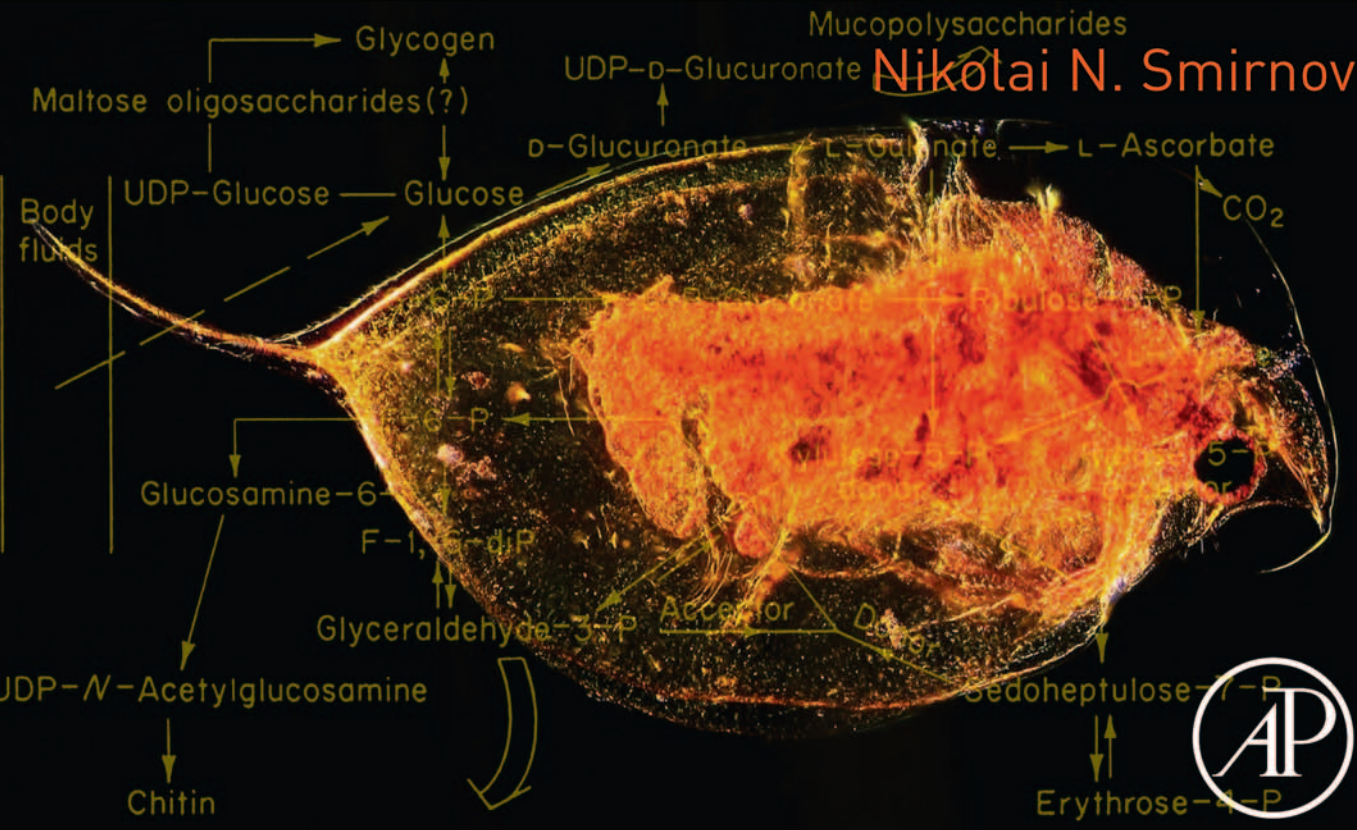
Second Edition

Physiology of the CLADOCERA

Nikolai N. Smirnov



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With Additional Contributions

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Contents

18. Notes on the Physiology of Embryogenesis

A.A. KOTOV

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Preface

Increasing and universal environmental pollution stimulated numerous recent investigations in the pathological physiology of the Cladocera that brought forth abundant information discussed in the present edition. In addition, studies in normal physiology progressed in various ways.

The structure of the previous edition is only slightly changed. However, new sections are added and numerous new findings are introduced throughout the text. As well, misprints and errors are corrected. The present edition is supplemented mainly with data from sources for recent years.

Reviewing the results of the worldwide investigations one may note that they remain mostly confined to daphnids which live in open water, especially to *Daphnia*, whereas representatives of other families coping with different life conditions are still awaiting investigation. The vast and specific world of littoral and bottom-living Cladocera is still waiting for due physiological assessment. Some data available for such species are discussed as well. Transformations of functions and the related structures in different families would be highly instructive and revealing. Probably, the present review will indicate what and how should further be studied in these animals which live very differently from pelagic species. Especially demonstrative would be, e.g., data on transformations of the system of muscles and of the involved skeletal structures performing different kinds of locomotion in representatives of various genera.

Another special point of studies in Cladocera physiology is that they are still fragmentary: some issues are now clarified and some are almost unknown.

Over 700 species of Cladocera (Crustacea: Branchiopoda) are known and representatives of this group are often dominant in the freshwater fauna, sometimes occurring in enormous quantities. They live in both small and large water bodies from arctic to tropical latitudes, in open water, on the bottom, in mud, among inshore vegetation, in acid pools on bogs, in small accumulations of water in epiphytic plants, in narrow aquatic spaces between moist sand grains. A few species even left the water and live in moist moss-like growth on tree trunks in tropical cloud forests. Some species are specialized for life in saline lakes and in the sea.

Cladocerans, especially *Daphnia* species, belong to the commonest animals in hydrobiology. They are counted, measured, weighed, cultured, their species lists are composed, distribution in space and time described. Their role is recognized as the food resource of fish and as water quality indicators. Species of Cladocera are described and redescribed nowadays in excellent morphological detail.

Usually, little more is taken into consideration than a cladoceran as a living object with individual, mostly external, traits that permit species recognition. There follows an attempt to summarize information showing that the Cladocera possess complicated and special metabolism and behavior which deserve knowing, as these data may explain how and why Cladocera species successfully live in their various media. The following summary is an attempt to contribute to a more profound understanding

of how and why they participate in the processes developing in inland waters. Particular chapters of cladoceran physiology are still covered very unevenly. Recently, numerous and more in-depth data on pathological physiology are being accumulated, comparing with older data that were confined mostly to longevity and amount of progeny as affected by xenobiotics.

Comparative physiological investigations of representatives of various families with their different ecology are urgently desirable and would make a promising field. Of course, sometimes more questions are raised than answers supplied. The present review of this vast field is rather an attempt at systematic assembling of the available data and demonstration of specificity of this group of crustaceans. Within the present context, the main attention is paid to data demonstrating which and how the metabolic links are influenced by particular natural and anthropogenic factors in the hope of revealing the reasons of this impact.

Investigations of aquatic invertebrates frequently endeavor to obtain answers why a certain species is present or absent, why it is abundant, why it lives in a certain habitat. Part of explanation is supplied by morphology (structural traits), e.g., thin skeletons, swimming appendages, oil drops, sometimes the presence of slime, etc., in planktonic forms. However, physiology makes a wide field which can be used for understanding causative relationships.

Daphnia is more and more frequently used in water-quality testing and as a model organism supposed to represent situations in natural or artificial ecosystems (Lampert, 2011; Seda and Petrusek, 2011).

Along with special discussions, introductory remarks are made whenever it seemed to be necessary to make the matter useful both for specialists and for nonspecialists.

The present review comprises studies made in the period starting from the second half of the 19th century. Completeness of information was checked against Zoological Record, part 10 (Crustacea), vols 1−150 (for 2015) and Russian Referativnyi Zhurnal (Biology, Zoology) (1992−2015). Some earlier sources are also added. Any incompleteness herein is because some fields are not yet investigated, some literature has not been found, and some points might have escaped the author's attention.

Acknowledgments

The present volume is an attempt at making a summary of work of many experts throughout the world in various fields using special methods. A substantial contribution to physiology of the Cladocera has recently been made by toxicologists.

Special thanks are due to Dr. M.J. Burgis (United Kingdom) who generously used her time and experience to make the manuscript of the first edition acceptable.

The present review is motivated by the author's observations on living, mostly littoral, cladocerans. Both the initial training and subsequent work of the author implied that hydrobiology is impossible without physiological data. Some recent observations are made at the Hydrobiological Station "Lake Glubokoe" (Russia). The author is grateful to his immediate colleagues from the "Cladocera team" for help and discussions: O.S. Boikova, N.M. Korovchinsky, A.A. Kotov, E.I. Bekker, and A.Y. Sinev. Dr. Kotov critically read the draft manuscript, suggested numerous useful additions, and used his skill for preparation of the manuscript and figures.

The author is obliged to many teachers at school and at the Linguistic University (Moscow) for training in European languages sufficient for direct understanding of original texts. Many librarians, mostly personally unknown to the author, retrieved numerous publications in different languages and times. Their care and labor are appreciated, including those of Ms. N.I. Gotovskaya and Ms. E.V. Morozova the Biological Department Library of the Russian Academy of Sciences (RAS). The facilities and library of the Moscow Society of Naturalists were very useful, especially for earlier sources. Dr. V.R. Alekseev and Ms. N.M. Sukhikh were very helpful in work with resources of the library of the Zoological Institute RAS (St. Petersburg). I am sincerely grateful to Professor G.A. Boxshall (FRS; UK) for help in getting rare publications.

My wife, L.A. Smirnova, Ph.D. (cited here as L.A. Luferova) is tolerant (mostly) toward using a big part of my time for such ventures as this.

Formulation of ideas included in this book and its composition was much favored by creative environment at the Institute of Ecology and Evolution of the RAS, and by personal attention of academicians D.S. Pavlov and Yu.Yu. Dgebuadze.

The authors of Chapters 16–18 described their special fields and made the subject much more advanced and complete.

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Abbreviations and Units

Standard abbreviations and units are used.

ACh	Acetylcholine
ATP	Adenosine triphosphate
DHA	Docosahexaenoic acid
DNA	Deoxyribonucleic acid
DW	Dry weight
EPA	Eicosapentaenoic acid
FAs	Fatty acids
GST	Glutathione S-transferase
h	Hour
Hb	Hemoglobin
HUFAs	Highly unsaturated fatty acids
IUs	International units
MF	Methyl farnesoate
mg%	mg per 100 g
min	Minute
MUFAs	Monounsaturated fatty acids
NADH	Nicotinamide adenine dinucleotide plus hydrogen
PCBs	Polychlorinated biphenyls
PUFAs	Polyunsaturated fatty acids
RNA	Ribonucleic acid
RQ	Respiratory Quotient
SAFAs	Saturated fatty acids
TBT	Tributyltin chloride
UVR	Ultraviolet radiation
WW	Wet weight

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CHAPTER

1

General

1.1 SYSTEMATIC POSITION

It is now thought that over 700 species of the order Cladocera exist in the world fauna, many of which develop enormous populations and thus play a big role in the biosphere. New species are still being described.

The Cladocera belong to the subclass Phyllopoda of the class Crustacea. Most Cladocera belong to the orders Anomopoda and Ctenopoda. Anomopoda principally comprise the families Daphniidae (e.g., the genera *Daphnia*, *Ceriodaphnia*, *Simocephalus*, and *Scapholeberis*), Moinidae (*Moina* and *Moinodaphnia*), Ilyocryptidae (*Ilyocryptus*), Macrothricidae (e.g., *Macrothrix* and *Streblocerus*), Acantholeberidae (*Acantholeberis*), Ophryoxidae, Eurycercidae (*Eurycercus*), Chydoridae (e.g., *Chydorus* and *Pleuroxus*), Bosminidae (*Bosmina*, *Bosminopsis*). Ctenopoda comprise the families Sididae (e.g., *Sida*, *Pseudosida*, and *Diaphanosoma*) and Holopedidae (*Holopedium*).

Others belong to the order Onychopoda (the freshwater *Polyphemus*, as well as a few marine and brackish-water species) and the order Haplopoda with the family Leptodoridae (*Leptodora*, with two species). Both anomopods and ctenopods produced species living on various substrata and planktonic species. Onychopods and haplopods comprise fewer species, all of which are planktonic predators.

For reliable identification of the subjects in physiological investigations, the keys to the worldwide fauna of Cladocera are available: "Guides to the Identification of the Microinvertebrates of the Continental Waters of the World" issues 1, Macrothricidae (Smirnov, 1992); 3, Ctenopoda (Korovchinsky, 1992), 11, Chydorinae (Smirnov, 1996); 17, *Simocephalus* (Orlova-Bienkowskaja, 2001), 13, The predatory Cladocera (Rivier, 1998); 21, *Daphnia* (Benzie, 2005); 22, Ilyocryptidae (Kotov and Štifter, 2006), and 25, *Eurycercus* (Kotov and Bekker, 2016). There are also newer, general worldwide resources for ctenopods, created by Korovchinsky (2004); *Leydigia* (Chydoridae), by Kotov (2009); and *Eurycercus*, by Bekker et al. (2012); as well as recent regional keys. As investigations into Cladocera are actively developing, the aforementioned summaries are rapidly becoming incomplete, and literature that is more recent should be considered.

1.2 GENERAL MORPHOLOGICAL BACKGROUND

As animal functions are linked to their form, some comments on the body structure and organs of Cladocera are provided here. Most of the animals attributed to the order Cladocera have the same principal structure, with various modifications present in different species. Investigations into comparative and functional morphology (such as, those by Fryer, 1968,

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1974, 1991, etc.) have revealed exciting data on particular species, permitting a better understanding of their lifestyles.

