no evidence supports cooperative hunting. Observations of opercular movements were used as a measure of oxygen consumption and showed that fishes in groups of eight had reduced energy consumption when compared to fishes in smaller groups or to individual fish. Reduced oxygen demand may also be advantageous in hypoxic Amazonian swamps (Queiroz and Magurran, 2004).

Many species spawn en masse, and, while these aggregations may or may not fit the definition of a school, they certainly bring individuals together at the same time and place. The possibility of a hydrodynamic advantage in which schooling fish utilize the vortices created by other fish in a school as a means of increasing the thrust of the tail and reduce drag has considerable support (Liao *et al.*, 2003; Weihs, 2004). Fish swimming at the rear of a school receive hydrodynamic advantage, similar to birds flying in formation which may offset reduced oxygen availability. Studies of fishes swimming in respirometers show that members of a group exhibit a reduced metabolic rate

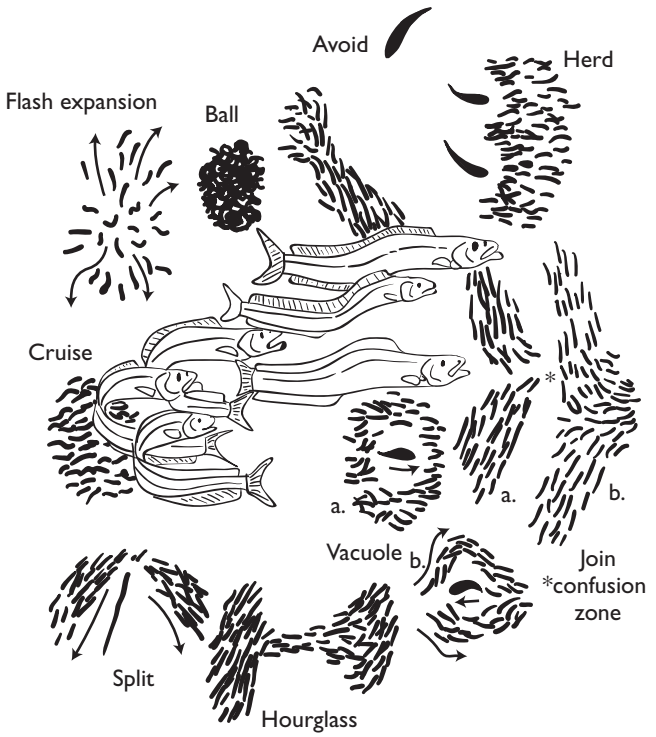

Figure 13.7 Schematic diagram of the repertoire of antipredator tactics in schools of sandeels (US, sand lance; Ammodytidae) under threat from hunting mackerel. The small school of turning sandeels is drawn in the foreground, evasion tactics are illustrated in plan view. Based on observations made in a 12 m area tank by Pitcher and Wyche (1983), figure adapted from Pitcher and Parrish (1993, p. 387).

(oxygen consumed per unit body weight) when compared to solitary fish. Other, less direct ways of estimating energy consumption, such as tail-beat frequency, also support the theory of energy conservation due to the hydrodynamics of swimming in a school (Herskin and Steffensen, 1998; Svendsen *et al.*, 2003).

The advantages of shoaling are clear, the disadvantages less so. In many cases the advantages and disadvantages maybe viewed as trade-offs. While there is safety in numbers, shoals are more conspicuous in conditions of good underwater visibility and from the air (possibly to avian predators; Connell, 2000). Limited food has to be shared. Usually shoals of clupeoids break to feed on particles when food is less dense, but schools remain intact during filter feeding when food is abundant. Particle feeding often leads to intense competition and a feeding frenzy, and the build-up of metabolites and noise may attract large predators. Oxygen may become limiting. A large stock of overwintering herring in a Norwegian fjord, estimated at 2 million tonnes, was recently reported to be living in very low oxygen levels of 1–2 ml litre^{-1}, close to their minimum tolerance level and schools of migrating mullet (*Mugil cephalus*) will exhaust the dissolved oxygen in the water they are swimming though before the entire school has passed. How the fish at the rear of the school

manage this stress is unknown, but since schooling also reduces per capita oxygen consumption, perhaps enough energy is saved to allow their survival briefly in the oxygen depleted waters.

Schooling is partly innate, partly learned. Very small fish typically do not associate with other fishes, possibly because their nervous and sensory systems are incompletely developed (Browman, 1989). As sensory systems develop, fish begin to swim in pairs and eventually larger groups in the regularly geometrical patterns characteristic of schools. How much of this is by choice (or instinct) and how much by happenstance – the fortuitous result of selecting the same water masses that are capable for transporting large numbers of the very small fish from the spawning to feeding grounds, is debatable.

13.4 Orientation, and Migration

Vertical migration

Throughout the oceans, seas, and lakes, many species of fish and invertebrates are found to make diel (with a 24-hour periodicity) vertical migrations usually toward the surface at dusk and toward the bottom at dawn. The most plausible general explanation for such a regular event is that phytoplankton is to be found in the euphotic zone, near the surface. Herbivores must visit these strata in order to feed, and since they can feed in the dark (unlike most carnivores) the best time to visit the surface layers is at night, while by day they are safer dispersed in deeper water. The carnivores follow the migrations of the herbivores, feeding on them at dusk and dawn when they are in dense concentrations and before the illumination has fallen below the carnivores' visual threshold. Thus, vertical migration is driven by the need to feed and to avoid predators. In particular, it is desirable for many larger species to avoid the surface waters by day where they are vulnerable to avian predators.

Upward vertical migration at dusk seems to be triggered by falling light intensity (Figure 13.8) and downward migration at dawn by increasing intensity. In high latitudes, in the polar summer and winter, vertical migration is less evident since there is a much reduced diel cycle of light. Vertical migration is also predictably influenced by bright moonlight (which tends to inhibit upward movement) and lunar or solar eclipses (which cause upward movements during the period of darkening; Neilsen and Perry, 1990).

The amplitude of vertical migration is limited in physoclist fish with closed swimbladders (p. 119). If a physoclist is near neutral buoyancy at a particular depth, rapid downward movements cause it to become negatively buoyant as the swimbladder volume decreases under the increased hydrostatic pressure. If the physoclist moves upward, the expanding swimbladder not only makes the fish positively buoyant but there is a danger that the swimbladder could burst if the fish moves up too far. The amplitude of movement (usually tens of meters in the cod, *Gadus morhua*, example) is determined by the rate of gas secretion for a downwardly moving fish and gas resorption for an upwardly moving fish. The "decompression schedule" for gadid fish is, coincidentally, similar to that for humans, a halving of the pressure followed by a pause. In physostomes such as herring, with swimbladders connected to the exterior, no such constraints occur and some oceanic herring may move up or down 100–200 m during the diel cycle, while a swordfish, *Xiphias gladius*, was shown

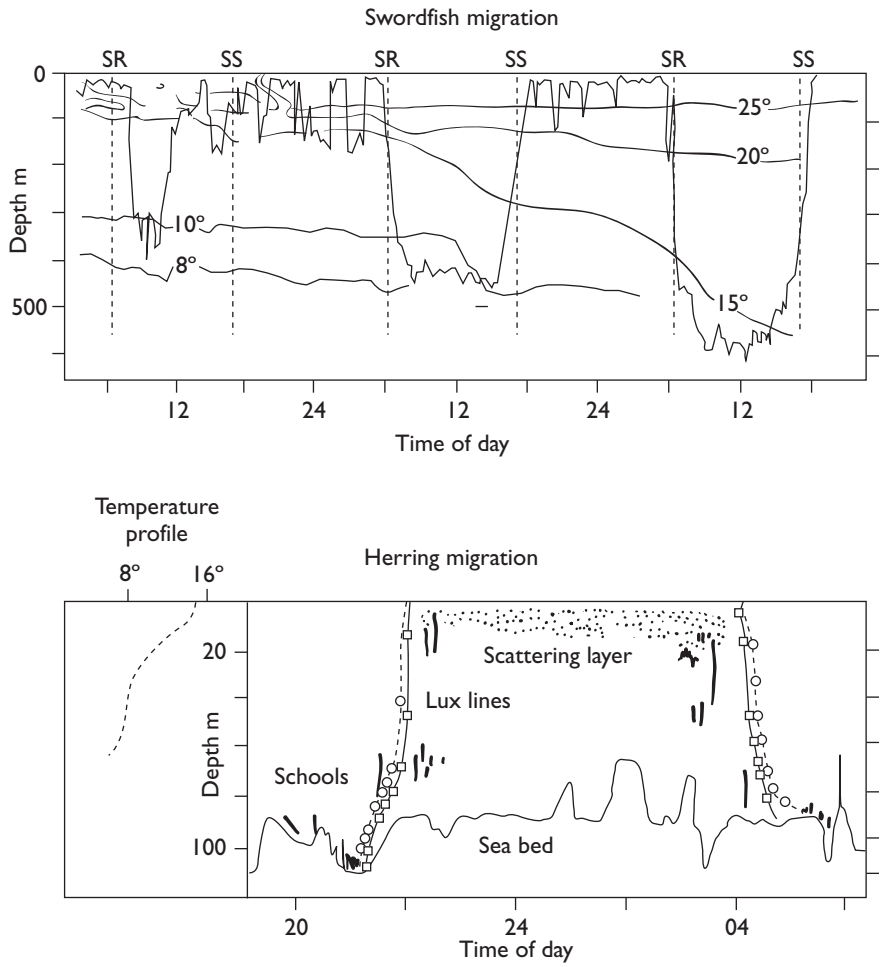

Figure 13.8 Upper figure: vertical movements of swordfish depending on the time of day. The depth is given in meters and the isotherms are shown. SR = sunrise; SS = sunset. Redrawn from Carey and Robison (1981). Lower figure: vertical movements of herring in the North Sea over the dusk, night, dawn period. Lines joining points of equal light intensity (isolux lines) are shown. Note how the schools disperse into a scattering layer during the night. Redrawn from Postuma (1957).

to migrate from the surface to 600 m between night and day (Carey and Robison, 1981; Figure 13.8). *Xiphias* is not a physostome, and unlike the other billfishes does not posses a compartmentalized swimbladder (an adaptation permitting rapid adjustments to swimbladder volume) so how rapidly it manages such depth transitions is not known at this time.

Horizontal migration

Daily vertical migrations are modulated by small-scale horizontal migrations involved in feeding and predator avoidance. Reef fish may move on off the reef with a 24-hour periodicity, feeding by day and hiding at night (some "hide" by

drifting in school-like aggregations away from the reef). Much larger seasonal horizontal migrations occur that are related to spawning and feeding. These are often depicted in the form of oscillatory triangular movements (Figure 13.9). For example, maturing Atlantic cod migrate to the Norwegian coast to spawn in the spring. After spawning they return to the offshore feeding grounds to recover. Herring in the North Sea move southward in the early summer. After spawning they tend to drift eastward, overwintering in the eastern North Sea. In the spring they migrate offshore to the west and north and start to feed and mature for a repeat of the spawning cycle (Figure 13.10). Plaice in the southern North Sea have distinct spawning grounds but wider areas in which they feed and recover after spawning (Figure 13.10; McCleave *et al.*, 1984).

Some oceanic species of tuna make huge regular trans-ocean migrations, as we know from recaptures of tagged fish. For example, albacore tuna (*Thunnus alalunga*) move from the mid-Pacific to the west coast of the USA or Japan. Bluefin tuna (*T. thynnus*) may migrate between Florida and Norway (Figure 10.19, p. 310). Blue sharks (*Prionace glauca*) also migrate across the Atlantic between the eastern USA and the South American and African coasts (Hasler, 1971).