Cladocerans have inherited from their ancestors a weakly segmented body covered with a chitinous, mostly bivalved, shell and bearing few pairs of appendages—antennules, antennae (biramous, with the single exception of female *Holopedium*), mandibles, maxillulae, maxillae (may be completely reduced), mandibles, and five or six pairs of thoracic limbs (Figs. 1.1–1.4). Cladocerans are mostly oval, compressed from the sides, but many are spherical. In the case of *Graptoleberis*, there is a curious and unique combination of lateral and dorsoventral compression. The appendages have numerous, but organized, setae. The posterior end of the body (postabdomen) is bent at a right angle to the abdomen, or may even be reversed. On the proximal dorsal side of the postabdomen, a pair of setae are present, traditionally termed setae natatoriae (shown, e.g., in Figs. 1.3 and 13.1 right). All structures tend to undergo morphological radiation, and

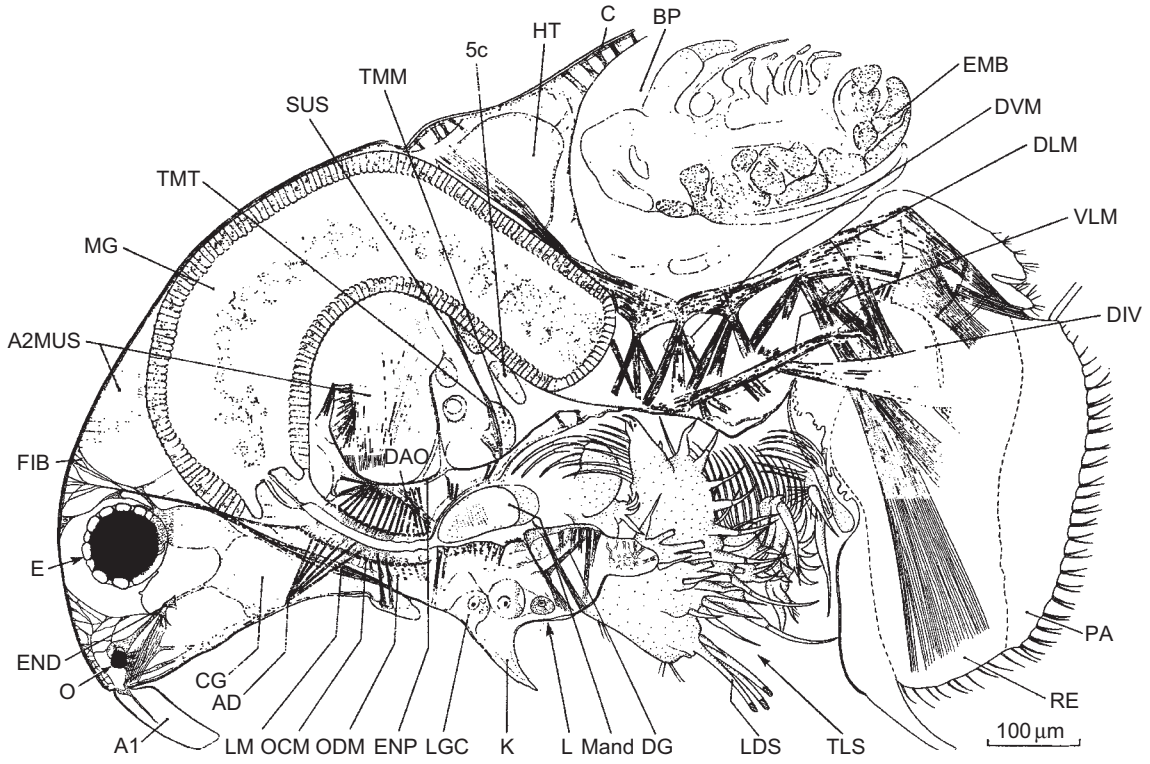

FIGURE 1.1 General anatomy of *Acantholeberis curvirostris*. *A1*, antennule; *A2MUS*, antennary muscles; *AD*, apodeme; *BP*, brood pouch; *C*, carapace; *CG*, cerebral ganglion; *DAO*, dilator muscle of atrium oris; *DG*, duct of labral glands; *DIV*, diverticulum; *DLM*, dorsal longitudinal muscles; *DVM*, dorso–ventral trunk muscles; *E*, compound eye; *EMB*, embryo; *END*, endoskeleton; *ENP*, endoskeletal plate; *FIB*, fibrils; *HT*, heart; *K*, keel of labrum; *L*, labrum; *LDS*, long distal setae of outer distal lobe of limb 1; *LM*, levator muscle of labrum; *Mand*, mandible; *MG*, midgut; *O*, ocellus; *OCM*, esophageal constrictor muscles; *ODM*, esophageal dilator muscles; *PA*, postabdominal lamella; *RE*, rectum; *SUS*, suspensory ligament; *TLS*, trunk limbs; *TMM*, 5c, transverse muscle of mandible; *TMT*, transverse mandibular tendon; *VLM*, ventral longitudinal trunk muscles. *From Fryer, G., 1974. Evolution and adaptive radiation in the Macrothricidae (Crustacea: Cladocera): a study in comparative functional morphology and ecology. Philosophical Transactions of the Royal Society of London, B: Biological Sciences 269 (898), 137–274.*

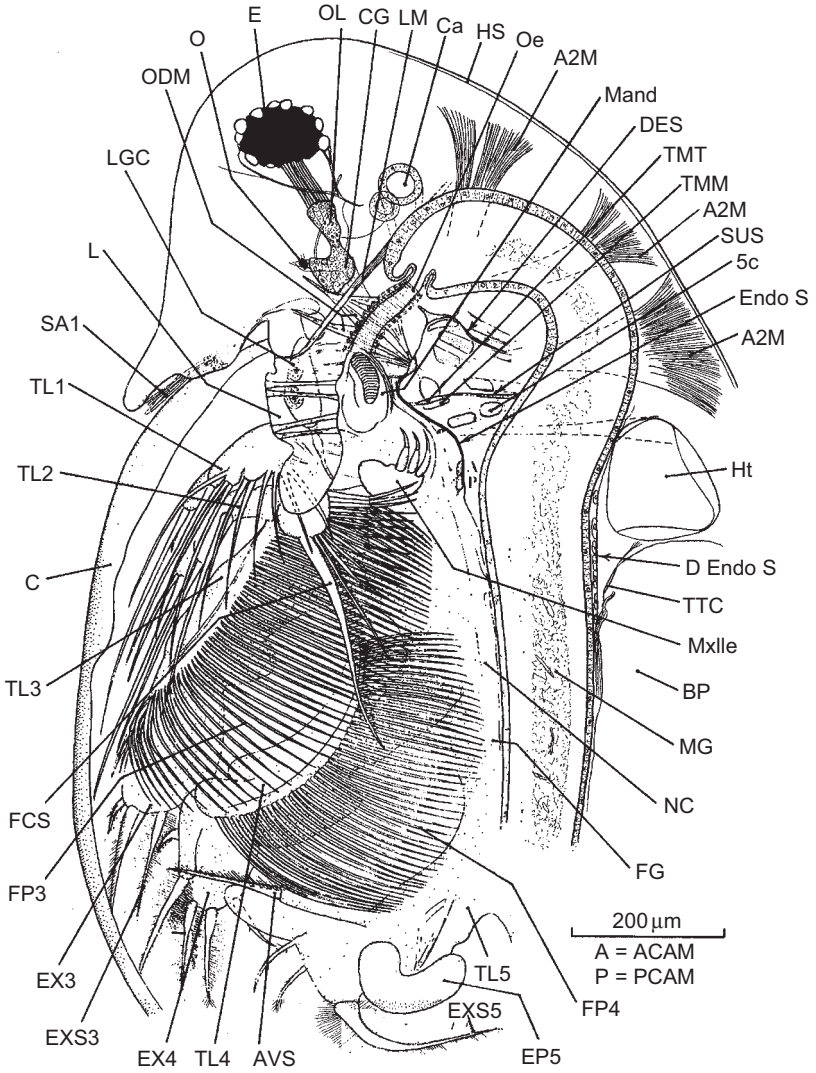

FIGURE 1.2 General anatomy of *Daphnia longispina*. *A*, anterior carapace adductor muscle; *A2M*, antennal muscles; *AVS*, anterior vertical seta of trunk limb 5; *Ca*, caecum; *D Endo S*, dorsal endoskeletal sheet; *DES*, dorsal extension of ventral endoskeletal sheet; *EndoS*, endoskeletal sheet; *EP5*, epipodite of trunk limb 5; *EX3, 4*, exopod of trunk limbs 3, 4; *EXS5*, exopod seta 5; *FCS*, filter-cleaning spine of trunk limb 2; *FG*, food grove; *FP3*, gnathobasic filter plate of trunk limb 3; *FP4*, gnathobasic filter plate of trunk limb 4; *Ht*, heart; *HS*, head shield; *LGC*, labral gland cells; *MxIIe*, maxillule; *NC*, nerve cord; *Oe*, esophagus; *OL*, optic lobe of cerebral ganglion; *P*, posterior carapax adductor muscle; *SA1*, sensory seta of antennule; *TL1, 2, 3, 4, 5*, trunk limbs 1, 2, 3, 4, 5; *TTC*, thickened trunk cuticle. Other abbreviations as in Fig. 1.1. *From Fryer, G., 1991. Functional morphology and adaptive radiation of the Daphniidae (Branchiopoda: Anomopoda). Philosophical Transactions of the Royal Society of London, B: Biological Sciences 331 (1259), 1—99.*

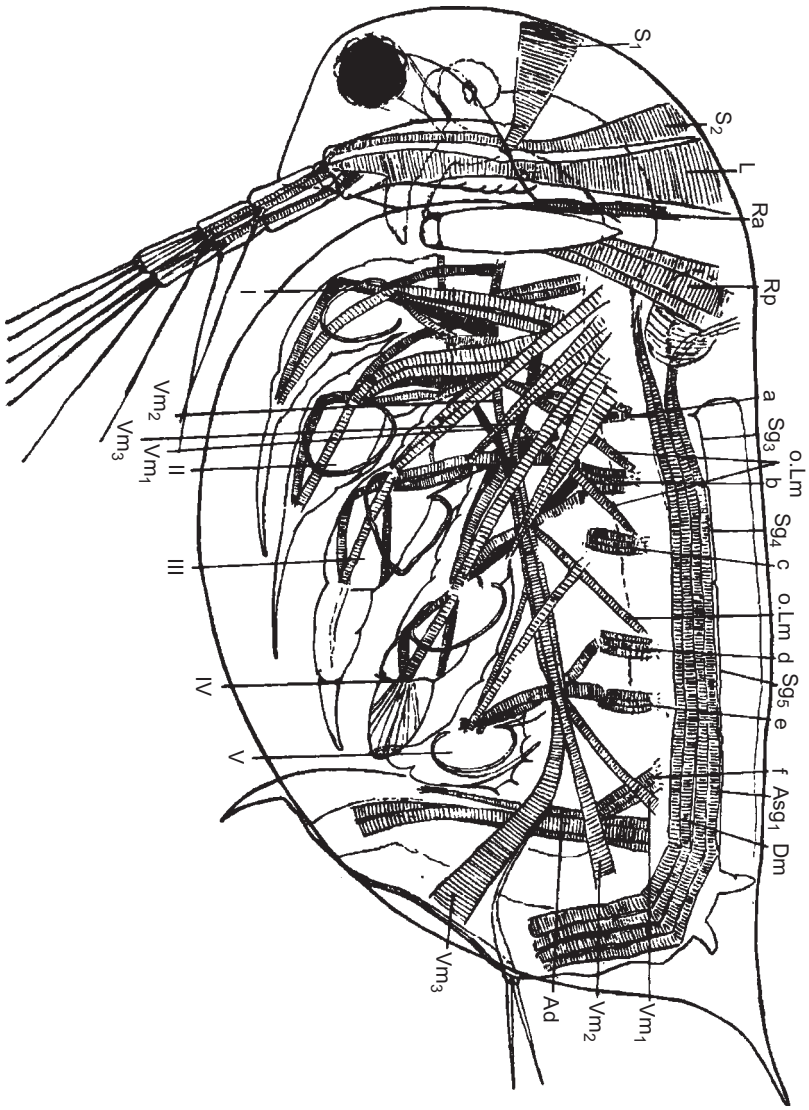

FIGURE 1.3 Muscles of *Daphnia magna*. *From Binder, G., 1931. Das Muskelsystem von Daphnia. International Review of Hydrobiology 26, 54–111.*

homologous structures may occur in different species in various forms, from the ancestral state to their complete disappearance or, in contrast, enlargement, and specialization.

The body in most species is covered by the head shield and valves. The outer surface of the chitinous shell may be smooth, reticulated, ciliated, or variously honeycombed (see, e.g., Kotov, 2013). The head shield of most species exhibits head pores (Frey, 1959; Olesen, 1996), leading to an organ the function of which is probably ion exchange. Littoral cladocerans, which live

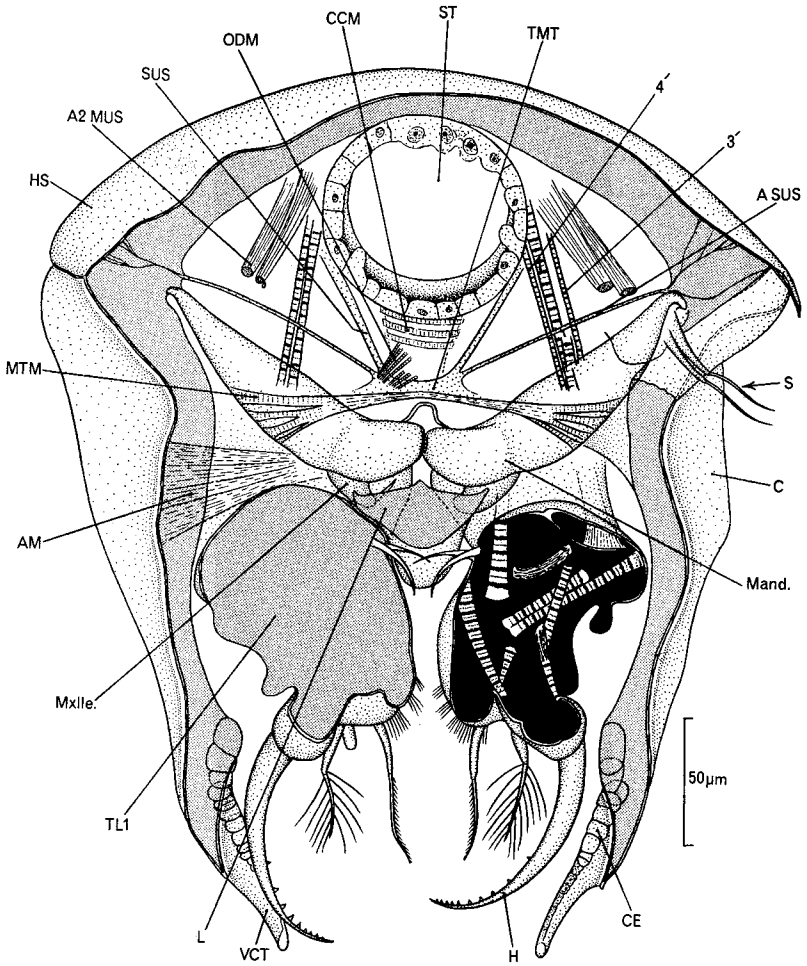

FIGURE 1.4 Transverse section of *Anchistropus emarginatus*. *3′*, promotor roller muscles; *4′*, remotor roller muscles; *AM*, adductor muscle of carapace; *ASUS*, accessory suspensory ligament; *CCM*, circular esophageal constrictor muscles; *CE*, chitinous elaboration within carapace; *HS*, cuticle of head; *MTM*, major transverse muscles of mandibles; *Mxille.*, maxillule; *S*, sensory setae of antenna; *ST*, stomach; *SUS*, suspensory ligament; *TL1*, trunk limb 1; *TMT*, transverse mandibular tendon; *VCT*, ventral carapace tooth. Other abbreviations as in Fig. 1.1. *From Fryer, G., 1968. Evolution and adaptive radiation in the Chydoridae (Crustacea: Cladocera): a study in comparative functional morphology and ecology. Philosophical Transactions of the Royal Society of London, B: Biological Sciences 254, 221–385, Fig. 115 on p. 341.*

among organic and mineral particles and require protection, are supplied with thick chitinous shells, most with sculpturing; this increases the durability of their shells. In pelagic species, the integuments are thin.

The dorsal space under the shell is the brood chamber into which eggs are laid.

The trunk segments are not numerous and the outer structure is rather simplified.

The inner organs are situated within the body rather loosely. The intestine may be straight or convoluted. Muscles do not form compact masses and most of them can be seen individually (Figs. 1.1–1.4). The largest muscles are

longitudinal bands stretching along the gut. Groups of muscles allow motion of the thoracic limbs and antennae. Small muscles rotate the eye and move the labrum and antennules. The intestine is supplied with circular muscles. The ovary (or testis) is paired and situated ventrally along the gut; this is also where the fat body is situated (Fig. 4.13), in contact with the ovaries (Jaeger, 1935).

There are two paired remains of the coelom: the antennal gland and the maxillary gland (shell gland; Fig. 7.1). The latter is the organ of excretion, whereas the antennal gland has no duct and no outer orifice.

The nervous system comprises a double chain of ganglia, with the brain located in the cephalic region. Nerve fibers reach all structures, including remote ones. Sense organs comprise the unpaired eye, the unpaired ocellus (Figs. 1.1, 1.2, and 13.2), sensory papillae situated on the antennule and on some thoracic limbs (Fig. 13.8), and numerous tactile setae. The eye or the ocellus, or both, may be absent in some species.