Equally impressive and precise migrations are those of the Atlantic salmon (*Salmo salar*), and eel (*Anguilla*). The anadromous Atlantic salmon migrates from the sea into the upper reaches of rivers to spawn in the autumn and winter. The eggs are laid in gravel beds or redds, and hatch after about 2 months. The young stages take 2 to 3 years to reach the smolt stage which returns to the sea. There the smolts grow rapidly on the abundant food, making long migrations as far as the southwest coast of Greenland. After 1 year, the most precocious fish migrate as grilse back to the home rivers in which they were spawned. More often they remain at sea for 2 or more years before spawning. The spawners that survive, known as kelts, return to the sea and may spawn

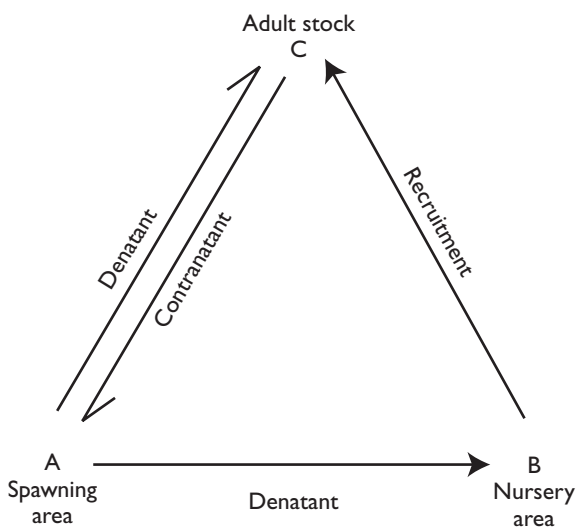

Figure 13.9 A simple triangular pattern of fish migration. From Harden Jones (1968).

again. The orienting mechanisms have been extensively studied but are still not fully known. The smolts are certainly imprinted with the odor of the home stream before they start their seaward migration. Their migrations in the ocean may involve residual current systems with probably some degree of crude orientation using the sun as a reference point (see below). This may

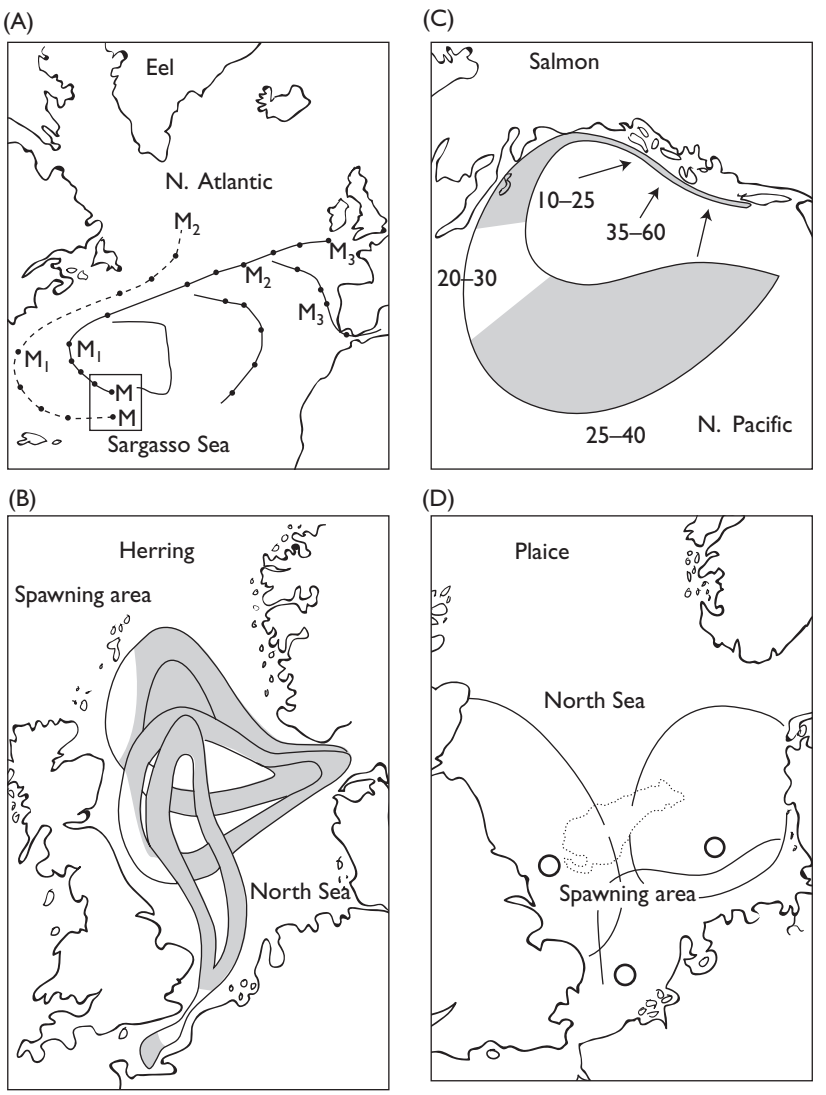

Figure 13.10 Migration paths of various species. (A) The drift of the leptocephalus stages of the eel across the North Atlantic. (B) The movements of herring of three spawning races around the North Sea. (C) Distribution and movement (arrowed) of Pacific pink salmon (*Oncorhynchus gorbuscha*) in the North Pacific. Lengths in centimeters are given against various shaded areas. (D) Three spawning groups of North Sea plaice with spawning area given (circle) and subsequent area of distribution of the adults. (A) and (D) redrawn from Harden Jones (1968); (B) and (C) redrawn from Tesch (1975).

bring the salmon back to areas where they can identify topographical, auditory or chemical landmarks. These fine-tune the migration to the point where the salmon recognizes the unique characteristics of its home stream, which it left as a smolt several years before.

In the Pacific there are several species of salmon (*Oncorhynchus*) each with rather different migration characteristics. Some stocks of coho or silver salmon (*O. kisutch*) are land locked. Chum salmon (*O. keta*) and pink or humpback salmon (*O. gorburischa*) spawn near the mouths of rivers and the young stages soon drift to the sea (Figure 13.10), and sockeye salmon (*O. nerka*) make long migrations into the far Pacific Ocean. The chinook or king salmon (*O. tshawytscha*) used to make extensive migrations of many hundreds of miles up the Columbia River before its spawning grounds were isolated by dams.

The catadromous European eel (*Anguilla anguilla*), and American eel (*A. rostrata*) migrate upstream as elvers a few centimeters long. They spend some years growing in freshwater habitats, becoming yellow eels, which turn silvery *before* they migrate to the sea. The French physiologist, Fontaine, felicitously called this pre-adaptation "anticipatory." Although it is not possible to trace their subsequent migrations, there is good circumstantial evidence that they spawn in the Sargasso Sea (Figure 13.10) in deep water. Pacific eels (*A. japonicus*) have similiarly been shown to migrate to deep-water spawning sites around sea mounts. The leaf-like leptocephalus larvae can be traced in plankton catches as they drift with the residual currents eastward and westward to the American and European coasts over a period of 1 to 3 years. At the coast they metamorphose to the elver stage, and it is likely that they make their way up the estuaries by riding the tides. Observations of elver activity suggest that they stay near the floor of the estuary on ebb tides but move into midwater on the flood tide, so making net movements upriver.

Riding the tides was fairly recently discovered by acoustic telemetry, and also occurs in fish such as plaice, cod, and dogfish in the open sea. In the southern North Sea, plaice migrating for spawning ride the southwardly flowing flood tide and rest on the ebb; after spawning they return northward by riding the ebb tide and resting on the flood. Other mechanisms may exist to allow fish to orientate their migrations. The active mechanism involved in riding the tide may be superimposed on a passive drift associated with residual current systems, the so-called *denatant* part of a migration cycle. *Contranatant* movement is best seen in salmonids swimming upstream to spawn in the upper (non-tidal) reaches of rivers (Gibson, 1965, 1992).

Of the visual mechanisms that may be involved, the most studied is the sun compass reaction. Fish such as coho salmon, centrarchid sunfish (*Lepomis gibbosus*), and the cichlid *Aequidens portalegrensis*, can be trained in circular tanks to escape on a particular compass bearing regardless of the time of day. This implies that they can not only move at an angle to the sun, but allow for the movement of the sun across the sky (Figure 13.11). More simply, changes in activity during different times of day coupled with swimming toward the sun could also lead to orientation in different directions (Figure 13.12), but there is no evidence that this mechanism is actually used by any fish. These mechanisms are not equivalent to coordinated navigation in which a fish might be able to monitor latitude and longitude by the altitude of the sun at

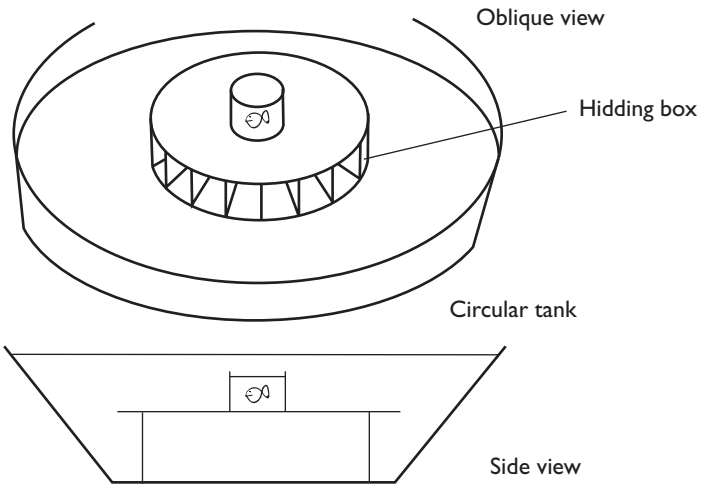

Figure 13.11 Oblique view (above) and side view (below) of an arena tank with a central circle of refuge boxes to observe the ability of bluegill sunfish (*Lepomis macrochirus*) to hide in a particular box using the position of the sun as a reference point. The fish was released from a central container at the beginning of each trial. Redrawn from Hasler (1971).

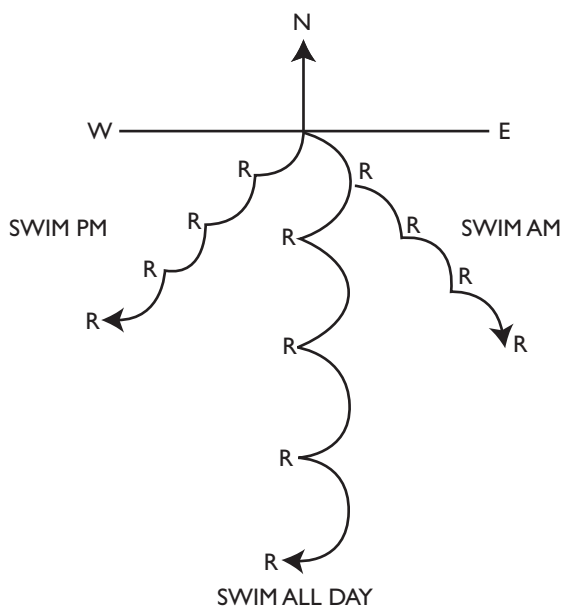

Figure 13.12 Diagram showing how a fish swimming towards the sun in the morning (AM) during the daylight hours (All Day) and in the afternoon (PM) and resting (R) at other times would make a net movement in a southeasterly, southerly, and southwesterly direction (in the northern hemisphere).

midday and the time of sunrise related to some fixed meridian. Neither coordinate, nor inertial navigation (in which an organism could determine its position by remembering changes of course and distances moved) have yet been demonstrated in fish.

Other visual mechanisms that have been postulated include the perception of the direction of passing waves which would give the direction of the wind, and the perception of the plane of the polarized light which is characteristic of the altitude and azimuth of the sun even if obscured by clouds. Olfactory mechanisms operate at relatively close range to a source of stimulation before the chemical stimulus becomes too dilute or chemical gradients are disturbed (Kruzhalov, 1990). The imprinting of salmonids is the best established olfactory mechanism and has been supported by neurophysiological evidence. In coho and chinook salmon, infusion of home-stream water into the nasal cavity causes a marked increase in the activity of the brain recorded by electroencephalograph (p. 337). There is evidence accumulating that skin mucus is at least one of the determinants of home-stream odor (Esteve, 2005). Obviously this mechanism would only operate if non-migrants were present upstream to scent the water.