Each homologous structure in representatives of various genera is a result of morphological radiation, ranging from the ancestral state to enlargement and specialization or to reduction (sometimes complete disappearance) (Smirnov and Kotov, 2009, 2010). On the basis of the general structural scheme, three kinds of specialization are formed: one used for collecting food from substrata (Fig. 1.1), another for filter feeding in open water (Fig. 1.2), and the last, predatory.

Further information on Cladocera may be obtained from www.cladocera-collection.cz, Lampert (2011); Kotov (1913).

1.3 GEOGRAPHIC DISTRIBUTION

Clear intercontinental differences exist in the composition of the Cladocera fauna. More precisely, zoogeographic regions are discerned as formed due to geologic history (see, e.g., Darlington, 1957). In a general way, they are: Palearctic (including North Africa), Nearctic, Oriental (South Asia), Australian (see Smirnov and Timms, 1983; Van Damme et al., 2007a,b), Ethiopian (Africa south of Sahara), and Neotropical regions. The Cape region is also clearly discerned (Smirnov, 2008). There are good reasons (eight endemic Cladocera species) to discern the Baikal region, as made by Starobogatov (1970) with reference to Mollusca.

With reference to ctenopods, Korovchinsky (2004) suggests the Boreal region with the Palearctic and Nearctic subregions, the Mediterranean–Asian region with the Mediterranean–West Asian and East Asian subregions, the Paleotropical region comprising the South Asian and Australasian subregions, the Central American–South American region comprising the Neotropical and Patagonic–Chilean subregions. Peripheral limits of geographic ranges of particular species are mostly unknown.

Some species are very widely distributed, whereas others are restricted to very small geographic areas (e.g., some endemics of the Cape region). Navigation resulted in cases of transcontinental transfer of Cladocera, for example, of *Bythotrephes* from Europe (Lake Ladoga) to North American Great Lakes (Bur et al., 1986). Circumtropical species obviously prefer high temperature, whereas some northern species do not expand their ranges to tropical latitudes.

Obvious differences exist in the intercontinental and latitudinal distribution of Cladocera species. Some species are clearly circumtropical and occur in latitudes where the limiting factor is a high water temperature. High water temperature is sometimes combined with slight salinity. Some species are confined to northern latitudes or occur in the area of minimum winter temperatures. Differences in the geographic ranges, obvious for many Cladocera species, may be confronted with the geochemical or hydrochemical provinces. It seems that little is done in this promising line.

Studies of the Quaternary history of Cladocera communities by skeletal remains in bottom deposits shed light on their state in the past and trends of development (Frey, 1959, 1962; Berglund, 1986; Smol et al., 2001; Smirnov, 2010; Desellas et al., 2011).

1.4 SPECIES-SPECIFIC EFFECT OF XENOBIOTICS

Different sensitivity to toxic substances was reported for different Cladocera species. Immobilization by copper tested for 44 species was different: EC_{50} (effective concentration determined in 48 h) was from 5.3 for *Scapholeberis mucronata* to 70.6 μg Cu/L for *Disparalona rostrata* (Bossuyt and Janssen, 2005). Comparative tests of Cd and Zn on *Daphnia magna*, *Daphnia pulex*, *Daphnia ambigua*, and *Ceriodaphnia dubia* demonstrated that *D. magna* is significantly more tolerant to these metals, or their combinations, than other daphnids (Shaw et al., 2006).

It was shown that *Moina macrocopa* was twice more sensitive than *D. magna* in 7-day toxicity test to perfluorooctane, sulfonic acid, and perfluorooctanoic acid (Ji et al., 2008). The highest sensitivity to the same concentrations of carbaryl and methomyl (carbamate insecticides) was manifested by *Ceriodaphnia reticulata*, the lowest—by *M. macrocopa* and *Scaphleberis kingi* (Mano et al., 2010). *D. magna* is more severely affected than *D. pulex* by diflubenzuron (Duchet et al., 2011). Sensitivity to insecticides imidacloprid and fipronil was different (in descending order): in *Ceriodaphnia*, *Moina*, and *Daphnia* (Hayasak et al., 2012).

D. magna was less sensitive than *Daphnia curvirostris* to veterinary antibacterials (Bona et al., 2014).

Mechanisms of reaction to toxic blue-green algae were found to be different in *D. magna* and *D. pulex* (Asselman et al., 2014).

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CHAPTER

2

Methods

Methods of investigating the physiology of Cladocera range from direct observation of living specimens to recording the physical and chemical manifestations of particular physiological processes.

2.1 METHODS OF COLLECTION

Cladocera may be collected with planktonic nets and dip nets in the littoral and pelagic zones of large and small water bodies. They should be looked for in ponds, pools, puddles, temporary pools, roadside ditches, acid bogs, fountains, all kinds of artificial basins, moist moss, and even in moist, moss-like growth on tree trunks. Usually, a catch of a planktonic net contains many specimens belonging to several genera. However, sometimes Cladocera may be absent or rare. The latter situation occurred, for example, in water bodies of the Yucatan; though the faunal list was rather long, large volumes of water had to be screened to collect a few specimens (Smirnov and Elias-Gutierrez, 2011). Quantitative sampling (per unit volume or unit surface) with special devices is used in limnology.

2.2 OBSERVATION OF LIVING SPECIMENS

Detailed examination of preserved or living specimens supplies useful information on their anatomical background and function, as well as on the composition of food in the intestine. Intravital staining aids observation of functioning in living cladocerans. Various kinds of intravital staining of the organ systems of Cladocera were originally elaborated by Fischel (1908). The salivary gland can be stained using neutral red and Bismarck brown (Cannon, 1922). In thoracic limb IV of *Eurycercus*, there is a slime gland the secretions of which are stained bright blue with Mallory's stain (Fryer, 1962, 1963). Gut contents have been stained with eosin, Congo red, methyl red, neutral red, and uranine for the determination of pH (Lavrentjeva and Beim, 1978). External slime may be distinguished by placing a cladoceran in diluted Indian ink. Histochemical techniques have been used in the analysis of *Holopedium* slime (Brown, 1970).

2.3 CULTIVATION

Cultures started from a single female are called clonal cultures and provide relatively uniform material. Culture methods, mostly for *Daphnia* and *Moina*, have been described by various authors either alone (e.g., Biotechnics of *Daphnia* Culture at Fish Farms, 1958; Dewey and Parker, 1964; Ivleva, 1969; Parker and Dewey, 1969; Bogatova, 1973, 1980; Lampert, 1975; Ten Berge, 1978; Goulden et al., 1982; Dodson et al., 1991) or in descriptions of particular

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experiments. Cultured pelagic Cladocera should be fed with algae and adequate algal cultures should be maintained for this purpose. Some species (*Daphnia magna* and *Daphina pulex*) are more easily cultivated than others.

Gajewskaja (1940, 1948) suggested a method of separating large-scale cultures of Cladocera and algae because cultivation of Cladocera and algae requires conflicting conditions. Since then, methods of large-scale culture have been developed further (Yalynskaya, 1961; Ivleva, 1969; Lampert, 1975; Bogatova, 1992). The combined culture of *Daphnia* with other cladocerans was suggested by Bogatova (1963).

A method was described for cultivation of *Pleuroxus hamulatus* individually in small vessels, frequently supplied with fresh food (Galtsoff et al., 1937, p. 220). A similar method was used for littoral Cladocera by Smirnov (1964, 1965a). Fresh detritus was given at least every other day (actually, a specimen was transferred to a dish containing fresh detritus). Organic debris (detritus) was collected at shore bottoms and screened to remove foreign animals.

Artificial detritus prepared from plants was also used in cultivation of Cladocera (see, e.g., Rodina, 1963; Esipova, 1969, 1971; Dekker et al., 2006). Sterilization was used to demonstrate the role of bacteria in the alimentation of Cladocera (Gajewskaja, 1938; Rodina, 1963; Esipova, 1969, 1971). The latter author used a diluted Lugol's solution to minimize the quantity of bacteria in food offered to Cladocera.

Suspensions of latex beads have also been used in investigations of feeding behavior (Burns, 1968b, 1969; Hessen, 1985).

Preferences for environmental factors, and for food, may be ascertained by experiments on living specimens. Examination of food composition is made easier by dissolving the soft tissues of cladocerans with 3% sodium hypochlorite (Infante, 1978). This treatment may also be useful for other purposes.

2.4 IMMOBILIZATION AND ATTACHING

Most cladocerans are very agile; therefore, high-speed photography has been used (e.g., Storch, 1929; Zaret and Kerfoot, 1980) to study them. Alternatively, methods to inhibit their movement have been devised. To retard quick motion, Fryer (1968) immersed living cladocerans in an nontoxic viscid medium ("cellulose nitrate"). Immobilization by narcotization is discussed in more detail in Section 12.5. Early techniques included attaching specimens to a substrate on a glass slide using wax dissolved in alcohol (Scourfield, 1900b; Peñalva-Arana et al., 2007). Porter and Orcutt (1980) used silicone grease to fix *D. magna* by its head shield to observe its feeding. For the purposes of his study, Jacobs (1980) attached *Daphnia* by the caudal spine to a plasticine bed. Specimens thus immobilized could be observed and the next instar was released into free water from the attached exuvium.

Using cyanoacrylate glue, Onbé (2002) attached *Pseudevadne* and *Evadne* to the tip of a glass capillary held in place by a stand to facilitate video recording. Peñalva-Arana et al. (2007) reported unbiased observations using computer recording combined with the immobilization system.

Methods of attachment were recently described by Seidl et al. (2002) and used by Pirow et al. (2004) for investigating oxygen transport processes in *D. magna*. The latter authors immobilized fasting animals by gluing their posterior apical spine with histoacryl to a bristle, which was then fixed to a coverslip with plastilin. Dye was then microinjected into the circulatory system from the dorsal side into the space "directly downstream of the heart." The coverslip then formed the base of a thermostated perfusion chamber in which an immobilized daphnid was able to freely move its antennae.

Ivanova and Klekowski (1972) achieved immobilization of *Simocephalus* by placing it into the bulb of a Cartesian diver (used for determining oxygen consumption) and leaving no free space around the animal, that is, it was too small to allow movement. Photographic recording of the heart rate and movement of thoracic limbs has been used (e.g., Kolupaev, 1988). Further on, computer recording of behavior is now used (Peñalva-Arana et al., 2007).

2.5 MICROSCOPY

Various kinds of microscopy, including scanning electron microscopy (SEM), can be used in investigations of Cladocera (Kotov, 2013). A variation of cladoceran preparation for SEM was suggested by Laforsch and Tollrian (2000). Modern video microscopy and digital image processing methods take advantage of the transparency of cladocerans (Colmorgen and Paul, 1995). Fluorescence analysis was originally used by Pravda (1950).

Surgical methods can be applied in investigations of regeneration, vision, and neurosecretion. These are described by Ermakov (1927), Angel (1967), and in Chapters 10 and 13.

2.6 BIOCHEMICAL AND SPECIAL PHYSIOLOGICAL METHODS

In biochemical and metabolic investigations, special procedures, such as homogenization (e.g., Guan and Wang, 2004b) and radiotracer or chemical methods, have been used. There are various respirometric methods (Fig. 2.1. See also Chapter 5). Studies on homogenates using modern sensitive methods have opened up the possibility of investigating metabolic pathways. Special techniques may be found in descriptions of the original investigations.

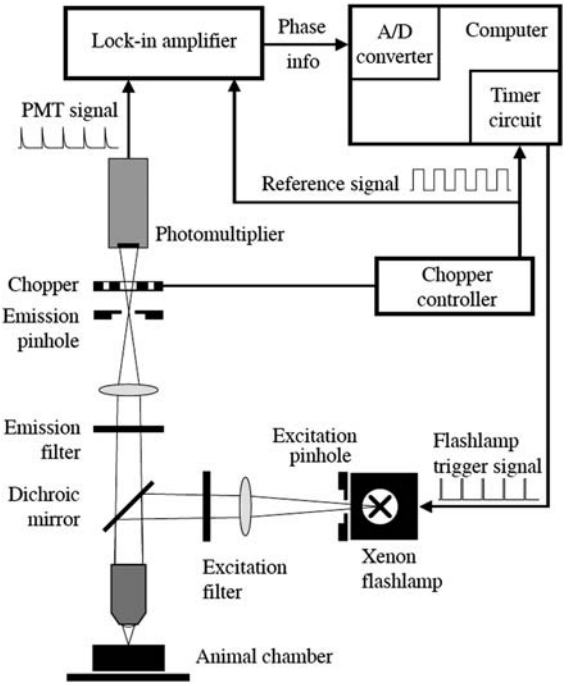

FIGURE 2.1 The microscopic setup for partial pressure of oxygen (pO_2) imaging. *A/D*, analog/digital; *PMT*, photomultiplier tube. *From Pirow, R., Wollinger, F., Paul, R.J., 1999a. The importance of the feeding current for oxygen uptake in the water flea Daphnia magna. Journal of Experimental Biology 202 (5), 553–562, Fig. 2.1 on p. 555.*

Spectrophotometry has been used for the identification of particular compounds, including hemoglobin (Hb) (Karnaukhov et al., 1986). Initially, Fox (1948) suggested that a quantitative estimation of Hb content in *Daphnia* in arbitrary units could be obtained against a wedge-shaped standard prepared from the worker's blood. Following this, cladoceran Hb was investigated using spectral (Hildemann and Keighley, 1955; Hoshi et al., 1968) and chemical (Hoshi, 1963a, 1963b; Smirnov, 1970) methods.

Recent toxicological studies have assessed the effects of xenobiotics on particular physiological processes.

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CHAPTER

3

Chemical Composition

3.1 LABILITY OF CHEMICAL COMPOSITION

The chemical composition of the Cladocera is labile. In their body, relative quantities of physiologically important constituents, those of no such importance, or of xenobiotics vary depending on the composition of the environment and of food. Cladocera may accumulate useless or toxic substances. Furthermore, the chemical composition fluctuates in the course of the molting cycle.

3.2 MOISTURE CONTENT AND CALORIFIC VALUE

The moisture content and calorific value of some Cladocera spp. are shown in Table 3.1. Moisture content is usually within 80–90% and calorific value within 3–6 kcal/g dry weight (DW). According to Sushchenya et al. (1990), the dry matter content in *Daphnia magna* ranges from 7.4% to 10.6%, increasing at higher temperatures and higher food concentrations.

The calorific value (kcal/g) is: *Daphnia hyalina*, 6.3; *Bosmina coregoni*, 6.3; *Chydorus sphaericus*, 6.1; and *Leptodora kindtii*, 5.8 (Vijverberg and Frank, 1976). Variations in the calorific value obviously depend mostly on the fat content. Thus, the calorific value of "lean" *D. magna* is only 60% of that of *Daphnia* containing more fat (Chalikov, 1951).

The caloric content also changes in cladocerans exposed to xenobiotics. Thus, it somewhat decreased in *Daphnia schodleri* exposed to hexavalent chromium (as potassium dichromate) due to restructuring of its chemical composition (Arzate-Cárdenas and Martínez-Jerónimo, 2012).

3.3 PRINCIPAL CONSTITUENTS

There have been various determinations of the chemical composition of some Cladocera (daphnids, *Moina*, *Bosmina*, and *Chydorus sphaericus*). The general chemical composition of some Cladocera is shown in Table 3.2. The protein content ranges from 30% upward, the fat content is 1–20%, and the carbohydrate content is 10–30%. Far more informative are reports on the dynamics of chemical composition, which depend on seasonal changes in a number of factors, on starvation, or on other particular factors. Often, scattering of the data indicates dependence on specific factors. Indeed, if arranged by season, chemical constituents demonstrate clear composition changes, as shown, e.g., for *Daphnia pulicaria* (in Fig. 3.2, from Heisig-Gunkel and Gunkel, 1982). The chemical composition of *Daphnia pulex* generally confirms these data but depends on the period of starvation, with relative quantities of carbohydrate and fat decreasing, and those of protein and ash increasing (Fig. 3.1) (Lemcke and Lampert,

http://dx.doi.org/10.1016/B978-0-12-805194-8.00003-9

TABLE 3.1 Moisture Contents and Calorific Values of Some Cladocera spp.

Species	Moisture Content (%)	Calorific Value (kcal/g DW)	Ash (% DW)	References
Bosmina longirostris	89	6.5	–	Sherstyuk (1971)
Bosmina longispina	–	9.9	4.8	Romanova and Bondarenko (1984)
Bythotrephes longimanus	84.7	8.6	5.2	Romanova and Bondarenko (1984)
Ceriodaphniaaffinis, adults	89.2	–	–	Stepanova (1967)
Ceriodaphnia affinis, juveniles	90.4	–	–	Stepanova (1967)
Ceriodaphnia pulchella	70–82	4–4.7	–	Sherstyuk (1971)
Ceriodaphnia quadrangula	–	4.9	4.6	Riccardi and Mangoni (1999)
Ceriodaphnia reticulata	87–96	2.4–5.5	7.2–23	Bobiatyńska-Kwok (1970) and Bogatova et al. (1971)
Daphnia cucullata	–	5.1–5.4	10.6–14.0	Riccardi and Mangoni (1999)
Daphnia hyalina	–	4.9–5.0	12.0–12.5	Riccardi and Mangoni (1999)
Daphnia longispina	82.6	4	22	Romanova and Bondarenko (1984)
Daphnia magna	86.4–95.6	2.4–5.6	–	Karzinkin (1951), Ostapenya et al. (1968), Schindler (1968), Bogatova et al. (1971), Stepanova (1968), Stepanova et al. (1971), Mityanina (1980) and Sushchenya et al. (1990)
Daphnia pulex	89–95	2.8–4.9	7.6–25	Birge and Juday (1922), Ivlev (1939), Karzinkin (1951), Malikova (1953), Ostapenya et al. (1968) and Stepanova (1974)
Bythotrephes longimanus	–	5.2	5.6	Riccardi and Mangoni (1999)
Leptodora kindtii	97.2	4.6	–	Sherstyuk (1971)
Moina macrocopa	95	5	–	Bogatova et al. (1971)
Moina spp.	87–88	4–4.3	–	Ostapenya et al. (1968), Stepanova (1974), and Zhao et al. (2006a,b)
Polyphemus pediculus	80–86	4.4–4.9	–	Sherstyuk (1971)
Sida crystallina	84	5.3	–	Sherstyuk (1971)
Simocephalus vetulus	82–92.4	3.65–4	–	Sherstyuk (1971), Stepanova (1968), and Stepanova et al. (1971)
Eurycercus lamellatus	80–88	4.1–4.3	–	Sherstyuk (1971)

TABLE 3.2 Composition of Some Cladocera spp. in % DW

Species	Carbon	Hydrogen	Nitrogen	Carbohydrate	Protein	Lipid	Author
Ceriodaphnia quadrangula	49.1	7	9.8	–	54.4	5.3	Riccardi and Mangoni (1999)
Daphnia cucullata	53.3–54.5	7.8–8	10.1	14.6	57.0–62.8	19.8–22.7	
Daphnia hyalina	50.2–52.3	7.6	11	21.3–22.9	62.2–64.0	13.1–16.5	
Daphnia pulex	–	–	–	3.3–10.9	36.4–61.7	2.8–27.9	Birge and Juday (1922)
D. pulex	–	–	–	1.13	60.4	21.8	Malikova (1953)
D. pulex	–	–	–	6.4	63.4	8.6	Stepanova (1968)
Moina brachiata	–	–	–	8.2	63.4	17.5	
Simocephalus vetulus	–	–	–	16.7	52.3	11.5	
Bythotrephes longimanus	51.2	7.6	11.1	22.0	63.9	14.1	Riccardi and Mangoni (1999)

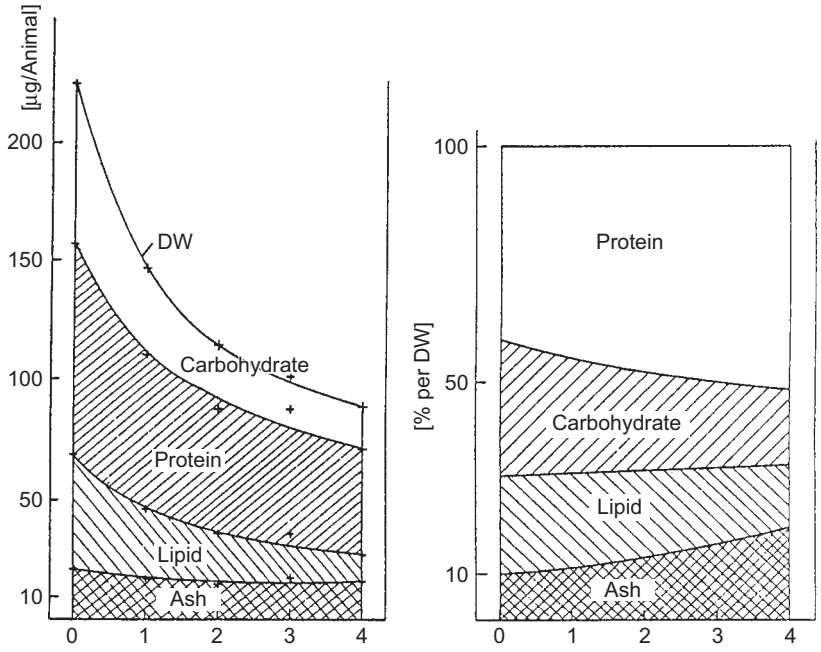


FIGURE 3.1 Chemical composition of *Daphnia pulex* and changes caused by starvation (left, per animal; right, % DW). Horizontal axis, days of starvation. *From Lemcke, H.W., Lampert, W., 1975. Changes in weight and chemical composition of* Daphnia pulex *during starvation. Archiv. für Hydrobiologie 48 (1), 108–137.*

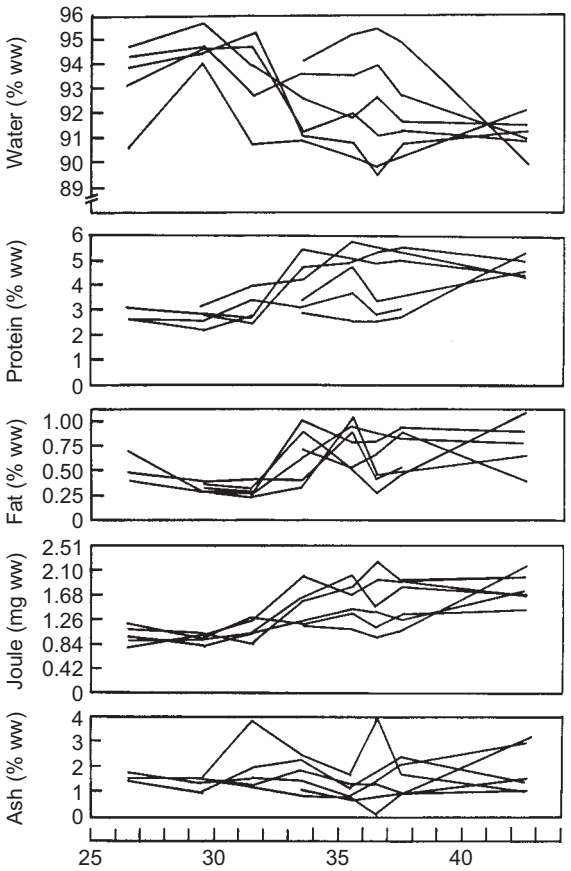


FIGURE 3.2 Seasonal changes of chemical composition of *Daphnia pulicaria* in six ponds, beginning from June. *From Heisig-Gunkel, G., Gunkel, G., 1982. Distribution of a herbicide (atrazin, s-triazine) in* Daphnia pulicaria*: a new approach to determination. Archiv für Hydrobiologie, Supplement 59 (4), 359–376.*

1975). Seasonal variations in the biochemical composition of *D. magna* (in Luxembourg) were studied by Cauchie et al. (1999). The variation (in mg/g DW) was: protein from c. 140 to 400; lipids, 120–180; chitin, 40–70; carotenoids, 70–230; and ash, 100–340.

To understand these variations, data on the dynamics of the chemical constituents in relation to growth are also useful. McKee and Knowles (1987) studied the levels of protein, glycogen, lipid, ribonucleic acid (RNA), and deoxyribonucleic acid (DNA) during the growth of *D. magna*. They found that the protein content (percentage DW from the first to the 21st day of life) varied within the range of 48–62% (maximal on day 8); glycogen content steadily increased from 2.4% to 7.5%; lipid content varied from c. 18% to c. 15%, decreasing to 6.4% on day 21. In addition, RNA content varied from 8.6% to 6.6%, decreasing to 4% on day 21, and DNA content generally decreased from 0.20% to 0.14%.

The chemical composition of *D. magna* is much influenced by the chemical composition of its food (Stepanova et al., 1971). Thus, the highest content of protein, fat, and carbohydrate was found in *Daphnia* fed on yeast.

3.3.1 Protein

The protein content of *Daphnia* and *Ceriodaphnia* increases with increased protein in their natural food (Guisande et al., 1991). It has also been shown that during culture the protein and lipid contents of *Moina macrocopa* decreased in comparison with those of the initial culture, from about 25 to 0.56 mg/g WW to about 18 and 0.26–0.33 mg/g WW, respectively (Romanenko et al., 2004).

The protein content was shown to decrease during chronic exposure of *D. magna* to chlordecone (an organochloride insecticide) (McKee and Knowles, 1986).

Amino Acids. The amino acid content of various Cladocera is shown in Table 3.3. According to Malikova (1953, 1956), the content of amino acids in *D. pulex* is (% of total protein): tyrosine 4.27, tryptophan 3.62, arginine 10.92, histidine 2.69, cystine 1.17, methionine 3.45. The amino acid composition of *D. magna*, *Ceriodaphnia reticulata*, and *Chydorus sphaericus* was determined by Sadykhov et al. (1975).

In the carotenoprotein complexes, the following predominant amino acids were found:

TABLE 3.3 The Content of Different Amino Acids in Some Cladocera spp.

Amino Acid	*Bosmina longirostris* (% protein) (Verbitsky, 1990)	*Daphnia magna* (mg/100 g WW) (Stepanova and Naberezhnyi, 1972)	*Daphnia pulex* (% total amino acids per DW) (Dabrowski and Rusiecki, 1983)	*Daphniopsis tibetana*, (g/100 g protein) (Zhao et al., 2006a)	*Ceriodaphnia* spp. (% total amino acids per DW) (Dabrowski and Rusiecki, 1983)	*Simocephalus vetulus* (mg/100 g WW) (Stepanova and Naberezhnyi, 1972)	*Moina mongolica* (g/100 g protein) (Zhao et al., 2006a,b)
Phenylalanine	1.3	228–833	3.76	1.79	3.82	107–157	3.40
Tyrosine	3.1	–	4.05	3.55	3.81	60	2.80
Leucine	3.3	222–725	3.74	5.76	4.61	425–625	5.30
Isoleucine	2.1	–	2.24	3.68	2.43	–	3.40
Methionine	–	–	1.44	3.64	1.40	–	1.50
Valine	2.9	–	3.71	3.78	3.49	–	3.90
Alanine	2.8	157–438	3.95	6.72	4.57	283–342	4.30
Glycine	2.6	194–239	2.85	3.91	2.69	236–296	3.20
Proline	2.2	211–358	2.53	4.53	2.76	100–250	2.70
Glutamic acid	5.0	317–458	5.46	7.40	6.29	522–619	8.00
Serine	1.8	205–357	2.57	3.27	3.07	255–285	3.00
Threonine	2.6	121–279	2.93	4.00	2.99	121–164	3.20
Aspartic acid	3.2	365–474	5.22	7.46	5.94	318–371	6.40
Arginine	1.9	200–401	3.40	2.85	3.45	244–348	4.30
Histidine	1.5	–	1.25	1.64	1.46	–	1.20
Lysine	3.8	–	3.78	4.78	4.36	–	3.40
Cystine	0.7	275–423	0.71	0.49	–	100–127	0.80
Tryptophan	–	–	–	0.43	–	–	1.20

in *D. magna*, alanine, glutamine, glycine, and leucine (Czeczuga, 1984); in *Moina micrura*, asparagine, glutamine, and glycine (Velu et al., 2003). The presence of free intracellular amino acids in *D. magna* was shown by Gardner and Miller (1981).

It was determined that in *Daphnia*, the amino acid composition during ontogeny is rather constant (Brucet et al., 2005). However, it may vary rather widely depending on the culture conditions, as has been shown for *D. magna* (Stepanova and Naberezhnyi, 1970, 1972) and *Moina* spp. (Kokova, 1982). In well-fed *Daphnia*, the content of amino acids is 6.29 g% WW (i.e., 6.29 g/100 g of wet weight), whereas in "lean" *Daphnia* it is 3.12–3.35 g% WW.

Amines. The following amines are found in *D. magna* (Ehrenström and Berglind, 1988):3,4-dihydroxyphenylalanine, dopamine, noradrenaline, adrenaline, tyramine, epinine, 3-methoxytyramine, 3,4-dihydroxyphenylacetic acid, L-tryptophan, 5-hydroxytryprophan, 5-hydroxytryptamine, and 5-hydroxyindolacetic acid. Diurnal variations of the contents are recorded for 3,4-dihydroxyphenylalanine, dopamine, and 3,4-dihydroxyphenylacetic acid (the catechols). The variations are recorded in *Daphnia* exposed to 12 h of continuous illumination.

3.3.2 Carbohydrates

Carbohydrates comprise glycogen and chitin.