Whether long-distance migrants, such as eels, could orientate using the Earth's magnetic field remains unclear, although it seems probable. Magnetite has been found in migratory species such as the eel, salmon, mackerel, yellowfin tuna, and herring, but also in the non-migratory carp and perch. The rainbow trout (*Oncorhynchus mykiss*) has the mineral magnetite linked by microtubule-like strands to a few mechanically gated ion channels in the membrane of receptor cells that communicate, via the trigeminal nerve, with the brain. This species, European and American eels, several other salmonid species, several sharks, and the stingray (*Urolophus halleri*) have been shown in experiments to detect weak magnetic fields and to alter their orientation in response to manipulation of the Earth's magnetic field (Meyer *et al.*, 2005). Although the discovery of cells sensitive to geomagnetic fields has been likened to finding a "magnetic needle in a haystack" (Walker *et al.*, 2002), recent technological advances in tracking have permitted the testing of models of navigation and behavior based on the detection of geomagnetic variations and various species of fishes as well as sea turtles, whales, and birds have been shown to behave in a manner consistent with the use of such a sense. The "needle in the haystack" was finally conclusively found in 1997 (see p. 309).

13.5 Symbiosis

Symbiosis comes from the Greek word that translates as "living together," although applicable to intraspecific aggregations, is more generally applied to associations between individuals of different species. It is possible to categorize these associations as part of a continuum ranging from free-living organisms that depend on others for food, to two organisms that cannot survive unless they are always together such as the alga and fungus that combine to form a "species" of lichen. If both (or all) individuals benefit from the association, it is termed "mutualism." If only one benefits, but the others are not harmed, then it is termed "commensalism." In the most extreme case, parasitism, one benefits, while the host species is harmed (Losey, 1978).

Many examples of commensalisms involve an exchange of food for protection. Some marine gobies, such as *Psilogobius mainlandi*, live in the burrows of shrimps (Figure 13.13). The shrimp, which often is sightless, maintains the burrow while the goby gives warning of danger and may provide particles of food for its symbiont. In a turn-about, the blind goby *Typhlogobius californiensis* lives in the burrows of ghost shrimp (*Callianassa* spp.). This exchange of food for protection also applies in the associations between fishes and coelenterates. The best-known associations are between pomacentrids, such as *Amphiprion*, and corals or anemones in which the coelenterates obtain nitrogen from the excretory products of the fish (Porat and Chadwick-Furman, 2005), but young whiting (*Merlangius merlangius*), carangids, and stromateids are frequently found in association with medusae such as *Cyanea* in the pelagic zones of the sea. Such associations have even been shown to occur between deep-water abysso-pelagic species as well (Drazen and Robison, 2004; Figure 13.14). The stromateid *Nomeus gronovii* seems to have a rather equivocal relationship with the Portuguese man-of-war (*Physalia*),

Figure 13.13 Several species of commensal gobies (e.g. *Amblyeleotris* spp.) inhabit the burrows of the blind or nearly blind snapping shrimp (*Alpheus* spp.). The shrimp dig and maintain the burrow while the goby acts like a watchman alerting the shrimp with a flick of its tail if any danger approaches.

sometimes living apparently unscathed among the stinging tentacles but at other times being observed as a partly digested corpse.

One of the best known, but least understood, examples of an association between different species is the association of remoras (family Echeneidae) with larger organisms including fishes, whales, sea turtles, and sirenians (Figure 13.15). Remoras are equipped with a sucker formed by a highly modified spinous dorsal fin by which they attach themselves to their hosts. There are several species in the remora family and it would seem that together they represent examples of the different stages of the symbiosis continuum (O'Toole, 2002). Among two closely allied families, the dolphins (Coryphaenidae) associate with drifting objects, and the cobia (Rachycentridae) with schools of sharks. Some species of echeneids are generalists, attaching themselves to just about any moving object including the occasional ship or other inanimate target, but others are found with only one or a relatively few types of hosts. While there is evidence that parasitic copepods are important in the diets of remoras with restricted hosts, producing somewhat of a benefit to the host as well as the remora, the more generalized species have much more varied diets, including eating the feces of the species which they follow and so would appear to provide little benefit to the host organism.

The best studied symbiotic behavior is cleaning symbiosis in which cleaner species, such as shrimps or other fish, remove ectoparasites, diseased or necrotic tissue from the host fish which often are predatory species such as grouper, sharks or rays (Figure 13.15). It is especially important in reef fishes where at least 50 species have been shown to make their living in this way. Curiously, this behavior is rare in freshwater except for a few cichlids and centrarchids. Such behaviors also have important practical applications. The introduction of cleaner species into culture systems has recently been utilized as a form of biological disease control in the salmon farming industry. Labriids such as the goldsinny wrasse (*Ctenolabrus rupestris*) are placed in salmon cages in seawater to remove sea lice from the skin of the salmon.

Cleaners establish highly specific territories, usually referred to as cleaning stations, to which as many as 100 potential hosts may be attracted each day (the nomenclature of cleaning symbiosis is a bit confusing, one might expect the cleaner to visit the host, but in fact it is the other way around). The number of individuals seeking cleaning may be so great as to require them to queue up, awaiting their turn. Some feel that *site-tenacity* on the part of both cleaners and host species alone can be used to explain the success of cleaning symbiosis and that the ability to recognize and respond differently to different individuals is not either unnecessary or unproven.

Although examples of cleaning symbiosis have been well documented for some time, the evolutionary benefits have only recently received much attention. At first glance, the observer would be tempted to say that the benefits were obvious. The host gets cleaned and the cleaner gets fed. But, not surprisingly, the actual situation is far less simple. For one, the cleaners regularly "cheat" – ingesting scales, mucus, and even taking bites of healthy tissue from their hosts. Host individuals may reciprocate by abandoning one cleaning station for another, but only if there are multiple stations within the host's own territorial range, so fishes with relatively small territories may be at a disadvantage here, being confined to just one cleaning station. Host individuals have

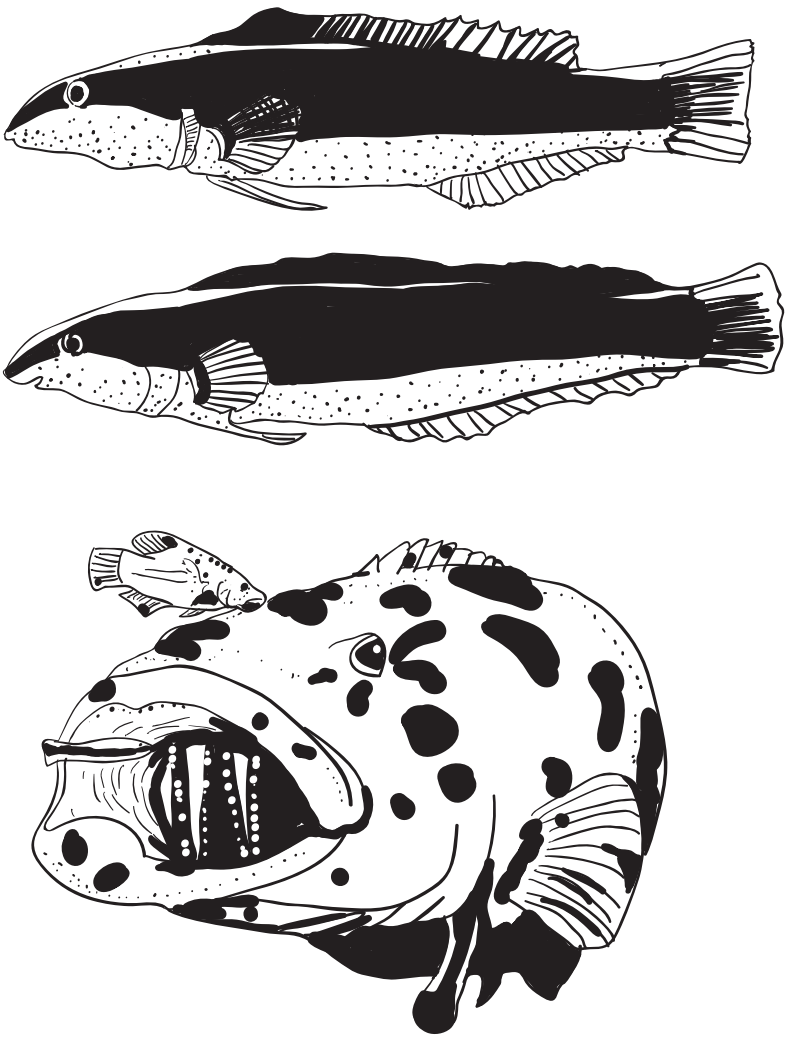

Figure 13.14 Cleaner wrasse *Labroides* and its aggressive mimic, *Aspidenotus*. Note the difference in the jaws between the two species. Lower figure shows a client species, the potato cod, *Epinephelus tukula*, being cleaned by two cleaner fish *Labroides dimidiatus* and an adult facultative cleaner fish, *Bodianus axillaris*. Aggressive mimics such as *Aspidenotus* would take advantage of the grouper's acceptance of fish resembling the cleaners.

also been observed taking agonistic action against overly aggressive cleaners, snapping at them or chasing them. The cleaner may then take refuge by associating itself with a larger host, one that itself represents a danger to the insulted host. Thereafter the cleaner may be more solicitous toward this individual, providing a more thorough cleaning or greater tactile stimulation, in order to regain the individual's confidence. Without intending to anthropomorphize, the responses of both cleaners and hosts resemble those of humans in situations where trust needs to be practiced.

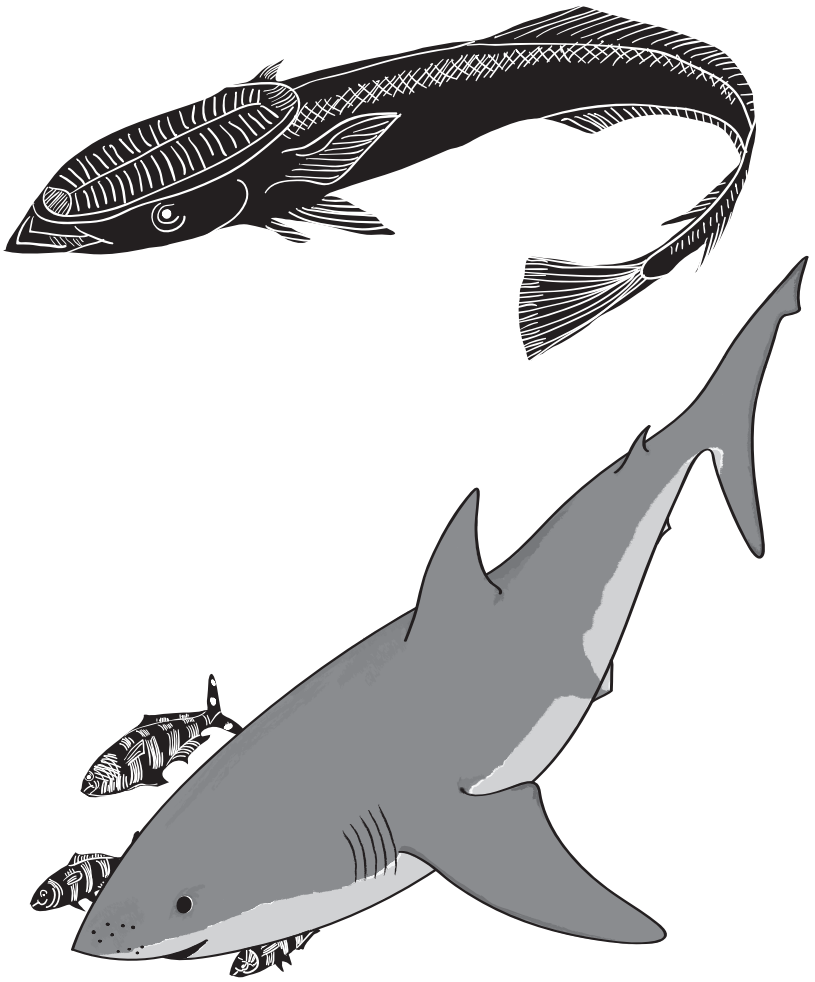

Figure 13.15 Members of the family Echeneidae (remoras, upper figure) are distinguished by the dorsal fin being modified into a laminated sucking disc by which they attach themselves to large fish or other objects. Lower figure shows a shark accompanied by attached remoras and leading pilot fish, another commensal.

A noted earlier in the chapter, the existence of cleaner species has also created an opportunity for specialized predators that closely resemble the cleaner species but which feed on skin, scales, mucus, or even small bites taken from unsuspicious hosts (Moland and Jones, 2004). So long as the aggressive mimic is relatively rare (like the instances of cleaners cheating on their hosts) hosts will continue to tolerate their presence. Such aggressive mimics are found virtually wherever cleaner species occur, taking on the appearance of even local variants of the cleaners, but assuming other patterns in the absence of cleaners. The reader is referred to the recent review of mimicry in marine fishes by J. E. Randall (2005) which includes excellent color photographs of numerous pairs of inoffensive fishes (not all of which are cleaner species) and their aggressive mimics.

The addition or removal of cleaner fish has also been shown to affect overall fish diversity in reef environments, not only among species that rely on cleaners, but rather unexpectedly also among species that do not regularly associate with cleaners (Bshary, 2003).

A final example of symbiotic behavior among fishes is the pattern of cooperative hunting as occurs between morays, or other eels, and groupers or octopuses. Small groupers "shadow" foraging eels or octopuses. As the eels search for their prey inside of coral heads small fishes or crustaceans seek to escape via alternate exits only to be grabbed by the grouper (Diamant and Shpigel, 1985). Jacks and wrasses similarly accompany shoals of feeding goatfish, although here the goatfish disturb buried prey from open sand flats, leaving them susceptible to attack by the predatory fishes.

Envoi

In one view, behavior represents the culmination of all the anatomical adaptations and physiological processes that occur within an organism. Recent attention has shifted from observation and quantification of behavioral responses to trying to understand the internal control mechanisms as well as external interactions with other individuals and species that share the same environment. As in other aspects of their biology, the study of cognition and learning in fishes presents opportunities to investigate commonalities in vertebrate behavioral systems in a phyletically "primitive" form but also demonstrate species-specific specializations that illustrate the vast anatomical, physiological and ecological diversity of fishes.

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14 Fisheries and Aquaculture

14.1 Introduction

This is a somewhat Cassandra-like chapter carrying dire but not always clear predictions, and it is important for all of us, not just for fish biologists and fisheries students. The references and further reading listed at the end of this chapter include a sampling of current opinion (which is not universally accepted) as well as a variety of more "classical" works. We saw on p. 16 that fish consist mainly of valuable protein, and present best estimates are that fish protein makes up around 15–16% of total world animal protein supplies, providing more than 2.6 billion people with 20% or more of their average daily protein intake. These figures are from the biennial review of world fisheries, published by the Food and Agriculture Organization (FAO) in its report on the *State of World Fisheries and Aquaculture* (SOFIA; FAO, 2007). There are naturally difficulties in collecting the statistics on which the report is based (one being that Chinese reported statistics may be biased high, and are thus considered separately) and as we should expect, there is a delay in the appearance of the report, that for 2006 being the most recent only contains data through 2004. Nevertheless, it is a very important and interesting document that the reader should be aware of and peruse. Table 14.1 shows the broad divisions of how fishes are taken and utilized, again from the SOFIA report. Most are taken from marine capture fisheries, a poor second from inland aquaculture, followed by marine aquaculture and lastly, inland capture.

The majority of these fishes are adult teleosts, although there are (somewhat controversially) elasmobranch fisheries, and a few small specialized fisheries for amphioxus, teleost larvae and sub-adults (for example eel elvers), and for lampreys. The world catch of fin fish, mainly teleosts, from all sources has steadily risen over the last two decades (Figure 14.1) to some 95.0 million tonnes per annum in 2004. This harvest is worth perhaps US$75 billion (about £40 billion) per annum at the fish market and many times that amount by the time it reaches the consumer. Most of the catch is taken by the high-seas fisheries from wild stocks that man has tried to manage, with singular lack of success. Almost all of the historically great stocks, such as the cod of the Grand Banks, are being, or have been, overfished.

Table 14.1 World fisheries production and utilization

	1998	1999	2000	2001	2002	2003[1]
(million tonnes)						
Production						
Inland						
Capture	8.1	8.5	8.7	8.7	8.7	9.0
Aquaculture	18.5	20.2	21.3	22.5	23.9	25.2
Total inland	26.6	28.7	30.0	31.2	32.6	34.2
Marine						
Capture	79.6	85.2	86.8	84.2	84.5	81.3
Aquaculture	12.0	13.3	14.2	15.2	15.9	16.7
Total marine	91.6	98.5	101.0	99.4	100.4	98.0
Total capture	87.7	93.8	95.5	92.9	93.2	90.3
Total aquaculture	30.6	33.4	35.5	37.8	39.8	41.9
Total world fisheries	118.2	127.2	131.0	130.7	133.0	132.2
Utilization						
Human consumption	93.6	95.4	96.8	99.5	100.7	103.0
Non-food uses	24.6	31.8	34.2	31.1	32.2	29.2
Population (billions)	5.9	6.0	6.1	6.1	6.2	6.3
Per capita food fish supply (kg)	15.8	15.9	15.9	16.2	16.2	16.3

Notes: Excluding aquatic plants.

[1] Preliminary estimate.

As well as the specific articles mentioned below, and other recent FAO SOFIA reports, such journals as the *ICES Journal of Marine Science* and *Fisheries Research* repay browsing.

14.2 Fish and People

Miller and Johnson (1989) define a fishery as a "union of aquatic organisms and humans" which they divide into three components: the resource, the environment that supports it, and the people who harvest the resource and interact with the environment. There is a long tradition of human interaction with fishes going back to prehistoric times as humans have utilized fish for food, for trade goods such as oils, skins and ornamentation, and for religious purposes.

Humankind has been fishing and eating fish, including shellfish, for about 90 000 years, as attested by the Paleolithic rock carvings of fish and

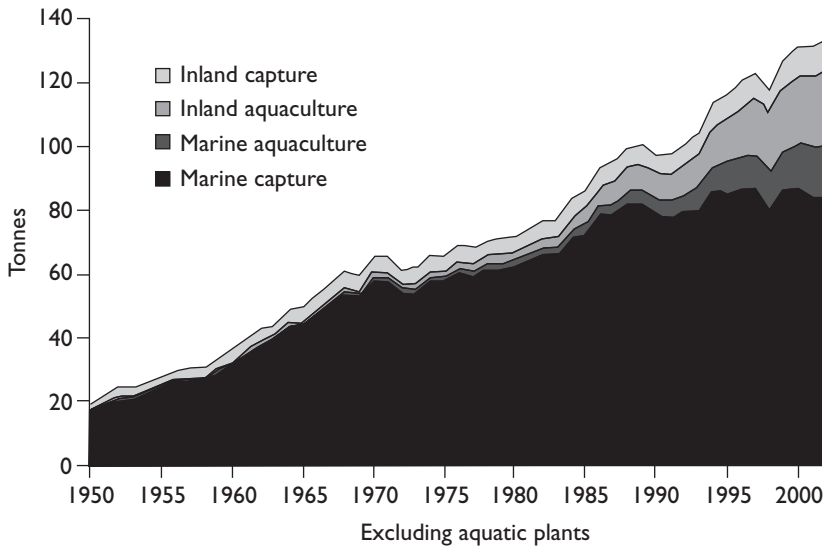

Figure 14.1 World capture and aquaculture production. FAO statistics for 2004 – total capture 95 mmt (marine 86): 15 mmt (17%) herrings and anchovies, 5.1 mmt (6%) cods, 4 mmt (4%) tunas and billfishes, 1.0 mmt flatfishes (1%), 0.8 mmt salmon (0.8%). Aquaculture 45 mmt (Fw 27, M 18), 18 mmt (67%) carps, 1.5 mmt (5.5%) salmon, 1.8 mmt (6.7%) tilapias. Based on data from FAO (2007).

the elaborate bone fishing instruments (in addition to fish bones and scales) found in many ancient archeological sites, as well as being documented in Egyptian paintings.

For nearly as long as 90 000 years, we have been human (many authorities date the appearance or "modern" *Homo sapiens* at about 100–125 000 years BP). In fact, some authorities regard the necessity for two fatty acids most commonly found in fish that are essential for brain development and function, EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid; Figure 14.2), as evidence that our earliest ancestors must have relied on a diet rich in fish, and that this diet may have been partly responsible for the rapid evolution in brain size and cognitive ability that literally made us what we are today – humans.

14.3 Fish as a Source of Food

Fish are an excellent source of food for people. Fish now account for 30% human protein supply in Asia, 20% in Africa, and 15% in Latin America and the Caribbean. Because they are neutrally buoyant, most fish have less need for a supporting skeleton, and consequently fishes have a higher ratio of muscle to bone than land animals. This makes them a very valuable source of protein. Because fish are low in fat they are considered a healthy alternative to other meats. They also contain minerals, vitamins, and essential fatty acids. Although there are exceptions, most fish pathogens are not usually hazardous to humans. A few species also naturally contain toxins that make them poisonous to

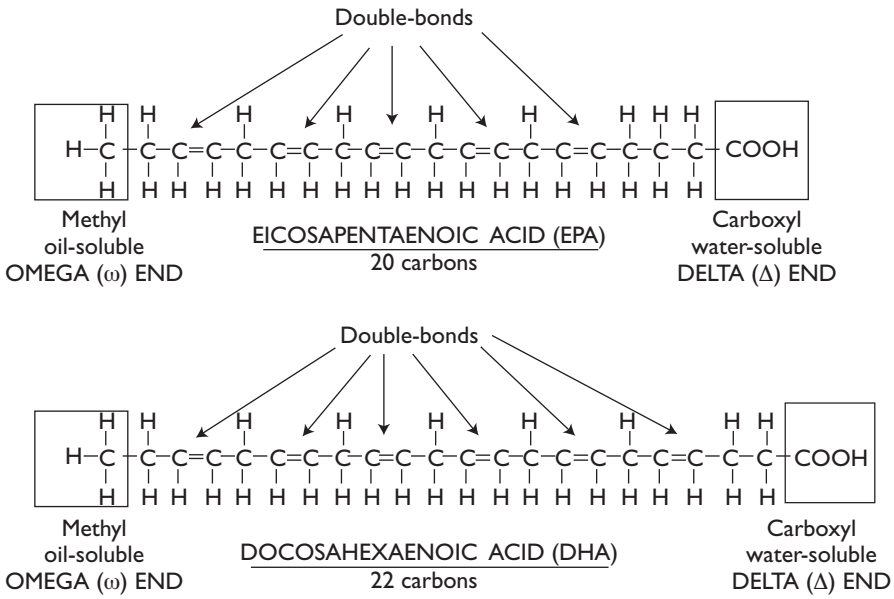

Figure 14.2 Essential fatty acids, most commonly found in marine fishes.

humans and the use (or overuse) of hormones, antibiotics, and genetically engineered feeds in aquaculture may also represent risks to human health.

14.4 World Harvests

The world catch of marine fin fish, mainly teleosts, had risen steadily since the 1950s before leveling off in the 1990s (Figure 14.1) at about 80–85 million tonnes per annum, a figure close to the best estimate of the maximum annual sustainable yield of the world's ocean of about 100 million tones first suggested by Gullard in 1971, but one that assumes the discovery and utilization of new, hitherto unexploited, stocks including many invertebrates. Freshwater yields and aquaculture, especially from Asia, continued to increase, allowing for continued growth in total fish production. Recently, however, it has been suggested that data from China, which accounts for about 18% of the world's capture fishery landings, have been routinely exaggerated (Watson and Pauly, 2001). Fishery landings are used as estimates of stock or population size and inaccurate reports of landings will result in imprecise stock assessments and management quotas that, in turn, can lead to overfishing.