Glycogen. Glycogen is a polysaccharide consisting of glucose bound to protein. Most likely, Smith (1915) was the first to demonstrate distribution of glycogen stained with neutral red over the gut and thoracic limbs of *Daphnia* females, in contrast to fat (Fig. 3.4). The content of glycogen was determined by Blazka (1966) (% of total composition, WW): 23% in *Bosmina longirostris*, 53% in *Ceriodaphnia reticulata*, 1–36% in *Daphnia* spp., and 33% in *Simocephalus* sp. Glycogen is present in developing embryos. The glycogen content in *Simocephalus vetulus*

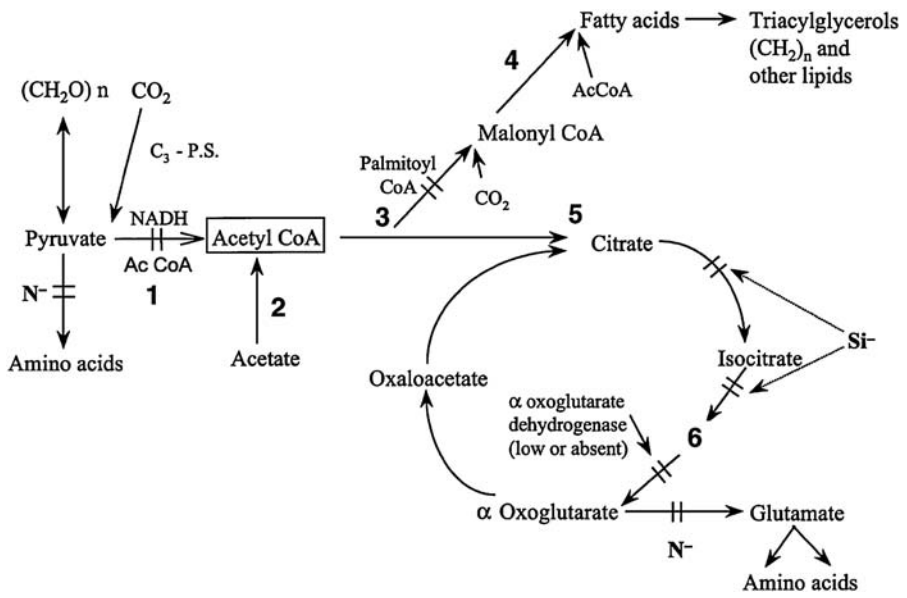


FIGURE 3.3 Schematic pathway from initial photosynthetic saccharides to lipids and amino acids in algae. C_3-*P.S.*, photosynthesis. *From Arts, M.T., Robarts, R.D., Evans, M.S., 1997. Seasonal changes in particulate and dissolved lipids in a eutrophic prairie lake. Freshwater Biology 38 (3), 525–537.*

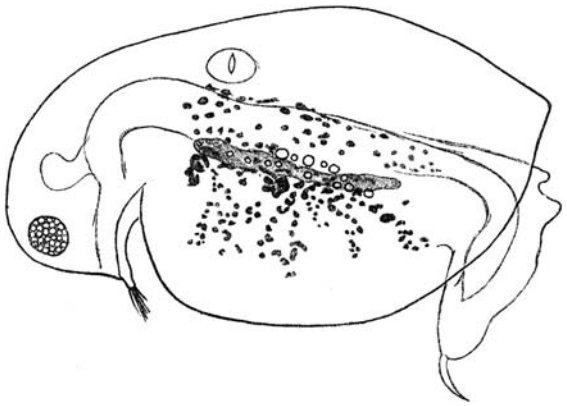


FIGURE 3.4 Glycogen in the body of *Daphnia* stained red (shown as dark patches). Fat globules are shown as circles. *From Smith, G., 1915. The life-cycle of Cladocera, with remarks on the physiology of growth and reproduction in Crustacea. Proceedings of the Royal Society of London B 88, 418–435, Fig. 3.5 on p. 425.*

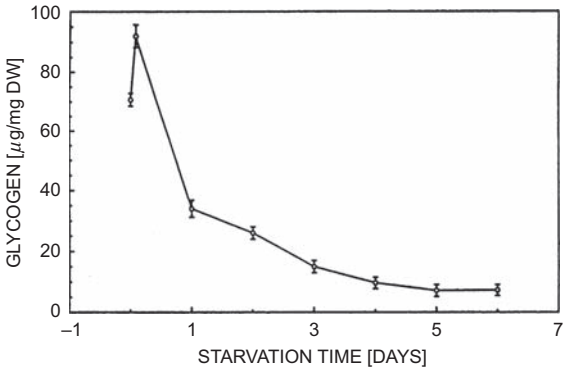


FIGURE 3.5 Change of the glycogen content of *D. magna* during starvation. *From Elendt, B.P., 1989. Effects of starvation on growth, reproduction, survival and biochemical composition of* Daphnia magna. *Archiv für Hydrobiologue 116 (4), 415–433, Fig. 3.7a on p. 423.*

was determined to be 0.7% in the gastrula, 0.91% in the nauplius, 0.71% in released young, 1.2% in the third instar, and 2.23% in the fifth instar (Hoshi, 1953).

In *D. magna* exposed to tetradifon at 0.44 mg/L during 120 h the content of glycogen was 0.24 µg/individual versus 0.64 in the control (Villarroel et al., 2009).

Chitin. Chitin is a major structural component of arthropods. The polysaccharide chitin is a polyacetylglucosamine [β-(1-4)-linked homopolymer of *N*-acetyl-D-glucosamine]. The acetamide group CH_3CONH is present in the chitin molecule. The chitinous covers of cladocerans are strong and nonwettable. In Cladocera, like in other Crustacea (Hohnke and Scheer, 1970), chitin is formed and secreted by the hypodermis underlying the shell. Secretion of chitin in the wound healing was studied by Anderson and Brown (1930) who found that chitin secretion starts 60% of the way through the intermolt period.

In resting eggs of *Ceriodaphnia quadrangula* 16–17% DW of chitin and 11% DW of chitosan are found (Kaya et al., 2014). Chitosan is a derivative of a linear polysaccharide, the macromolecules of which consist of randomly bound β-(1–4)-D-glucosamine units and *N*-acetyl-D-glucosamine.

Cladocera, being abundant in nature, produce enormous quantities of chitin. The content of chitin in the body of *Daphnia* is approximately 15% (Chalikov, 1951) or 7% DW (Andersen and Hessen, 1991). In *D. magna*, it is 2.9–7% DW (Cauchie et al., 1995) or c. 30–70 mg/g DW (Cauchie et al., 1999). The total annual chitin production by various cladocerans in Europe is estimated as: *D. magna*, 11.5 g/m^2 (4.6 g/m^3); *Daphnia galeata*, 3.2 g/m^2 (0.16 g/m^3), *Daphnia hyalina* and *Daphnia cucullata* combined, 0.14–0.30 g/m^2 (0.09–0.2 g/m^3) (Cauchie et al., 1995).

In contrast to chitin from copepods, chitin from dead Cladocera is not decomposed in bottom sediments (or at least is not fully decomposed). After their death, it accumulates at the bottom of water bodies, sometimes forming a high proportion of the total mass of the sediment. Bottom sediment with dominant chitinous remains was termed *chitin gyttja* by Lundqvist (1927).

Chitin is not derived directly from food: it must be formed by the process of metabolism. Although chitin is specific to arthropods and abundantly accumulated by cladocerans, the metabolic pathways of chitin formation were not clearly described. For insects, Kuznetsov (1948, p. 336) noted that "there are no actual data on metabolism yielding chitin from the cycle of transformations; even more, there are no data on the metabolism intermediate as to chitin." It was noted by Hackman (1964, p. 499) that "the biological synthesis of chitin in insects (or other animals and plants) has received little attention."

In other crustaceans, chitin is formed from glucosamine chiefly derived from glycogen, with the acetyl group furnished by oxidation of fatty acids (Vonk, 1950; Hohnke and Scheer, 1970). The latter authors suggested a general scheme of crustacean carbohydrate metabolism yielding, inter alia, chitin (Fig. 3.6).

An inhibitor of chitin synthesis, larvicide diflubenzuron is highly toxic for *D. magna*, Median Effective Concentration at 48 h exposure is 0.06 μg/L (Abe et al., 2014).

3.3.3 Mucopolysaccharide (Slime)

Slime may abundantly surround the animal (as is the case with common forms of *Holopedium*) or make a thin layer over the body of a cladoceran (except antennae) (as in *Sida* and *Ophryoxus gracilis*) (Montvillo et al., 1987). The chemical composition of slime was determined for the jelly capsule of *Holopedium* (Brown, 1970). Sulfated mucopolysaccharide was found to be present, as well as mucopolysaccharide modified by carboxyl groups. It has been suggested that the jelly capsule is produced by the mechanism that produces the exoskeleton at each molt.

Slime is also produced by salivary glands situated within the labrum (Fig. 4.1) and stored in

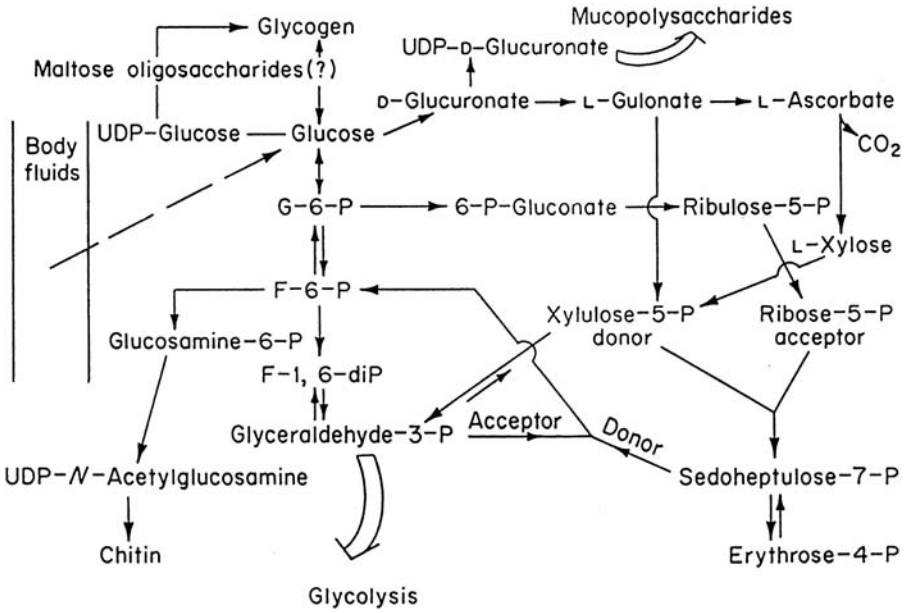


FIGURE 3.6 Carbohydrate metabolism in Crustacea. *UDP*, uridine diphosphate. *From Hohnke, L., Scheer, B.T., 1970. Carbohydrate metabolism in Crustacea. Chemical Zoology, vol. V. Arthropoda, Part A. Academic Press, New York, London, pp. 147–197.*

paired reservoirs letting the secretion into the lumen of the esophagus. Slime glands are found also in some thoracic limbs (Fig. 4.2), as has been shown for *Eurycercus* (Fryer, 1962, 1963) and for *Alonopsis elongata* (Fryer, 1968), but not yet for other cladocerans. The salivary glands may be shown by intravital staining with neutral red or Bismarck brown (Cannon, 1922).

3.3.4 Phosphorus-Containing Substances

The content of phosphorus in *D. pulex* was determined as 1.49% DW (Malikova, 1953). In *D. galeata*, 35–69% of the total phosphorus content is associated with nucleic acids (Andersen and Hessen, 1991; Vrede et al., 1999). Nucleic acids (RNA, DNA) are P-containing high molecular weight compounds (polynucleotides). The RNA content was determined to be c. 2–6% DW in *Daphnia* spp. and the phosphorus content to be c. 0.7–1.5% DW (Kyle et al., 2006). The nucleic acid content comprises 4.7–5.2% of the DW of *Moina* spp. (Kokova, 1982), below 3% in *Alona affinis* (Bullejos et al., 2014). In *D. magna*, RNA content varied from 8.6% to 6.6% DW, decreasing to 4% on day 21 of its growth; and DNA content generally decreased from 0.20% to 0.14% (McKee and Knowles, 1987).

Ribosomal DNA contains a significant fraction (c. 49%) of total body phosphorus.

(Acharya et al., 2004). The growth of Cladocera involves the requirement for a greater amount of ribosomal RNA, and especially of phosphorus (Main et al., 1997).

In juvenile *D. pulex*, an elevated DNA content was measured in the postmolt period, followed by an increase in RNA during the intermolt and premolt periods (Gorokhova and Kyle, 2002). It was found that the ratio of RNA:DNA in *D. galeata* increased in response to an increase of P:C ratio in its food (Vrede et al., 2002). Use of this ratio has been suggested (Markowska et al., 2011) for evaluating the condition of *D. pulex*, assuming that higher values correspond to a "good" condition. Specimens with a lower RNA:DNA ratio have lower metabolic rates and greater longevity. The RNA:DNA ratio is more than 8 times higher in Cladocera than in Copepoda (Bullejos et al., 2014).

Accumulation of messenger RNA (mRNA) products in daphnids was the highest under induction by piperonyl butoxide, chlordine, 4-nonyphenol, Cd, chloroform (Hannas et al., 2011). It was also found that presence of dissolved humic substances in the environment caused methylation of DNA in *D. magna* and *M. macrocopa* (Menzel et al., 2012).

3.3.5 Introductory Remarks About Lipids

Lipids are a major and complex group of organic compounds controlling biological processes at cellular, tissue, organismal, and cenotic levels. Recent investigations have supplied abundant new information on lipids in freshwater organisms [Lipids in Aquatic Ecosystems (2009)].

Oil droplets are often clearly seen in the bodies of cladocerans. According to Jordão et al. (2015) the core of a such droplet consists of neutral lipids (triacylglycerols and cholesterylesters), it is surrounded by a monolayer of phospholipid and cholesterol with associated specific proteins.

It is notable that the fat content is especially variable. Up to 17.5% DW was measured in *Moina rectirostris* (syn. *Moina brachiata*) (Stepanova and Vinogradova, 1970), 17–22% in *D. magna*, 10–40% in *D. pulex*, 10–19 in *Bythotrephes cederstroemi* (Bilkovic and Lehman, 1997). Variations in the content of lipids in Cladocera are shown in Figs. 3.1 and 3.5. It was found that the content of triacylglycerols decreased with growth in immature *D. magna* (Bychek and Gushchina, 1999). The fatty acid composition of Cladocera spp. is indicated in Tables 3.4–3.8; that of *Ceriodaphnia quadrangula* was reported by Farhadian et al. (2012), of

TABLE 3.4 The Total Content of Lipid and Percentage Lipid Components in Some Cladocera spp.

Species	Total Lipid (%DW)	Phospholipid (%)	Triglyceride (%)	Cholesterol (%)	Cholesterol Esters (%)
Bosmina obtusirostris	30.6	70	13	3	12.8
Holopedium gibberum	52.4	57	8.7	18.8	17
By. cederstroemi	16.4	55	11.3	5	11.3

DW, dry weight.
From Lizenko, E.I., Bushman, L.G., Nefedova, Z.A., 1977. Content of lipids in plankton of some Karelian lakes. Gidrobiologicheskii Zhurnal 13 (3), 74–80.

TABLE 3.5 Content of Fatty Acids (as % of Total Fatty Acid Content)

Fatty Acids	*Daphnia* spp.	*Daphniopsis*	*Bosmina*	*Holopedium*	*Leptodora*	*Bythotrephes*
SATURATED						
C12:0	0.7–2.3		–	0.8	0.1–2.1	0.4
C14:0	4–10	2.92	8.9	12.0	2.2–7.4	4.4
C15:0	0.6–3	0.99	1.1	0.9	0.6–2.8	0.9
C16:0	21–36	11.4			24–36	22–26
C17:0	20.6	0.64	17.3	15.4		19.1
C18:0	0.3–9.9	2.57	–	–	6.5–17.6	6.7–9
MONOUNSATURATED						
C16:1ω7	6.7	5.81	4.1	3.5		2.9
C18:1ω6 + 18:1ω9	9.2		12.3	8.1		10.3
C18:1ω7	4.1		5.0	8.1		6.0
C20:1ω9	–		1.2	2.3		–
POLYUNSATURATED						
C18:3ω3	6.8	26.5	7.6	6.7		5.3
C18:4ω3	11.8		10.4	11.0		4.1
C20:5ω3	11.8	1.41	14.4	17.5		23.0
C22:6ω3	0.9		2.6	2.1		2.1
C18:2ω6	4.6		5.3	4.1		3.6
C18:3ω6	1.6		0.6	0.8		0.6
C20:4ω6	3.5	0.63	4.7	6.8		9.3

–, not detected.
Daphnia *spp.*, Bythotrephes longimanus *from Bychek, E.A, Guschina I.A., 2001. The transfer of fatty acids in a freshwater planktonic foodweb of the Kuibyshevskoe Reservoir (middle reaches of the Volga). Hydrobiologia 442, 261–268;* Daphniopsis tibetana *from Zhao, W., Huo, Y.-Z., Gao, J, 2006. Analysis and appraisement of nut rient compositions for* Daphniopsis tibetana *Sars. Journal of Fishery Sciences of China 13 (3), 446–451;* Leptodora kindtii *from Bychek, E.A, Guschina I.A., 2001. The transfer of fatty acids in a freshwater planktonic foodweb of the Kuibyshevskoe Reservoir (middle reaches of the Volga). Hydrobiologia 442, 261–268;* Bosmina coregoni *and* Holopedium gibberum *from Bychek, E.A, Guschina I.A., 2001. The transfer of fatty acids in a freshwater planktonic foodweb of the Kuibyshevskoe Reservoir (middle reaches of the Volga). Hydrobiologia 442, 261–268.*

TABLE 3.6 Content of Fatty Acids (% DW)

Fatty Acids	*Daphnia cucullata*	*Daphnialongispina*	*Bosmina longirostris*	*Simocephalus vetulus*
C14:0	7.0	2.5	2.5	3.5
C14:1	0.8	3.0	2.3	3.8
C14:2	0.1	0.8	2.8	1.4
C16:0	16.4	11.7	14.0	12.8
C16:1	5.7	14.6	10.3	12.1
C16:2		0.8		
C18:0	6.8	3.7	7.4	5.1
C18:1	8.3	10.1	19.3	11.8
C18:2	15.5	4.2	4.2	6.0
C18:3	8.2	11.3	5.7	6.8
C18:4	6.3	15.8	2.8	7.8
C20:2	1.1	0.7	1.7	2.0
C20:4	6.7	1.3	5.4	6.6
C22:1		0.6		
C20:5	7.6	17.4	21.7	18.9
C22:5	5.4			0.4
C22:6	4.1	1.5		1.0

From Herodek, S., Farkas, T., 1967. Gas chromatographic studies on the fatty acid composition of some fresh-water crustaceans. Annales Instituti Biologici (Tihany) 34, 147–152.