Freshwater fisheries are also probably fully exploited at about 9 million tonnes per annum and, while aquaculture at about perhaps 40 million tonnes per annum has assumed much greater importance in the last three decades of the twentieth century, it is unlikely to compete with hunting techniques on wild stocks in terms of weight. In many countries, however, the value of aquaculture production may exceed that of the fisheries on wild stocks and in 2000 Chinese aquaculture biomass production exceeded fisheries' landings by 3:2. Not all of the world's fish production is for human

consumption, some 32 million metric tonnes are used annually for animal feed, 15 million tonnes of this for fish feed in aquaculture.

Productive areas and species

Continental shelves and upwelling areas

The largest fisheries occur over the continental shelf (less than 200-m depth) where nutrient turnover occurs in winter, or in areas of upwelling of deep water as occur on the coasts of Peru, Chile, and West Africa (Figure 14.3). This nutrient-rich water from the deeper layers replenishes the nutrients used in the primary production of the phytoplankton at the base of the food chains, ultimately supporting the reproduction and growth of fish stocks. The yield from the open ocean is low, except for migratory species such as tuna, and the biomass in deep oceanic water is very low except close to the ocean bed.

But these areas of high production are not immune from the dangers of overfishing and environmental changes. One of the most historically important shelf fisheries in the North Atlantic has been the Grand Banks off Newfoundland. In 1990 finfish stocks on the Grand Banks were at levels only 1% of those seen in 1950. Similar upwelling areas located off West Africa are still considered one of the world's richest fishing grounds, although stocks there are now down 80% compared to pre-exploitation levels, and are regarded as depleted as those of the North Atlantic.

The world's largest fishery is located in upwelling areas off the coasts of Peru and Chile, where cold, nutrient rich waters from the Humboldt Current rise to mix with warm equatorial waters. Such high levels of productivity are not

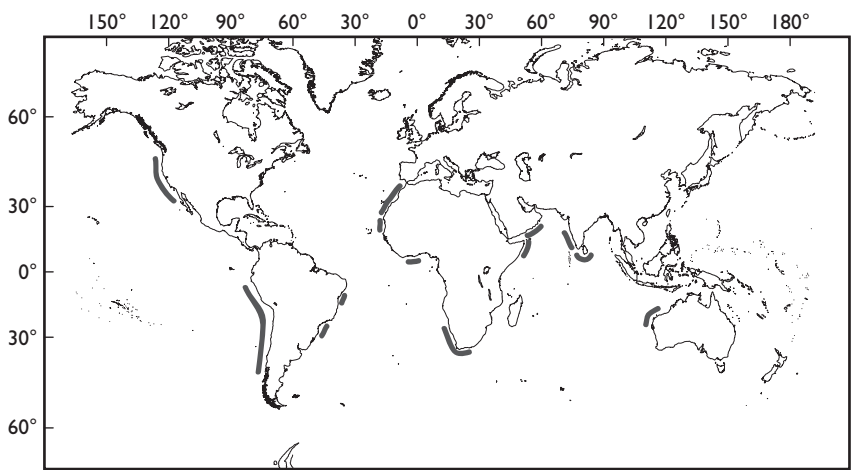

Figure 14.3 Principal upwelling zones of the world's oceans as indicated by phosphatic deposits. Major regions shown are the California Current and Humbolt Currents off California and South America respectively, the Canary Current off West Africa, and the Benguela Current off South Africa. Minor upwelling regions in the southwest Atlantic, Gulf of Guinea, and Indian Ocean are also indicated. After Cushing (1969).

constant; the ENSO (El Niño Southern Oscillation) characterized by changing environmental conditions affects the abundance and species composition of fisheries most dramatically in the southern east Pacific resulting in "sporadic fisheries" characterized by drastically fluctuating biomass levels in which periods of great abundance alternate with periods of collapse or depletion of the resource.

Slope areas and sea mounts

With the decline or disappearance of shallow-water fisheries, fishing fleets must range further offshore into deeper waters, but this engenders new problems. Deep-sea (continental slope) species are long-lived, some exceeding 200 years in age, and are slow growing, meaning they do not reproduce until late in their lives. It is estimated that orange roughy can live for 150+ years and do not mature until their mid-20s or 30s (Figure 14.4).

Deep-water fisheries have a very low long-term sustainable basis. That for orange roughy has been estimated to be in the order of 1.5 to 2% of the pre-exploitation (virgin) biomass, yet initial exploitation of these stocks has been as high as 85%. Because of these features, Merrett and Haedrich (1997), and many other fishery biologists, regard deep-sea stocks as essentially nonrenewable resources.

14.5 Species

Fisheries based on clupeoids, herrings, and their allies: anchovies, menhadens, sardines, and sprats which feed mainly near the base of the food chain on zooplankton or phytoplankton, often by filter-feeding (p. 211), have been among the most important and productive (in terms of weight, if not of value) throughout human history. The North Atlantic and North Sea herring (*Clupea*

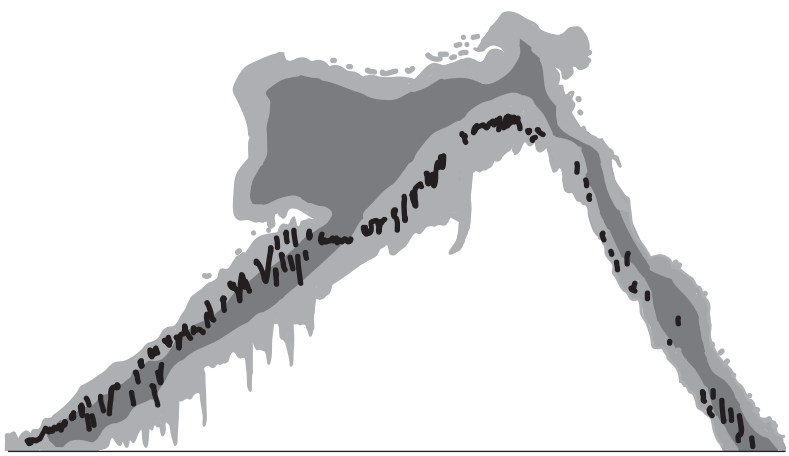

Figure 14.4 "Cloud" of orange roughy surrounds the top of sea mount off New Zealand. Actual "fish finder" sonar image is colored to indicate concentration of biomass. Image courtesy of S. McClatchie and G. Macaulay, NIWA, http://www.niwa.co.nz.

harengus L.) fisheries have provided northern European peoples with food for centuries, while in the Mediterranean, the southeast Atlantic, and Pacific, anchovies and sardines have represented some of the most important fisheries. In recent years, the total world clupeoid catch has been near 25 million tonnes, about one-quarter of the world catch, the yield being maintained by the wealth of species and stocks with high biomass.

Northeastern Atlantic herring are divided into 13 stocks (with yet more occurring in the northwestern Atlantic and Pacific Oceans), each with its own migratory and reproductive characteristics (for instance some spawn more than once during the year), so any discussion of this fishery must take this differentiation into account. The Atlanto-Scandian herring fishery is at present the largest one in the world. Management of fisheries based on this stock has been especially difficult since it migrates between the 200-mile zones of a number of countries, and is also accessible on the high seas outside 200 miles. Other stocks are found in waters surrounding the British Isles and into the Baltic where declines in herring were noted as early as 1400 AD – probably due to climate change rather than overfishing.

The North Sea herring is a stock notorious for its variability. The herring North Sea catch reached a peak in 1913 at about 600 000 tonnes (Figure 14.5). The reduced amount of fishing in the North Sea during the First World War allowed the fish stocks to replenish themselves, but the introduction of refrigeration and trawling, as opposed to drift netting, in the decades following the war resulted in a rapid decline in the population. Again, hostilities between the nations of Europe intervened and the virtual cessation of fishing in the North Sea during the Second World War allowed the herring stocks to recover. Many fishing trawlers had also been fitted with sonar as they were used to detect mines and so once the war was over they could use this sonar to track shoals of fish and the introduction of the purse seine allowed these to be surrounded and netted. The replenished stock and the use of sonar led to a fishing boom during the 1950s. However, this boom led to the overfishing of many species including herring. The catch fell abruptly in the late 1960s and did not recover on a significant scale until very recently (after 1990). The abrupt fall in the catch was associated with a steep decline in the stock, which is generally believed to have been caused by overexploitation. Figure 14.5 shows a very steep rise in the catches in the 1960s. This rise was caused by a leap in fishing technology. A device called the "power block" made it possible to haul purse seines mechanically instead of by hand, which in turn made it possible to use much larger boats and seines. Over just a few years the fishing capacity of the fleet was increased many times over and by more than the herring stock could sustain. This was well before the 200-mile economic zone and before any serious joint management of fish stocks was initiated; there was no control whatsoever of overfishing capacity, effort, or the total catch.

The Peruvian anchovetta, *Engraulis ringens*, once supported the world's largest single-species fishery. In the 1960s and 1970s, the annual catch of this species was as high as 10 million tonnes but it fluctuated on a yearly basis depending on El Niño. Following several consecutive El Niño years in the 1970s, anchovetta stocks were greatly reduced (Figure 14.6), but are now recovering as fishermen have increased exploitation of stocks of other species, such as the South American pilchard (*Sardinops sagax*) also known as the California

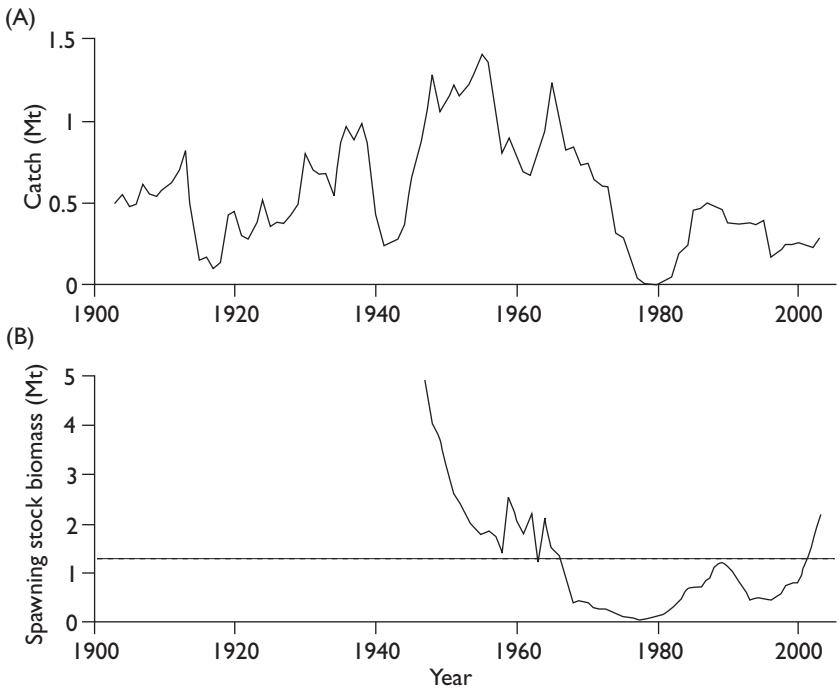

Figure 14.5 (A) Catch of North Sea herring and (B) spawning stock biomass of the autumn spawning herring. The dashed line in panel B is the target spawning stock of 1.3 Mt recommended by the ICES. Adapted from Saville and Bailey (1980).

sardine, although this fish is sometimes assigned its own species or subspecies (*S. caeruleus*). The catches of these two species are seen to vary inversely with one another (Figure 14.5). This points to climatic conditions, such as the El Niño, as the source of these variations and in such a way that the carrying capacity of nature is alternatively utilized by either of these species. To the extent these variations are caused by variations in natural conditions it would clearly be inappropriate to take declining catches as a sign of overexploitation, but an interesting and intriguing question is whether overexploitation of one species rather than natural forces might cause such shifts in stock abundance. Evidence compiled by Francisco Chavez (Chavez *et al.*, 2003) of the Monterey Bay Aquarium Research Institute in Moss Landing, California, and colleagues suggests that the Pacific undergoes a physical and biological shift about every 25 years. The oscillation, similar in some ways to El Niño, seems to coincide with boom-or-bust cycles of the sardine population. The sardine fishery began in California early in the twentieth century and continued to grow until its peak in the 1936–1937 season when 790 000 US tons (~717000 tonnes) were fished. The subsequent decline and its impact on Monterey's Cannery Row was made famous in 1945 by novelist John Steinbeck. In the early 1950s the bottom fell out of the California sardine industry. There is a significant amount of research that suggests that over fishing of the California sardine had significantly changed the age structure of the population. Without a significant

amount of reproductive aged adults the sardine was unable to maintain its numbers as fishing continued. Research points to the failed spawnings of 1949 and 1950 as the cause of the collapse of the sardine population in the North American Pacific. Since the age range of the population had been altered, the failures in reproduction in those 2 years were critical and resulted in a serious decline in the quantity of the species. There were no longer sufficient reproductive class populations to continue the population. The failed spawnings of those years would not have been so critical had the sardine been fished at sustainable levels.

Gadoids – cod, haddock, hake, pollock, saithe, and whiting – are species which tend to feed higher up the food chain than the clupeoids and are also a large and important group in terms of weight and value. The Atlantic cod was once one of the world's great fisheries. Concentrated first in the North Sea and later on the Grand Banks, cod represented one of the oldest European fisheries (Armstrong *et al.*, 2004). Declining numbers of cod have resulted in international disagreements (the "cod wars" of the mid-twentieth century) and forced many nations to all but abandon pursuit of these fish. Today, another species, the Alaskan or walleye pollock (*Theragra chalcogramma*) from the North Pacific and Bering Sea now supports one of the most important world fisheries, yielding about 6 million tonnes per annum (while all cods together contribute about 9 million tonnes).

The bleakest story is told by the fishery for Northern cod off Newfoundland. This used to be one of the richest fisheries in the world, and European nations (the British, the French, and the Spanish) fought over the access to this fishery in the past. Figure 14.7 shows the catches from this stock from 1850 and until this fishery collapsed in the early 1990s. There was an enormous increase in the catch in the 1960s, due to the development of deep-sea factory trawlers, mainly from the Soviet Union and its satellite states in eastern Europe. This was probably not sustainable, although the collapse of the stock came many years later and well after most of the stock had come under the jurisdiction of

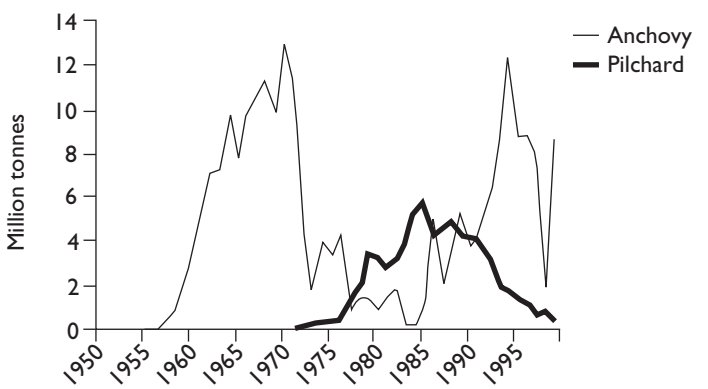

Figure14.6 Long-term variations in Peruvian anchovetta (anchovy) and South American pilchard catches. Pilchard abundance and landings increase in years when the anchovetta are scarce. Based on data in Gréboval (2002).

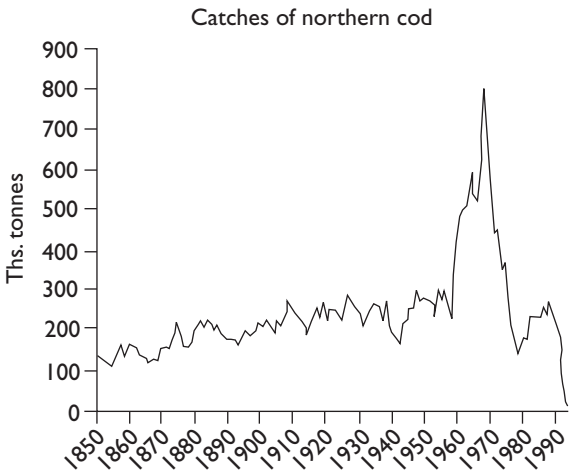

Figure 14.7 The North Atlantic cod fishery largely existed as hook-and-line and trap (pot) fishery until 1950s, however heavy exploitation by a trawl fishery in 1960s and 1970s resulted in the virtual elimination of large fish (spawning) in many stocks. Based on data in Gréboval (2002).

Canada (part of the stock was still accessible in the open sea on the so-called nose and tail of the Grand Banks of Newfoundland).

The collapse of the stock has by many fisheries biologists been attributed to faulty communication between biologists and the Canadian authorities. The biologists responsible for giving advice on the management of the stock did not discover the stock decline early enough, and the Canadian government was slow in cutting back the total permitted quota, because of the adverse effects this would have on job opportunities in Newfoundland. As in other cases, the causes of the cod decline are not indisputable. Cooling of the ocean off eastern Canada may have been a contributing factor, and some would argue that this was the decisive factor. Increased herds of seals feeding on cod, due to the near halt of the seal hunt, has also been mentioned as a possible reason for the collapse, and for the fact that few signs of recovery have been detected over the nearly 10 years that have passed since the fishery was closed down.

Groundfish fisheries have been conducted off the Alaskan coast for nearly 150 years chiefly for Pacific cod, Pacific halibut and sablefish in the inside waters of southeastern Alaska (Rigby *et al.*, 1995). Beginning in the 1950s foreign fishing efforts began to have an effect in the northeastern Pacific Ocean. Russian, and, to a lesser extent, Japanese and Korean trawl vessels, heavily exploited Pacific ocean perch (a slope rockfish species) in the early 1960s and 1970s, with a peak catch of 350 000 tonnes in 1965.

With the passage of the Magnuson Fishery Conservation and Management Act of 1976, the US declared management authority over the Exclusive Economic Zone (EEZ) to 200 nautical miles offshore, and the total allowable level of foreign fishing was limited to that portion of the optimum yield that was not expected to be harvested by domestic vessels. Americanization was

promoted by a "Fish and Chips" amendment to the Magnuson Fishery Conservation and Management Act in 1980, which significantly raised foreign fishing fees and tied foreign fishing privileges to commitments to purchase products by the developing US industry. Despite considerable catch fluctuations in recent years, the northeastern Pacific remains one of the most productive and economically valuable fisheries in the world.

The flatfish are another valuable group of mainly benthic feeders. Fish such as brill, flounder, halibut, lemon sole, plaice, sole, and turbot are caught on the sea bed of the continental shelf in many parts of the world, especially in more temperate areas. Tuna and mackerel are pelagic predatory schooling species usually supporting migratory fisheries of high value and in some cases substantial weight. Tropical waters in many parts of the world support a considerable snapper–grouper fishery. These species often change sex as they grow, complicating management efforts, as most large individuals, as would be preferred by a fishery tend to be predominately one sex or the other. Clearly, a fishery that targets one sex will run the risk of affecting the reproductive potential of the population more than a fishery that distributes its efforts over both sexes. Figure 14.8 illustrates the distribution of individuals of each sex in a population of protogynous grouper. Although the largest individual was female, most large fish are males and are relatively rare in relation to the more numerous smaller females. A fishery that concentrates on larger fish would remove a disproportionate number of males, reducing the probability of successful reproduction. Furthermore, the few large females that are also targeted

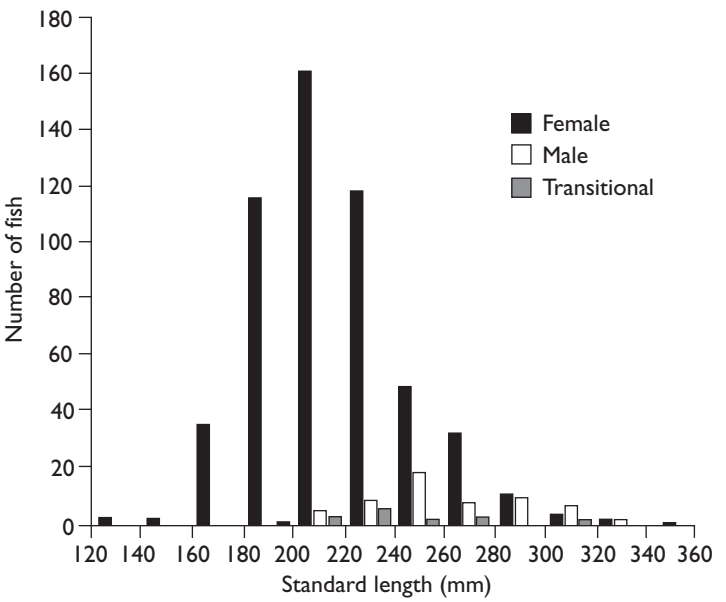

Figure 14.8 Size structure of a protogynous grouper population. All groupers mature first as females. Sex reversal begins after several years' maturity separated by a transitional state that may last 1 or more years. Relatively few males are necessary. Note there are still a few very large females, in fact the largest fish is female – perhaps all the really big males have been caught? Shapiro *et al.* (1993, Figure 1, p. 1153).

by this fishery, because egg production is exponentially related to length, have a reproductive potential equivalent to that of many small females.

Deep-water fisheries

Over the continental slope there are large stocks of blue whiting (*Micromesistius poutassou*) and on the ocean floor rat-tails (macrounds), and morids, related to the cod, are fairly abundant. It has been found that the orange roughy (*Hoplostethus atlanticus*), until recently the object of a considerable fishery off Australasia, is present in the North Atlantic to the west of the British Isles. But after only a few years' exploitation Atlantic stocks of this species have declined even more precipitously than those in the Pacific did. A recent addition to the menus of many seafood restaurants is the "Chilean sea bass," more correctly, but less appetizingly, known as Patagonian toothfish. This large, pelagic notothenid is another example of a long-lived, slow-growing species upon which basing a sustainable fishery is unlikely.

There are other important commercial and sport fisheries for migrating species such as the salmon in the North Pacific and North Atlantic and for sturgeon (*Acipenser*) and eels. Salmon and sturgeon move into freshwater to spawn (although some populations are landlocked) and the eels into the deep part of the Sargasso Sea in the Central Atlantic (p. 425). Salmon are now vulnerable to capture during their high-seas phase of growth (though this was not traditionally a high-seas fishery), as well as in estuaries and rivers. The elasmobranch fishes – sharks, dogfish, rays, and skates – have traditionally held much less commercial value but some support important sport fisheries throughout the world.

In freshwater, the fisheries are supported by the relatives of the salmon – rainbow, brown and steelhead trout, charr (*Salvelinus*), and whitefish (*Coregonus*) – and by a huge group of fish such as carp, perch, roach, and ruffe. In the brackish waters of the Black Sea and Caspian Sea, sprats comprise an important fishery.

14.6 The Fisheries, Economics, and Politics

The rise and subsequent leveling of world fisheries in the last quarter of the twentieth century was the result of two trends: the steady increase in marine fisheries up until the 1990s, and the dramatic growth in freshwater fisheries and aquaculture (some 10% per annum) through the 1990s. As marine landings have leveled off or even declined, two opposing developments have occurred in high-seas fishing fleets. Some countries, such as the UK, have contracted toward their coasts as the traditional distant or middle-water grounds were closed by changes in fishery limits, for example those of Iceland and Norway. The Common Fisheries Policy of the EC has, however, opened up near-water fisheries to other countries of the European Community increasing the pressure on the near-water stocks. Other countries such as Japan, Russia, and eastern European countries have sent fishing fleets, consisting of mother or factory ships and smaller catchers, to new grounds, for example in the southeast Atlantic where they remain for many months. Littoral, estuarine, and freshwater fisheries are important in many parts of the world where they can be prosecuted by small boats. In the Great Lakes of North America and the inland seas of

the former Soviet Union, serious pollution problems have influenced the fisheries that have the potential for good yields in optimum conditions.