TABLE 3.7 The Content of Phospholipid and Neutral Lipids in Three Age Stages of *Daphnia magna*

Types	Lipids	Newborn	3 Days Old	Mature
Phospholipids	Phosphatidylcholine	14.40	8.75	4.06
	Phosphatidylethanolamine	9.15	3.94	3.21
	Sphingomyelin	1.84	0.66	0.65
Neutral lipids	Triacylglycerols	566.2	283.2	452.1
	Diacylglycerols	Traces	Traces	74.1
	Free sterols	183.6	86.2	35.5
	Free fatty acids	146.9	128.0	60.8
	Wax esters	Traces	Traces	63.4

All values in μg/100 mg WW.
From Bychek, E.A., Guschina, I.A., 1999. The age changes of lipid composition in Daphnia. *Biokhimiya 64 (5), 652–655.*

TABLE 3.8 The Content of Fatty Acids (as % Total Fatty Acids) in Three Age Groups of *D. magna*

Fatty Acids	Newborn	3 Days Old	Mature
C14:0	0.9	2.3	3.5
C14:1	0.5	1.5	0.9
C14:2	0.6	1.7	2.2
C16:0	13.2	21.2	24.3
C16:1	4.7	4.7	4.5
C16:2	1.5	5.5	0.3
C16:3	1.2	3.4	7.7
C16:4	1.7	1.8	3.3
C17:0	1.2	Non det.	2.8
C18:0	3.4	7.7	5.8
C18:1	53.9	9.9	9.8
C18:2	6.7	22.0	18.9
C18:3	4.6	7.7	7.7
C20:4	2.8	6.1	3.8
C20:5	0.5	1.2	1.4

From Bychek, E.A., Guschina, I.A., 1999. The age changes of lipid composition in Daphnia. *Biokhimiya 64 (5), 652–655.*

D. pulex grown under different conditions was determined by Mims et al. (1991).

Cladocera synthesize de novo a minor fraction of total lipid which they obtain from food. As well, they possess a very limited capacity to transform lipids obtained with food.

Among the algae, diatoms are a prominent group that synthesize and store lipids: after photosynthetic production of a carbohydrate, they transform it into lipids and the stored lipid is seen as oil drops. The diatoms are one of the dominant groups on the bottom substrata and in phytoplankton. They are not the only lipid-producing group, but the metabolism of other algae is less well known. The lipid composition of algae is highly variable and depends on many environmental factors (Guschina and Harwood, 2009).

In temperate latitudes of the Northern Hemisphere, the spring peak of diatoms producing lipids as their reserve substance is followed by an accumulation of abundant oil drops by Cladocera. The content of particular fatty acids noticeably varies at different photoperiods, thus the content of saturated fatty acids and monounsaturated fatty acids in *Ceriodaphnia quadrangula* was maximum at 6Light:6Dark at feeding with *Chlorella* (Farhadian et al., 2013). In this species the content of α-linolenic acid (C18:3ω3) significantly increased during formation of resting eggs.

Due to their high-energy value, lipids are a prominent storage substance in Cladocera. Lipids are also used in the construction of biological membranes and hormones.

The lipid pathway in the aquatic environment starts with lipid formation by plants from initial products of photosynthesis (monosaccharide). According to Harwood and Jones (1989, p. 12), in algae "de novo synthesis requires the concerted action of acetyl-CoA carboxylase and a type II (dissociable) fatty acid synthase." The chain length of the product may then be elongated and unsaturated bonds may be introduced. Fig. 3.3 shows the schematic pathway from initial photosynthetic saccharides to lipids and amino acids in algae.

Lipids are present in water in both a particulate (i.e., within algae) and a dissolved state (Arts et al., 1997). In Cladocera, they are used as energy source, in the construction of cell membranes, and in metabolism; further, derivatives of lipids act as sex hormones. To understand the next sections, it is first necessary to state some basic ideas and definitions.

There are several classifications of lipids (e.g., Kucherenko and Vasilyev, 1985). In one of these, the following four groups are distinguished:

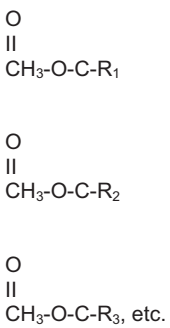
1. *Simple lipids*, which are esters of fatty acids with glycerol (fats) or aliphatic alcohols

(waxes). Waxes comprise true waxes and esters of cholesterol, vitamin A, or vitamin D.

2. *Complex lipids*, which are esters of fatty acids with other alcohols, e.g., phospholipids, glycolipids, sulfolipids, lipoproteins, or lipopolysaccharides.
3. *Lipid derivatives* (*lipoids*), which include fatty acids (saturated and unsaturated), monoglycerides, diglycerides, steroids alcohols with β ionic ring (vitamin A group), phosphatides, and carotenoids. Phosphatides are esters of polyatomic alcohols, fatty acids, and phosphoric acid. They comprise lecithins, which consist of radicals of glycerol, phosphoric acid, choline, and higher fatty acids (saturated or unsaturated).
4. *Various others*, including vitamins E and K, and aliphatic carbohydrates.

Functionally, the lipids are storage lipids (triglycerids and wax esters) and structural lipids (phospholipids and sterols) (Goulden and Place, 1993).

Generally, fat is represented as a triglyceride connected to various fatty acids (R):



The fatty acids may be saturated, i.e., containing no double bonds between the carbon atoms, and unsaturated, i.e., containing double bonds. The latter are described in the format *x:y* ω *z* (see, e.g., Ahlgren et al., 1990), in which *x* is the number of atoms, *y* is the number of double bonds (ω), and z is the position of the first double bond from the methyl end of the molecule. For example, 20:0 in which 20 is the number of carbon atoms and 0 (or a certain digit) is the number of double bonds in the molecule of a fatty acid. Some names of lipids corresponding to these designations are listed:

C14:0 myristic fatty acid
C16:0 palmitic acid
C16:1 palmitoleic acid
C18:0 stearic acid
C18:1 oleic acid
C18:2ω6 linoleic acid
C18:3ω3 α-linolenic acid
C18:3ω6 γ-linolenic acid
C18:4 octadecatetraenoic acid
C18:4ω3 stearidonic acid
C20:1 eicosanoic acid
C20:3 eicosatrienoic acid
C20:4ω6 arachidonic acid
C20:5ω3 eicosapentaenoic acid
C22:5 docosapentaenoic acid
C22:6ω3 docosahexaenoic acid

The following abbreviations are currently used; SAFAs—saturated fatty acids, MUFAs—monounsaturated fatty acids, PUFAs—polyunsaturated fatty acids, HUFAS—highly unsaturated fatty acids (the latter, namely C20: 4ω6, C20: 3ω3, C20:5ω3, C22:6ω3, overlap the range of PUFAs, i.e., they are also PUFAs).

Essential Fatty Acids

It is thought that almost all polyunsaturated fatty acids (PUFAs) are obtained by animals from plants and are not synthesized by animals. Thus, linoleic acid (C18:2ω6) and α-linolenic acid (C18:3ω3) are essential fatty acids, whereas eicosapentaenoic acid (20:5ω3) is not strictly essential. Becker and Boersma (2007, p. 463) arrived at the conclusion that "although dietary fatty acids can be used for energy purposes, specific fatty acids (namely, PUFAs) are required to build new biomass."

Lipids in Cladocera

Oil drops are easily observed in Cladocera and are often mentioned in the literature (e.g., Flückiger, 1951). They seem to be distributed throughout the body in some regular way, although this has not been sufficiently described for representatives of various genera living in different environments. They are mostly orange in color and their size ranges from quite small to very large. Tessier and Goulden (1982) recorded an abundance of oil globules in *Daphnia* by the visually estimated lipid index (LI). Hoenicke and Goldman (1987) estimated the lipid-ovary index in scores (based on visual scoring) in *Daphnia* and *Holopedium* and found that this index varies depending on the composition of their natural food. Dodson (1989) observed that in presence of predators the fat content in the body (LI) decreases, which might be related to defense from predators.

The following components are found in the fat of *D. magna* (Jaeger, 1935): butyric acid, sodium oleate, triolein, cholesterol oleate, cholesterol stearate, stearic acid, sodium stearate, tristearin, lecithin, linseed oil, and linoleic acid. Tessier et al. (1983) found that the major lipid types in *Daphnia* are triacylglycerols and wax esters. Arts et al. (1993) confirmed this prevalence and indicated that the next most dominant lipid class is phospholipids and sterols. It has been determined that Cladocera predominantly contain (12−23%) eicosapentaenoic acid, 20:5ω3 (EPA), a highly unsaturated fatty acid) (Persson and Vrede, 2006) (Fig. 4.5), in contrast to copepods. This difference is assumed to be a result of their phylogenetic origin.

It is likely that Goulden and Place (1993) were among the first to discuss the quantitative distribution of lipids in Cladocera. Taking into consideration that the fatty acids may be either derived from food or synthesized de novo in the body and that acetate (derived from the breakdown of carbohydrates or amino acids) is necessary for their synthesis, they used [^{14}C]acetate or $^{3}H_2O$ precursors and determined that the lipids synthesized by well-fed *Daphnia* (following incubation of up to 4 h) make up no more than 1.6% of the accumulated fatty acids.

Goulden and Place (1993) also indicated that lipids in daphnids consist of storage (triglycerides and wax esters) and structural (phospholipids and sterols) lipids, and that most of the lipids are transferred to the ovaries and then into eggs and used in the development of embryos.

Daphnia growth (in Schösee, Germany) was shown proportional to the available EPA (20:5ω-3) (Fig. 4.6) (Brett and Müller-Navarra, 1997) and is seasonally controlled by availability of EPA (Brzezińsky and von Elert, 2007). As algal polyunsaturated fatty acids make a major trophic resource for Cladocera, it was shown that in eutrophic lakes their growth and reproduction may be limited by EPA as was shown experimentally for *D. pulex* (Ravet et al., 2012). Limiting effect of EPA extends to predators. It was shown that *Bythothrephes longimanus* kept in the laboratory did not release broods as they were impoverished in EPA comparing with filed−collected specimens; having received EPA-enriched *Daphnia* as food they became heavier and had larger clutch size (Kim et al., 2014a,b).

It was found (Wacker and von Elert, 2001) that EPA (C20:5ω3) and α-linolenic acid (C18:3ω3) are not mutually substitutable resources for *D. galeata*, that their physiological functions are likely to be different, and that the former is not limiting for the growth of *D. galeata* cultivated on seston.

Bychek et al. (2005) found that *D. magna* is capable of high rates of de novo lipid radiolabeling; *D. magna* also makes direct use of dietary components (such as the PUFAs linoleate and α-linolenate). In addition, *D. magna* tolerates 24-h fasting with little change in lipoid metabolism. It has also been shown that *Daphnia* (specifically with reference to *D. magna*) cannot

synthesize linoleic acid (C18:2ω6) or α-linolenic acid (C18:3ω3) de novo (Persson and Vrede, 2006) (Fig. 4.5). Thus, they depend on fatty acids produced by plants (including essential fatty acids, e.g., EPA).

The content of fatty acids varies with age (Tables 3.7 and 3.8). In neonates of *M. macrocopa* percentage of myristic, palmitic, and stearic acids was 67% of the total fatty acids, in adults it decreased to 26% (Gama-Flores et al., 2015).

Having accumulated their fat reserves, *Daphnia* propagate, consume this resource, and then decline (Goulden and Hornig, 1980). Normally, the fat present in the body may be used by starving daphnias, as was observed in *D. magna* by Flückiger (1951), and short-term (24-h) starvation does not lead to profound changes in lipid metabolism (Bychek et al., 2005).

In addition to their basic trophic role, some lipid compounds may have exceptional properties. Pérez Gutierrez and Lule (2005) dried large numbers of *D. pulex* at room temperature, ground them, and produced 3 kg of fine powder. By extracting and fractionating it they obtained four glyceroglycolipids, all of which were found to be cytotoxic.

In *D. magna* exposed to tetradifon at 0.44 mg/L during 120 h the content of lipids decreased to 1.87 μg/ind. versus 18.36 in the control (Villarroel et al., 2009).

Further data are presented in Section 4B (Digestion).

3.3.6 Introductory Remarks About Steroids

Steroids are a major group of organic constituents of Cladocera controlling and channeling biological processes. They are derivatives of cyclopentano-perhydro-phenantrene. They comprise:

1. Sterols and their derivatives including ergosterol ($C_{28}H_{44}O$) and cholesterol ($C_{27}H_{46}O$). In the course of metabolism the latter is transformed into progesteron. Cholesterin also takes part in synthesis of ecdysons. Sterols are commented in detail by Martin-Creuzburg and von Elert (2009a,b);
2. Ecdysons (molt hormones; their antagonists are juvenile hormones), in the course of metabolism producing ecdysterone;
3. Steroid hormones—sex hormones: testosterone, androsterone (male hormones), estradiol, estron (progesterone) (female hormones), and hormones of carbohydrate metabolism—cortisol, hydrocortisone;
4. Vitamins of D group.

Ecdysteroid (ecd) concentration in whole *D. magna* is c. 200 pg ecd equivalent/mg DW (Bodar et al., 1990b) (see also Chapter 11).

In addition to PUFAs, sterols limit the growth, as Cladocera do not synthesize them de novo (Martin-Creuzburg and von Elert, 2009a,b). Sterols take part in formation of membranes and are precursors of steroid hormones, cholesterol being the most prominent. When *D. magna* obtain sterols in sufficient quantities for growth, eicosapentaenoic acid (EPA, 20:5ω3, a highly unsaturated fatty acid) becomes limiting (Martin-Creuzburg et al., 2008). Somatic growth of *D. magna* is mainly limited by the absence of sterols whereas egg production—by absence of long-chain PUFAs (Martin-Creuzburg and von Elert, 2009a,b).

In diatoms, cholesterol and C_{28} sterols are the major sterols (Soma et al., 2005). A low content of sterols in blue-green algae constrains C assimilation and cholesterol synthesis by *Daphnia* (von Elert, 2002, 2003; Martin-Creuzburg et al., 2008) and thus growth and reproduction (von Elert et al., 2002). Heterotrophic bacteria are scarce in sterols and thus limited growth, if fed with bacteria supplemented with cholesterol, *D. magna* demonstrated increased somatic growth (Martin-Creuzburg et al., 2011).