The gill nets and small beam trawls, used by sailing boats and lightly powered vessels of the nineteenth century (Figure 14.9), have gradually been superseded by much larger beam trawls and otter trawls fishing on the sea bed, and by pelagic trawls and purse seines fishing in mid-water (Figure 14.10). Although an effective means of catching fish, bottom trawling is now being viewed as analogous to clear-cutting terrestrial forests, not only is the target species removed, but all other biomass as well, while the physical habitat upon which recovery of the ecosystem is dependent is also destroyed. Such trawling also results in the capture of large numbers of unwanted species, referred to as by-catch. Some estimates place by-catch at 20% of the biomass of the target species, other estimates have by-catch figures that are much larger, sometimes exceeding the biomass of the target species. Some by-catch may be returned to the water, where some may survive. Other times by-catch may be utilized for alternative markets such as animal food, fertilizers or other non-food products but the majority of by-catch biomass is viewed as a wasted resource and high levels of by-catch are seen as a serious problem in trawl fisheries. Gill (entangling) nets and baited lines are of relatively minor importance in terms of weight of fish caught; however, abandoned or lost gill nets and long-lines can continue to catch fish, a phenomenon known as ghosting, which also receives public attention as another example of the wastage of our marine resources.

14.7 Aquaculture

Fishes have been farmed in China for over 3000 years and that country continues to dominate aquaculture producing some 69% of the world's aquaculture output (down from 83% just a decade ago). India comes in a distant second, contributing only 5.5% followed by Thailand, Bangladesh, Vietnam, Indonesia,

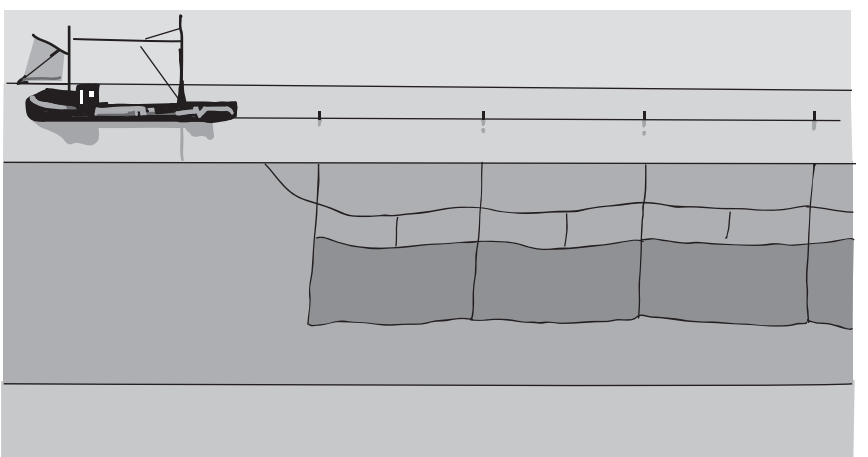

Figure 14.9 Early twentieth century drift netting for herring. Net panels of about 30 m length were combined to create drift nets up to 5 km in total length.

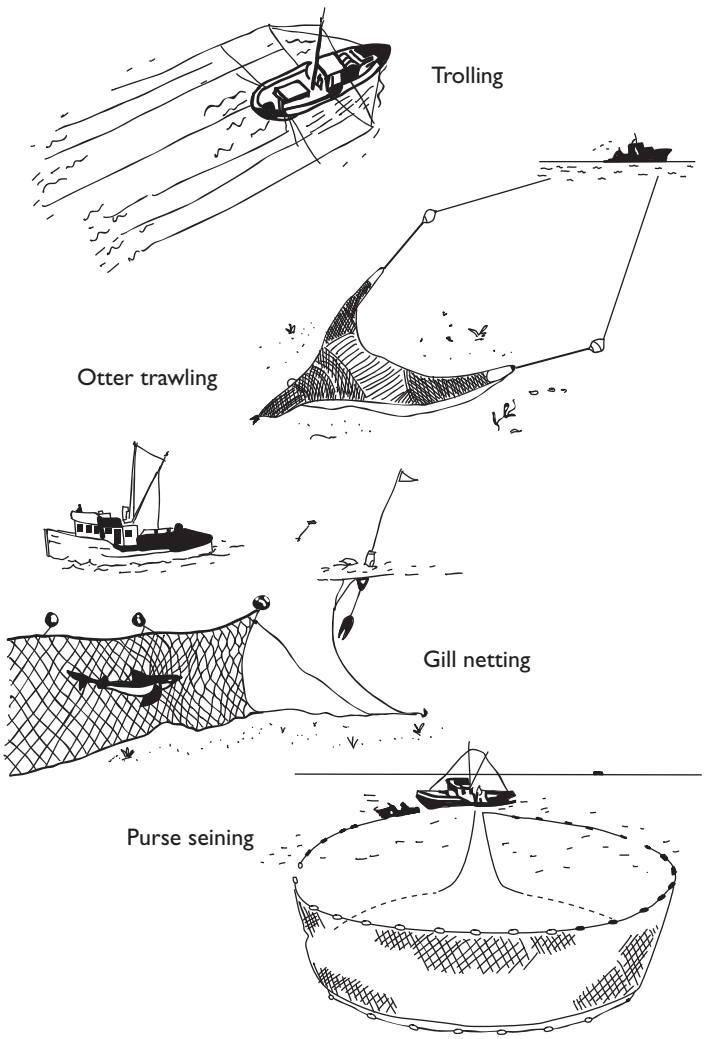

Figure 14.10 Various types of fishing gear. Based on drawings in Rounsefell (1975).

and Japan. The USA with only about 1% of the world's aquaculture production, ranks eighth.

Fish are valuable as a food resource because they have a high food conversion value (FCV) – that is the amount of food that it takes to produce a unit weight of the product. It requires only 1.9 units of feed to produce one unit of fish. Poultry are nearly as efficient, with a FCV of 2:1, and pork and beef are much less efficient, 4:1 and 8.5:1 respectively (Pullin *et al.*, 1993).

In 2000 the worldwide production of fish from aquaculture was 35 million tonnes, nearly a four-fold increase since 1990, of which 6.6 million tonnes was produced in developing countries. This represents about 9% of terrestrial production of animal protein. About 85% of production is of herbivorous fish especially cyprinids but also tilapias, milkfish and mullets. China produced just over 25 million tonnes, mainly of cyprinids like the carp.

Aquaculture has been practiced for many centuries in Africa and the Far East but also in some parts of Europe where carp (*Cyprinus carpio*) were reared in the ponds of medieval monasteries. Technologies for rearing other species have been developed more recently. The channel catfish (*Ictalurus punctatus*) is now farmed on a large scale in the southern USA. Other important species are eels, especially in Japan, the cichlid tilapias, with a worldwide production of at least 1.2 million tonnes per annum and the milkfish (*Chanos chanos*). More recently, there has been an explosion of interest in seawater cage farming of rainbow trout and salmon, especially in Norway, Scotland, Canada, and Denmark (Figure 14.11). Total annual production is now approaching 1.5 million tonnes and has reached the point where future growth may be limited by the difficulties of controlling disease and environmental concerns. The culture of high-value marine species such as bass, bream, halibut, sole, and sturgeon is now developing mainly due to breakthroughs in rearing of the young stages (which has not been a problem with salmonids). This technology is only beginning to reach an economically viable phase.

Much of aquaculture is based on herbivores like the grass-eating carp (*Ctenopharyngodon idella*) and milkfish, and on omnivores such as common carp and tilapias that feed low in the food web. Most high-priced species are, however, fed on fish-based diets. This makes high-conversion rates essential since cheaper protein is being upgraded to more expensive protein with little room for saving if the price of the final product falls.

Because many cultured species are dependent on a diet of wild fish, fish meal, and fish oil from natural stocks represent principal ingredients in many fish feeds used in aquaculture. The aquaculture industry consumes 70% of the global production of fish oil and 34% of total fishmeal, much of it as pellets for salmon and trout farming. This, itself, is seen by many as threatening the wild stocks of small ocean (pelagic) fish but the impact on fish stocks is partially offset by the use of species not normally consumed by humans or the use of by-catch, offal, or other waste protein that would otherwise be discarded.

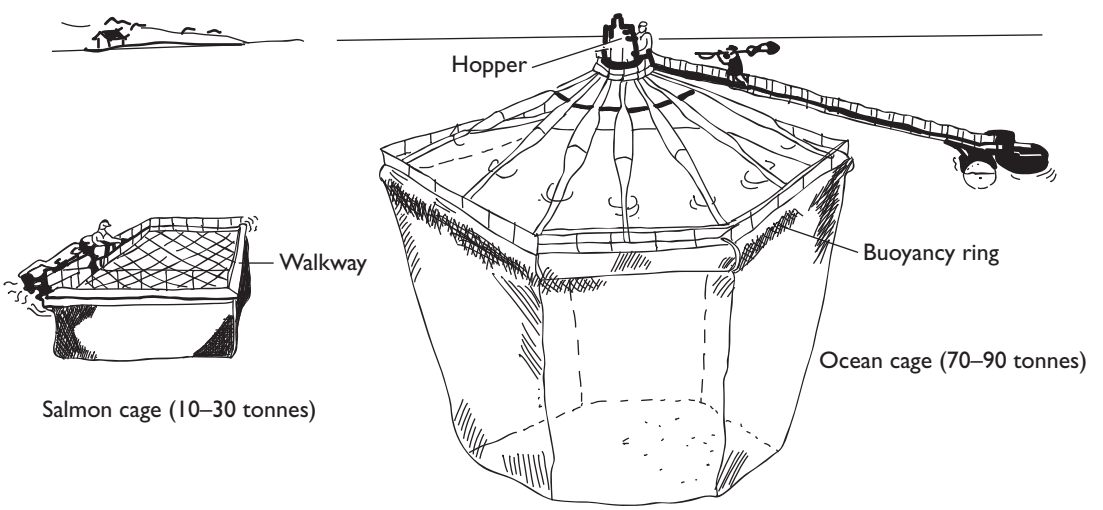

Figure 14.11 Two types of cage used in salmon farming.

Genetic manipulation in aquaculture is becoming commonplace (Purdom, 1993). In addition to simply breeding for desirable characteristics, it is now possible to produce unisex populations by chromosome manipulation (gynogenesis), appropriate hybridization, and hormonal treatment. In salmon and rainbow trout, for example, it is advantageous to rear all-female populations; because the males often mature early at a small size, become aggressive with a correspondingly lower growth rate, and have unpalatable flesh. In the tilapias, the males are faster growing than the females and all-male populations are optimal. Hybrids of salmonids, temperate bass (*Morone*), sturgeon, and other species, often combine the best qualities of the parents used. Although they are likely to be sterile, hybrids may be useful in aquaculture or in stocking sport fisheries. Sterility in non-hybrids can be induced by pressure or cold shock of newly fertilized eggs which induces triploidy. Triploid salmon, for example, are sterile and triploid females are especially useful since they do not mature sexually and maintain their growth until ready for harvesting. Another advantage of using sterile salmon is that escaped fish cannot interbreed with wild stocks. Interbreeding has been a matter of some concern to anglers and managers over the past few years (Cloud and Thorgaard, 1993), not only with salmon, but also with tilapias such as *Oreochromis mossambicus*.

14.8 Ranching

The release into the wild of species reared in shore facilities is analogous to ranching cattle as long as there is some guarantee of the fish being available for later harvesting. Since the cost of feed is the greatest financial burden in aquaculture, ranching is likely to be viable even with substantial losses through straying and predation. Such practices, also called stock enhancement, is used not only for food fishes, but also to maintain viable sports fisheries.

Salmon smolts can be imprinted with the characteristics of their home area (p. 338) and after one or more years in the sea can be expected to return for harvesting. At present, salmon ranching is only being practiced actively in Iceland. Sturgeons are also reared and allowed to migrate into the sea in the hope of an enhanced return later in life. Japan has tried stock enhancement of a variety of fish and invertebrate species since the 1890s; however, results suggest that stock enhancement is of limited value.

14.9 Management

According to Charles (1992) fishery management must consider three contrasting "fishery world views:" those of conservation (which for most contemporary fisheries is primarily followed in theory, if not in practice), rationalization (the maximization of economic efficiency and exploitation), and social community (which seeks to maximize social benefits to individuals engaged in the fishery).

Traditional fisheries management, throughout most of the last half of the twentieth century, has been based on the concept of *maximum sustainable yield* (MSY), the level at which a fish population can be exploited indefinitely without diminishing its numbers (Rounsefell, 1975). To achieve MSY the average

harvest should equal the average population growth minus natural mortality, taking advantage of the fact that moderate exploitation results in increased growth and production over what is seen in unexploited (or overexploited) populations. Population growth of a stock is a result of recruitment of young fish and their subsequent growth. Recruitment, growth, and harvest can generally be measured by experimental fishing and by recording the catches from commercial vessels or sampling their catches at the fish market, but the rate of natural mortality must be estimated (Mullin, 1993; Steele and Henderson, 1984).

What is meant by a *stock* of fish? It seems that some commercial species may be divided into stocks that should be considered independent of other stocks of the same species for management purposes. These stocks usually separate out during spawning but often mix outside the spawning season, a classic case being the North Sea herring. For many years, fisheries scientists have attempted to distinguish between stocks using identifying characteristics such as growth patterns determined from the scales and otoliths (p. 249) (L'Abee-Lund and Jensen, 1993), and counts of meristic characters such as vertebrae and finrays. Stocks of fish can sometimes be identified by parasites that are specific to particular areas of the sea. Genetic techniques allow stocks to be identified by the electrophoretic isozyme pattern obtained from tissues such as the muscles and eye lens. Some cod and salmon released into the Norwegian fjords and rivers for restocking can be identified by a special isozyme pattern. Genetic fingerprinting utilizing nuclear or mitochondrial DNA should also make it possible to identify individual fish or particular parental lines, although such techniques are neither quick nor cheap.

Beginning in the 1970s many nations established 200-mile EEZs in which fishing by other nations was restricted or forbidden. Examples of laws regulating fishing within the 200-mile EEZs are the 1976 US Marine Fisheries Conservation and Management Act (MFCMA) also known as the Magnuson Act, and the Common Fishery Policy (CFP) of the EU established in 1970. The United Nations Convention on the Law of the Sea (1982) codified the international jurisdictional basis for these zones.

Many of these newer regulations include criteria beside purely biological, most recently increased emphasis has been afforded to human sociological as well as economic factors. The MFCMA defines an OSY as "the amount of fish that (1) will provide the greatest overall benefit to the United States, with particular reference to food production and recreational opportunities, and (2) is prescribed as such on the basis of MSY from such a fishery as modified by relevant ecological, economic and social factors requiring fishery biologists to work with economists and sociologists (or anthropologists) to inform the policy makers". The 1996 Sustainable Fisheries Act of the USA requires managers to take necessary actions to rebuild depleted fish stocks.

Total allowable catches in which the annual sustainable catch for any stock is estimated by fishery biologists and divided up into quotas among the interested fishing nations have also been introduced. This measure, in use currently in many fisheries, is only partially successful because there is a lag in collecting statistics that creates difficulties in determining when a quota has been reached. Furthermore, discards of fish that cannot be landed because the quota for that species has been reached (or of undersized fish) are an appalling source of loss of future marketable fish.

Most recently the term "responsible fisheries" has been used to describe sustainable fisheries in which a more conservative approach is taken to guarantee the preservation and recovery of depleted fishery stocks and recognizing that even sustainable fisheries will not always be compatible with maintaining many natural marine communities (Pauly *et al.*, 2002).

Concern over overfishing is not new. In 1376, during the reign of Edward III, "great complaints" were expressed about the use of the beam trawl net called "wondyrchoun" (wonderous machine). According to the Rolls of Parliament for that year the fear was that this device led to wastage of the resource and would eventually destroy all sea life. "The Great and Long Iron of the Wondyrchoun Runs so heavily and hardly over the ground when fishing that it destroys the flowers of the land, below the water and also the spat of Oysters, Mussels and other fish upon which the great fish are nourished. By which instrument in many places the fishermen take such quantity of small fish that they know not what to do with them and they feed and fat their pigs with them, to the great damage of the common's of the realm and the destruction of the fisheries" (cited in Dyson, 1977). Some 500 years later Thomas Huxley, best known as "Darwin's Bulldog," and perhaps a better defender of natural selection than fishery manager, in response to an inquiry from Parliament in 1883 expressed his belief "that the cod fishery, the herring fishery, the pilchard fishery, the mackerel fishery, and probably all the great sea fisheries, are inexhaustible; that is to say, that nothing we do seriously affects the number of the fish. And any attempt to regulate these fisheries seems consequently, from the nature of the case, to be useless" (Huxley, 1883). In defense of Huxley, he did qualify his statement by setting up his context a few sentences before: "... in relation to our present modes of fishing ..." but within a decade the introduction of steam trawlers, and steam-powered capstans early in the twentieth century changed the game completely. One of the first fisheries bodies, the International Council for the Exploration of the Sea, was established nearly a century ago in response to declining catches in and around the North Sea. Individual fish stocks have waxed and waned over the years, sometimes spectacularly. In 1972, the annual yield of the Peruvian anchovetta fishery fell from over 10 million tonnes to less than 2 million tonnes, as a result of overfishing during adverse oceanic conditions.

While concern over overfishing is not new, the impacts of it are. Prior to the last century the fishing fleets were not powerful enough to deplete stocks below a sustainable level. With the introduction of steam- and then diesel-powered engines and winches, the boats could travel further and faster and use more effective gear. Fish-finding techniques such as sonar and the use of satellite transmitted oceanographic data have also made fishing practice more efficient. On-board processing and refrigeration allow vessels to range further and remain longer at sea. It is by estimated by the FAO that presently 75% of the world's fishery stocks are either fully or over-exploited, or depleted, while only 1% are in recovery. Overfished stocks are characterized by reduced size and age or length at maturity (known as tropicalization), as well as increased catch variability (uncertainty). The reasons for overfishing are complex and varied. Developing countries regard fishery resources as valuable for maintaining foreign trade surplus as well as providing food and jobs locally, while in developed countries government subsidies encourage over-capitalization and

over-exploitation. The obvious solution is to reduce the size of the fleets in many countries and allow the remaining boats a larger share of the national quota, but this has severe social implications.

In a recent paper in *Nature* (Watson and Pauly, 2001), it was suggested that the state of global fisheries is even worse than is generally believed. The paper claims that (mainland) China has consistently and significantly over-reported annual production to the Food and Agriculture Organization (the international body responsible for compiling global fishery figures), thereby inflating world fishery production. While China is by no means the only country implicated, the fact that she is a major fishing nation (accounting for nearly 30% of the world's fish production) means that her figures have a disproportionately large impact on estimates of global production. These estimates are used to judge the state of world fisheries and to make decisions about management and the appropriate levels of investment in fishery operations by governments, banks, and private enterprise. In other words realistic production figures are critical for sustainable resource use.

Daniel Pauley (again) and others (Pauly and Christensen, 1995; Pauly, 2000; Pauly *et al.*, 2002) have estimated that the sustainable yield of the world's oceans is about 100 million tonnes per year. Projections of world fishery production in 2010 made by the FAO range between 107 and 144 million tonnes, of which about 30 million tonnes will probably be reduced to fish meal and oil for non-food use. Estimated quantities which will be available for human consumption range between 74 million tonnes and 114 million tonnes. Most of the increase in fish production is expected to come from aquaculture that is growing rapidly. The contribution from capture fisheries will depend on some further development and also on the effectiveness of fisheries management. Improved management of currently overfished stocks could provide an increase of between 5 and 10 million tonnes, whereas continued overfishing will lead to declining production, as reflected in the pessimistic scenario in the table.

Early management techniques consisted of increasing the mesh sizes of nets to allow small fish to escape and by forbidding the landing of undersized fish, to allow the smaller fish to grow to a more optimal size before being harvested. These measures were not successful, mainly because of the difficulty of agreeing mesh sizes with the industry – it was a classical instance of too little, too late. Attempts by fishery managers to use ever increasingly complex models have not met with much success as fishery stocks continue to decline.

One result of management (or mismanagement) has been a decline in the mean trophic level of the world's fisheries, a phenomenon referred to as "fishing down food webs" a result as the more desirable, carnivorous species become depleted and fisheries are shifting to species once regarded as less desirable, simply because they are available. A consequence of this is reduced diversity and biomass that means greater annual variations and greater dependence on annual recruitment (Figure 14.12).

Given these problems, new ideas and approaches to traditional fisheries management approach, which considers each fish stock in isolation or several fish species but not the wider marine environment, are needed. One concept considered in recent years is that of Ecosystem Based Fishery Management (EBFM) which ideally should take a more holistic and integrative view of fisheries management. Simpler models, based on ecosystem management rather

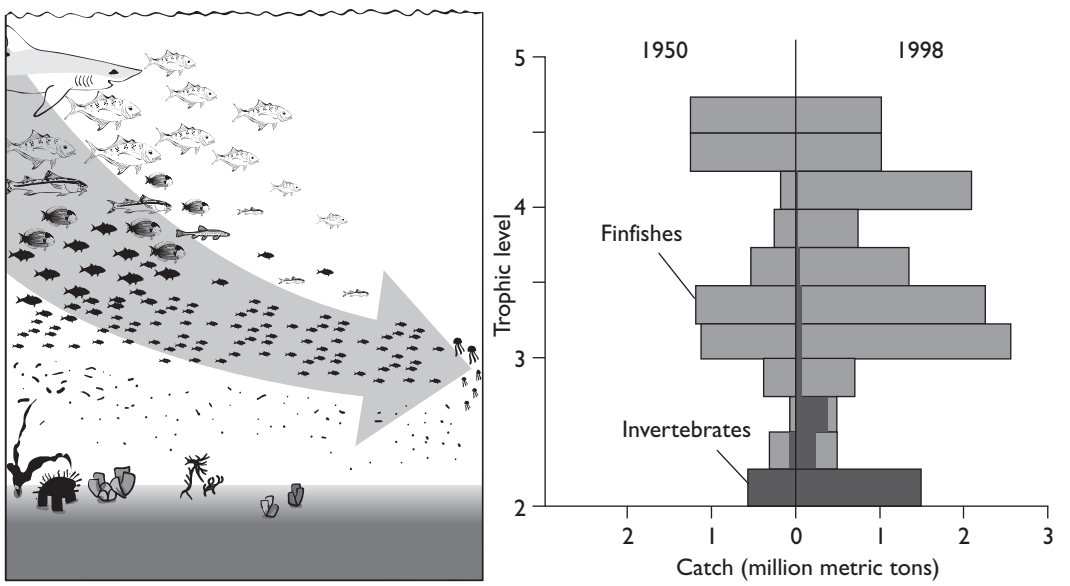

Figure 14.12 Fishing down food chains. Left figure shows diagrammatically the reduction in the average trophic level in heavily exploited fisheries. The right figure provides a more qualitative demonstration; the longer bars for 1998 showing the greater contribution of lower trophic levels to the overall community. From Pauly and Maclean (2003, Figures 16 and 17, pp. 51, 52).

than single species-models, such as large-scale protected (no fishing) zones are promising.

Envoi

Humans have depended on fish as food for a very long time. In even the recent past, the supposedly inexhaustible nature of fishery resources was exploited without regard. However, beginning in the early twentieth century, declining fishery catches prompted the application of scientific investigations to the study of growth and recruitment as well as the technology of finding and capturing fishes. Advances in the practices of fish farming and ranching are today offering the promise of greater availability of fish for human consumption and other purposes, however, consideration of environmental impacts of these practices are presenting further challenges to the development of aquaculture. In the immediate future, human societies must find answers to the questions of sustainable fisheries and aquaculture if they are to continue to depend on fish as a important source of human nutrition.

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