In newborn *D. magna*, the content of free sterols and phospholipids is high (phosphatidylcholine, phosphatidylethanolamine, and sphingomyelin)

(Bychek and Gushchina, 1999). These authors also found that the content of triacylglycerols decreases with the growth of *D. magna* fed on *Chlorella*. Fatty acid desaturation is lower in newborns, and wax esters are only detectable in adults.

The threshold concentrations of sterols in food are found to be from 3.5 to 34.4 μg/mg C (Martin-Creuzburg et al., 2014). Phytosterols are differently efficient in supporting somatic growth of *D.magna* (fucosterol and brassicasterol being more efficient). The limiting sterol level was higher in *D. galeata* than in *D. magna* (Martin-Creuzburg et al., 2005). The cholesterol content in *D. magna* increased with the increasing dietary cholesterol, as well as at increasing temperatures (15, 20, and 25°C) Martin-Creuzburg et al. (2009). At higher temperature (25 vs. 20°C), the cholesterol content in eggs increased.

The dietary sterol conversion into cholesterol by *D. magna* is also demonstrated (Martin-Creuzburg et al. 2014).

Martin-Creuzburg and von Elert (2009b, p. 50) also mention presence in *D. galeata* of "an efficient C-24 dealkylating system".

3.3.7 Pigments

Pigments are derived from food or produced during the course of metabolism. In Cladocera, they comprise orange (carotenoid) pigments, red hemoglobin (Hb) or bacterial carotenoids in cases of infestation by *Spirobacillus cienkowski*, green (carotenoprotein in hemolymph), and dark (e.g., ommochromes of eyes, or tanned protein formed at high pH, i.e., melanins) (Green, 1966b, 1971).

Generally, littoral Cladocera are brownish, whereas planktonic species are colorless. Orange or red coloration is also observed in some species. Rarely is a species brightly colored. Blue spots occur in *Eurycercus lamellatus* on the postabdomen, on the dorsal side of the trunk, at the base of the mandibles, and on the esophagus (Weismann, 1878; Behning, 1941; Smirnov, 1971, 1974). *Pseudochydorus* has large brown spots on its valves. Newly molted *P. globosus* are colorless, but during the intermolt period a brown spot appears and increases in intensity. At excessive solar irradiation, Cladocera are blackish (melanistic) as are their ephippia containing latent eggs.

Leydig (1860, p. 56) noted that the blood of Cladocera may be colorless, yellowish, reddish, bluish, or greenish. Green (1957a,b) reported his observations of *Daphnia* with pale green blood, *Simocephalus* with green blood, and *Megafenestra aurita* with blue blood. These colors are caused by carotenoid proteins, as they produce an orange color when treated with desaturating agents.

The coloration of cladocerans (especially of parthenogenetic eggs) may depend on the coloration of the food consumed. Information on the pigments of Cladocera, as related to their different ecology, and the transformation of ingested pigments is rather scarce. This is an open field for further useful investigations.

Carotenoids. Carotenoids are lipid derivatives. The cladocerans receive carotenoids with algal food (Green, 1966a), which contains significant quantities: green algae, 7–51 mg% WW (i.e., 7–51 mg/100 g WW); cryptomonads, 17–162 mg% WW, blue-green algae, 14–52 mg% WW (Lavrovskaya, 1965). The content of carotenoids in *Daphnia* and *Bosmina* was determined as 0.45–2.1 mg% WW (Lavrovskaya, 1965).

With reference to *Simocephalus*, Green (1955) found carotenoids (orange) in a free state in fat globules, in the gut wall, in fat cells, linked to proteins in the cytoplasm, ovary, and eggs, and as carotenoprotein (green) in blood.

Green (1966b) reported the principal pathways of carotenoid transfer, with reference to *Simocephalus* (Fig. 3.7). Carotenoids obtained from food are passed into the blood, and from there to fat cells, to the carapace epidermis, to the ovary, and then to the eggs. A female may pass half of its total carotenoids to her eggs. In

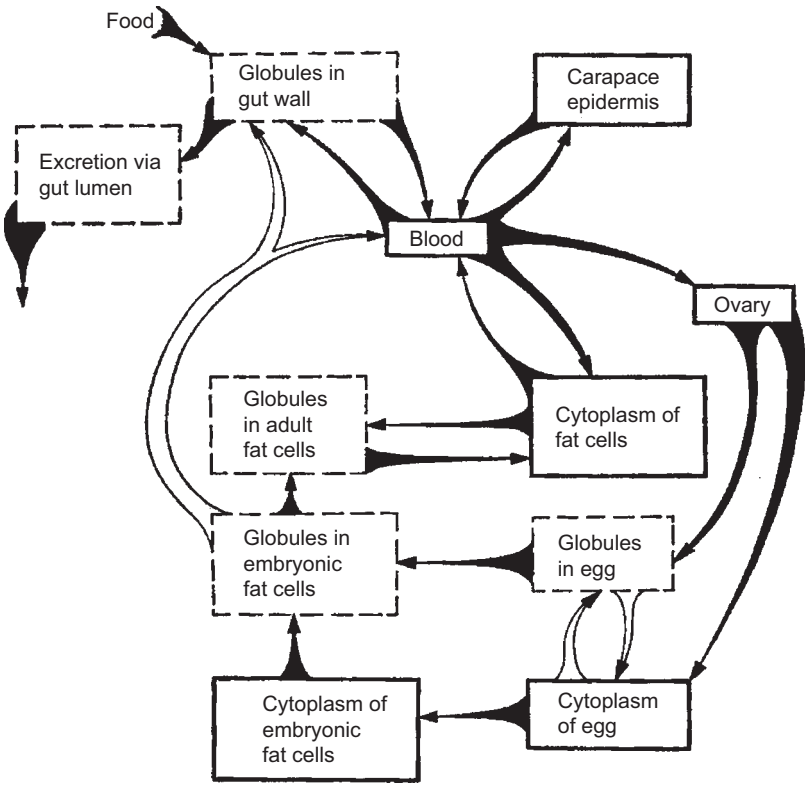


FIGURE 3.7 Pathways of carotenoid transfer in *Simocephalus*. Quadrangles of broken lines indicate stages in which carotenoid is free. *From Green, J., 1966b. Variation in carotenoid pigmentation of Simocephalus vetulus (Crustacea: Cladocera). Journal of Zoology 149 (2), 174–187.*

Simocephalus, carotenoids are either present in the cells of the gut wall and fat body or associated with proteins of fat cells, epidermis cells, the ovary, and eggs. Carotenoid pigments were found to be dissolved in oil drops or bound to cytoplasmic proteins in *Daphnia* (Green, 1957a,b) and *Simocephalus* (Green, 1966b). An intermolt cycle (Fig. 3.8) and a seasonal cycle (Fig. 3.9) of carotenoids were demonstrated (Green, 1966b).

In various cladocerans, particular carotenoids were found (indicated in Table 3.9) (Herring, 1968). Carotenoids are not found in *Leptodora* (Farkas, 1958). The following carotenoid pigments found in the gut wall, fat cells, and the ovary of *Daphnia* were reported by Green (1957a,b): astaxanthin, β-carotene, γ-carotene, and lutein. *Daphnia* grown in the light contain much more carotenoid than those grown in the dark.

Later, Herring (1968) found the following carotenoid pigments in *D. magna* and other cladocerans: astaxanthin, canthaxanthin, β-carotene, echinenone, and an unidentified ketocarotenoid. Lutein, supposed to originate from food, was present only in the midgut wall. Herring also fed *D. magna* pure carotenoids with yeast and found that the ingested β-carotene is transformed into echinenone, canthaxanthin, and astaxanthin. Foss et al. (1986) investigated in detail the

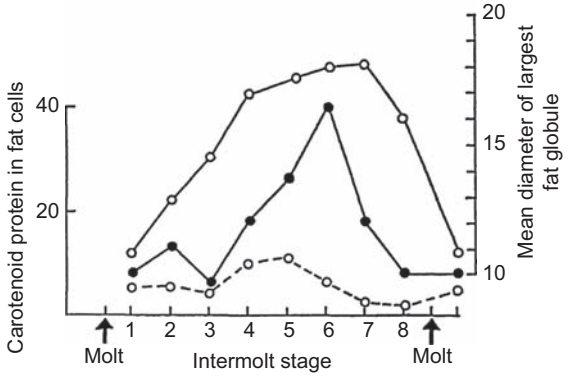


FIGURE 3.8 Intermolt cycle of carotenoid pigmentation of *Simocephalus* fat cells. Lower and middle lines, in cytoplasm (different years); upper line, diameter of largest fat globules. *From Green, J., 1966b. Variation in carotenoid pigmentation of Simocephalus vetulus (Crustacea: Cladocera). Journal of Zoology 149 (2), 174–187.*

composition of carotenoids of *D. magna* and found two new ketocarotenoids.

In *Ceriodaphnia reticulata*, Czeczuga (1976) found astaxanthin (quantitatively dominant), astacene, canthaxanthin, cryptoxanthin, 4-keto-4-hydroxy-β-carotene, lutein-5,6-epoxide isozeaxanthin, and astaxanthin ester. In *D. magna*, Czeczuga (1984) also found β-cryptoxanthin, isocryptoxanthin, isozeaxanthin, lutein epoxide, and zeaxanthin. According to his determinations, the total concentration of carotenoids is c. 4 g/g DW, of which astaxanthin forms 27% and β-cryptoxanthin 25%. In *M. micrura*, astaxanthin and canthaxanthin predominate (Velu et al., 2003).

In *Holopedium*, the carotenoid astacin was found by Sørensen (1936) and Goodwin (1960, p. 127) noted that *Holopedium* accumulates a lot

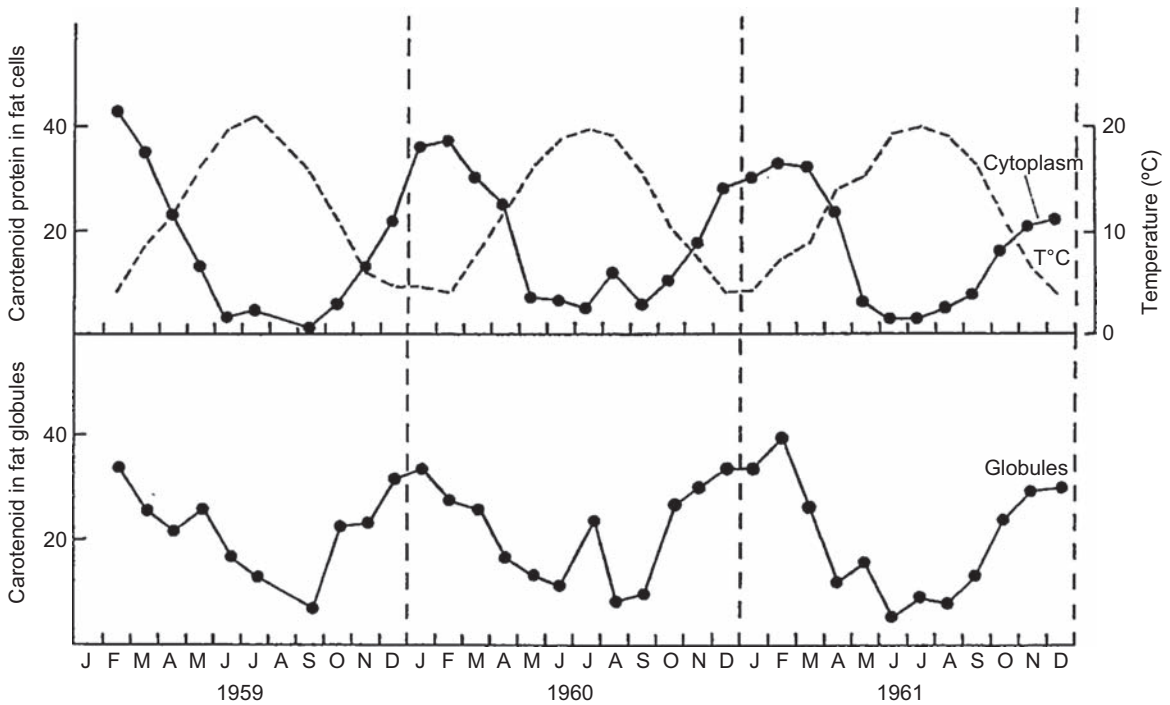


FIGURE 3.9 Seasonal variation of carotenoids in *Simocephalus*. Upper, in fat cells. Lower, in fat globules. Letters designate months. *From Green, J., 1966b. Variation in carotenoid pigmentation of Simocephalus vetulus (Crustacea: Cladocera). Journal of Zoology 149 (2), 174–187.*

TABLE 3.9 The Percentage Composition of the Carotenoid Pigments in Wild Cladocera spp.

Species	B-Carotene	Echinenone	Canthaxanthin	Ketocarotenoid	Astaxanthin	Lutein	2nd Ketocarotenoid
Ceriodaphnia megalops	5	6	5	3	72	5	2
Daphnia pulex	4–12	7–11	5–19	5–20	24–66	4–20	Trace
Daphnia longispina	6	5	2	0	56	13	3
Eurycercus	7	0	16	0	47	19	0
Moina micrura	0.43	–	4.60	–	39.82	21.21	–
Simocephalus vetulus	6	7	8	0	38	21	0

18.65% β-cryptoxanthin and 15.29% violaxanthin have also been found in *M. micrura* (Velu et al., 2003).
From Herring, P.J., 1968. The carotenoid pigments of Daphnia magna *Straus. I. The pigments of animals fed* Chlorella pyrenoidosa *and pure carotenoids. II. Aspects of pigmentary metabolism. Comparative Biochemistry and Physiology 24 (1), 187–221; Moina micrura, after Velu, C.S., Czeczuga, B., Munuswamy, N., 2003. Carotenoprotein complexes in entomostracan crustaceans (*Streblocephalus dichotomus *and* Moina micrura*). Comparative Biochemistry and Physiology B 135 (1), 35–42.*

of astaxanthin and that its "de novo synthesis is probably ruled out." As it is hardly possible that much astaxanthin is present in the food, Goodwin suggested that it is produced by oxidation of the β-carotene and its derivatives (including zeaxanthin) ingested with diatoms.

It seems that the excess of carotenoids ingested with food serves no physiological purpose. Having received carotenoids with their food, cladocerans just transfer them over to their eggs and progeny. Herring (1968) found that in *Daphnia* no requirement exists for dietary carotenoids or vitamin A. Moreover, during development there is no marked change in the total carotenoid content of the eggs. Green (1957a,b, 1966a) found that about half of the mother's carotenoids are transferred to the eggs of each brood, but the developing embryos do not utilize this pigment. The presence of free or conjugated carotenoids does not favor growth or the viability of eggs or adults.

Melanins. The term "melanins" relates both to brown and black pigments. Dark cuticular pigmentation is caused by melanins (Hebert and Emery, 1990), including that of ephippia (Gerrish and Cáceres, 2003). They are thought products of the metabolism of amino acids (tyrosine) (Goodwin, 1960, p. 133): "Chemically, the melanins are probably polymerized indole quinones formed by the action of the enzyme tyrosinase on the aromatic amino acids." Melanins are also present in the eyes. The ommochromes of eyes are rapidly dissolved with 10% alkali solution in both fresh and preserved specimens.

Arctic and high mountain populations of *Daphnia* are blackish (Hebert and Emery, 1990). In addition, chydorids collected in shallow rock pools in the hot climate of Western Australia are dark brown, as reported by B.V. Timms and M. Jocqué (personal communication). Such dark pigmentation is thought of protective value. *Scapholeberis* and *Dadaya* living under the surface of water are also black. Hobaeck and Wolf (1991) observed melanic populations of *Daphnia* at altitudes of 1200–1600 m above sea level inhabiting clearwater lakes and ponds, whereas transparent populations came from ponds with slightly humic water. Melanin was deposited in the dorsal area of the carapace, the head shield, and the antennae. Its absorption maximum was c. 249 nm. See also Section 13.4 on the protective role of melanins.

Hemoglobin. Hemoglobin is a red pigment, a compound protein consisting of globin and

heme (porphyrin with Fe^{2+}). Hemoglobins reversibly bind molecular oxygen. Populations of *Daphnia* have also been observed colored brown by Hb in combination with hematin, which contains three-valent iron in the heme moiety (Fox, 1948; Goodwin, 1955). Hematin is supposed to be either an alternative heme compound in the blood or an excretory product of Hb.

Extensive literature is available on hemoglobin (Hb) in Cladocera. Special investigations into Hb in Cladocera were made by Fox (during 1945–1955), Hoshi (during 1949–1974), and Green (1955). Hb may be measured chemically or spectroscopically.

In contrast to Malacostraca (Prosser and Brown, 1967 (1962)), Cladocera synthesize Hb, which may be present in their hemolymph, muscles, central nervous system, fat cells, ovaries, or dissolved in hemolymph of parthenogenetic eggs (Fox, 1955; Green, 1955).

The molecular weight of *Daphnia* Hb is 400,000, with an admixture of a small amount of 34,500 (Goodwin, 1960). In *M. macrocopa*, molecular weight of Hb was estimated 670,000, the minimum molecular weight 17,600, the iron (Fe) content to be 0.317%, and the number of Fe atoms per Hb molecule to be 38 (Hoshi et al., 1967). These authors conclude that each Hb subunit with one Fe atom corresponds approximately to human Hb. A variety of Hb having the molecular weight 69,000 (erythrocruorin) was also found in *Daphnia* (Svedberg and Eriksson-Quensel, 1930).

The role of Hb is considered further in Chapter 5, "Respiration."

Myoglobin. Some of the Hb is present in muscles as myoglobin. Goodwin (1960) warned against the use of the latter term for Crustacea, as its constitution was then unknown. In some of my formalinized samples of filamentous algae and Cladocera kept for a long time, the muscles of the cladocerans are stained blackish, thus indicating the probable presence of myoglobin. This may be attributed to tannic substances, which are known to occur in filamentous algae and produce black ink with Hb iron. However, attempts to stain similar samples with tannin failed, although further attempts may be worthwhile.

Later, the presence of myoglobin in the muscles of *Simocephalus* was directly confirmed by microspectrophotometry (Fig. 3.10) (Karnaukhov et al., 1986). Myoglobin was completely absent from oil drops.

Other Pigments. Cytochrome was shown by Fox (1955) to be present in muscles of *Daphnia*, absorption maxima at 566 and 500 nm, and by Hoshi and Akizawa (1979) in *Simocephalus*, at 603, 563, 550, and 532–522 nm.

Coloration of *Simocephalus* and *Chydorus* results from a combination of myoglobin,

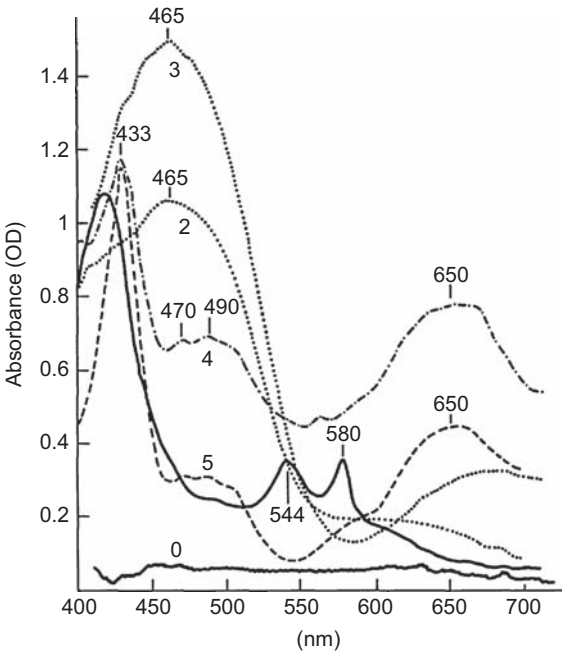


FIGURE 3.10 Levels of different components in *Simocephalus*: *1*, myoglobin in muscles; *2, 3*, lipid in tissues; *4*, green granules in eggs; *5*, yellow-green granules in eggs. *OD*, optical density. *From Karnauckhov, V.N., Del Rio De Valdivia, M.L., Yashin, V.A., 1986. Comparative spectral studies of pigmented organs and tissues of two cladocera species. Comparative Biochemistry and Physiology 85B 1, 279–284.*

carotenoids, and carotene protein, as shown microspectrophotometrically by their absorption bands (Karnaukhov et al., 1986).

Biliverdin has been discovered only in the eye of *Polyphemus*, and not in the eye of *Daphnia* (Green, 1961a).

3.3.8 Content of Particular Elements or Compounds

The content of some elements in Cladocera spp. is shown in Table 3.10. The content of mineral substances in the bodies of Cladocera depends on mineralization of the environment; in *D. magna*, it ranges from 12% to 37.6% DW; and in *Simocephalus vetulus*, from 10% to 19.4% DW (Stepanova et al., 1971).

The vitamin content was assessed as: vitamin A, 0.519 mg% WW (i.e., 0.519 mg/100 g WW); vitamin B_1, 0.255 mg% WW; vitamin B_2, 0.569 mg% WW (*D. pulex*, Malikova, 1956), vitamin B_{12}, 1.66–1.34 μg/g DW (*Moina dubia* and *Bosmina longirostris*, Stepanova and Borshch, 1970), vitamin C, 4–24 mg% WW; vitamin E, 0.6–7 mg% WW (*Daphnia* and *Bosmina*, Lavrovskaya, 1965). Previously, the content of vitamin B_{12} in *D. pulex* was indicated as 3.8 mg% WW (Hashimoto and Sato, 1954).

TABLE 3.10 Content of Some Elements in *Daphnia magna* (in Brooks in the Netherlands), mg/g DW

Ca	67–97
Na	6,8–130
Mg	1.1–4.2
K	5–14
Fe	0.7–57
Mn	0.12–5.2
Cd	0.19–20
Co	0.78–65
Cu	31–250
Ni	2–210
Se	0.53–5.8
Zn	98–520

From Verschoor, A.J., Hendriks, J., Vink, J.P.M., de Snoo, G.R., Vijvery, M.G., 2012. Miltimetal accumulation in crustaceans in surface water related to body size and water chemistry. Environmental Toxicology and Chemistry 31 (10), 2269–2280.

Essential and Nonessential Elements

Cladocera need some elements, whereas others may be physiologically nonessential or even toxic. In addition, essential elements [e.g., copper (Cu) or zinc (Zn)] may be toxic at concentrations higher than are normally necessary. Cladocera may also accumulate, without any physiological use, substances that just happen to be around (Table 3.11).

Generally, tissues of Cladocera consist of biogenic elements (C, H, O, and N), macroelements (Ca, Na, K, P, S, Mg, Mn, Fe, Cl, and Cu), and microelements (Zn, Co, I, Se, Mo, Li, and some others).

Carbon (*C*). Carbon is one of the principal macroelements. Although the content of carbon in most Cladocera is generally about 47–48% DW, there are variations (e.g., c. 53% in *Scapholeberis mucronata*) (Hessen and Lyche, 1991). Data on the content of carbon in representatives of particular genera are available. For example, the content of carbon in the bodies of *Diaphanosoma*, *Holopedium*, *Bosmina*, and *Daphnia* was found to be 48% DW, with little seasonal variation (Andersen and Hessen, 1991). It increases with increased body length in *D. hyalina*, from 42.8% total C in DW in newborns to 44% in adults (Baudouin and Ravera, 1972).

Direct determination of the total carbon content (using the dry-combustion method of Pregl-Roth) yielded the following results (von Metz, 1973). By measuring the uptake and release of radiocarbon (γC), *D. pulex* fell into two groups: 1.5–10 γC/ind. and 2–22 γC/ind. The radiocarbon content in *Daphnia* with eggs was twice that of *Daphnia* without eggs. About 52% of carbon was in the eggs, irrespective of

TABLE 3.11 Bioconcentration Factors (i.e., the Accumulation of Various Substances Over Time)

Substance	Species	Factor (× Original Concentration)	Time	References
Arsenic trioxide	*Daphnia magna*	219	21 days	Spehar et al. (1980)
Cesium-137	*D. magna*	84	5 days	Nilov (1983)
$HgCl_2$	*Ceriodaphnia affinis*	2000	In 20th generation	Gremiachikh and Tomilina (2010)
^{203}Hg (as $HgCl_2$)	*Daphnia carinata*	3300	24 h	Nilov (1980)
Manganese	*D. magna*	65	8 h	Kwassnik et al. (1978)
Neptunium	*D. magna*	32	48 h	Poston et al. (1990)
Nickel	*D. magna*	25	48 h	Pane et al. (2003)
Anthracene	*Daphnia pulex*	760	3 h	Herbes and Risi (1978)
Benz(a)anthracene	*D. pulex*	10,000	24 h	Southward et al. (1978)
Benzo(a)pyrene	*D. magna*	1000–8000	24 h	McCarthy (1983) and Oikari and Kukkonen (1990)
Chlordanes	*D. pulex*	24,000	24 h	Moore et al. (1977)
DDT	*D. magna*	23,000	24 h	Crosby and Tucker (1971)
Estrone	*D. magna*	228	16 h	Gomes et al. (2004)
Naphthalene	*D. pulex*	100	24 h	Southward et al. (1978)
Pirimicarb[a]	*D. magna*	50	48 h, per DW	Kusk (1996)
Triphenyltin chloride (0.1 mg/L in water)	*D. magna*	290	3 h	Filenko and Isakova (1979)
Water-soluble oil fraction	*Daphnia*	>500	19 h	Mikhailova et al. (1986)

DDT, dichlorodiphenyltrichloroethane.
[a] *2-(diethylamino)-5,6-dimethyl-4-pyrimidinyl dimethyl carbamate.*

the number of eggs. The minimum carbon content per egg was 1–1.5 γC.

The content of organic carbon was determined in *Polyphemus pediculus* (Butorina, 1973): parthenogenetic females, 3–7% WW, 40–50% DW; gamogenetic females, 3.4–7.5% WW, 41–48% DW; males 4.4–5.8% WW, 42–47% DW. It was 44.6% DW in *D. hyalina* and 49.05% DW in *L. kindtii* (Lin and Liu, 1985).

The content of stable isotopes was determined in *Daphnia longispina*: ^{13}C ranged from 39.9‰ to 28.2‰, ^{15}N–from 0.9‰ to 1.5‰ (Lee et al., 2011).

It was observed that the elemental composition may be changed by bacterial infection. *Pasteuria ramosa* was found to cause increase of C content and reduction of P content in *D. magna* the end of 28-day experiment (Frost et al., 2008).

Nitrogen (*N*). Nitrogen is an obligatory constituent of amino acids and protein. This element is always deficient in natural water bodies as it is avidly consumed by algae. The nitrogen cycle in lake waters is described by Hutchinson (1957).

In the bodies of Cladocera, the content of nitrogen (in % DW) was found to be 9.7 in newborn *D. hyalina* (Baudouin and Ravera,

1972); c. 9 in mature *D. hyalina* (Baudouin and Ravera, 1972; Lin and Liu, 1985); c. 9–10 in *Diaphanosoma*, *Holopedium*, *Bosmina*, *Daphnia*, and *Leptodora*, with little seasonal variation (Andersen and Hessen, 1991; Hessen and Lyche, 1991); 12.7 in *L. kindtii* (Lin and Liu, 1985); and c. 7.9 in *Scapholeberis mucronata* (Hessen and Lyche, 1991), in five species of Cladocera it was c. 8% (Main et al., 1997). The content of nitrogen (N) was determined by Hessen (1990) as 8.18% DW, with little variation; the lowest values were determined in starved specimens.

Phosphorus (*P*). In aquatic environments, there are only traces of free phosphorus because, similarly to N, it is avidly assimilated by algae. The phosphorus cycle in lakes is described by Hutchinson (1957).

Phosphorus content (as P_2O_5) in the bodies of Cladocera was found to be 3.4–3.6% DW in *D. pulex*; 3.5% DW in *Leptodora* (Birge and Juday, 1922). The content of phosphorus (P) in eight species of Cladocera (as summarized by Gutelmakher, 1986) was 0.68–1.89% DW, c. 1% DW in *Diaphanosoma*, *Holopedium*, *Bosmina*; c.1.4% DW in *Daphnia* (Andersen and Hessen, 1991; Vrede et al., 1999), in five species of Cladocera was c.1.4% (Main et al., 1997), and 1.1% DW in *D. magna* (Sterner and Schwalbach, 2001), with little seasonal variation (Andersen and Hessen, 1991, 1999). Brett et al. (2000) indicated 0.8–1.8% P DW for various species of *Daphnia*, the highest being 1.8 for *D. pulicaria*.

A major pool of phosphorus in *Daphnia* is present in the body (67%), a lesser part in the carapace (14%) (Vrede et al., 1998). Hessen and Rukke (2000a) also noted a significant fraction of P in the exoskeleton of *Daphnia*. The phosphorus content was especially high in juvenile *Daphnia*, which were thus more dependent on sources of phosphorus in their food (Main et al., 1997). P content was found to be higher in filter feeders (>1.5%) and lower in carnivorous cladocerans (c. 0.5% P DW) (Hessen and Lyche, 1991). The highest phosphorus content, c. 2%, was found in *Scapholeberis mucronata* and *Ceriodaphnia quadrangula*.

When the external Ca concentration increased from 0.5 to 200 mg/L, the P content in *D. magna* (obtaining P-sufficient food) decreased from 1.43% to 1.05% DW (Tan and Wang, 2009c).

Calcium (*Ca*). The ranges of calcium are different for different species (Mäemets, 1961). The lowest level of calcium in water in which *D. magna* live has been recorded is 0.1–0.5 mg/L (Hessen and Rukke, 2000a, 2000b), and 0–2 mg/L for *D. galeata* (Rukke, 2002). There are also interpopulational differences in tolerance to a low ambient calcium concentration, as has been determined for *D. galeata* (Rukke, 2002). The lowest calcium threshold for the growth of *D. pulex* juveniles was measured as c. 1.5 mg/L (Riessen et al., 2011). In *D. magna* raised in solutions with Ca its content in the body increased from 0.91% to 3.75% of DW, obtaining Ca mostly from solution (Tan and Wang, 2009a,b,c,d). *Ceriodaphnia dubia* and *M. macrocopa* with a low or intermediate Ca content in the body are better adapted to low Ca concentrations in water than *Daphnia* with high Ca content (Tan and Wang, 2011).

In littoral forms the content of calcium ranges from 3.6 mg/g in *Acroperus* to 23.2 mg/g in *Disparalona* (Shapiera et al., 2011). In planktonic forms the content of calcium ranges within 2–9.9% DW in *D. pulex*, 3.2% DW in *Leptodora* (as CaO in ash) (Birge and Juday, 1922), in *D. pulex* 9.6% DW (Malikova, 1953), and within 0.8–4.4% DW in *Daphnia* spp. in Norwegian lakes (Wærvågen et al., 2002).

The level of Ca in natural water influences metabolism of Cladocera. The content of Ca in *D. magna* decreases from 4.2% to 1% DW at lower concentrations of Ca in water (Alstad et al., 1999), a large part of the calcium is lost with the exuvium at molting. The content of P decreases from 1.43% to 1.05% DW when the Ca concentration in water increased from 0.5 to 200 mg/L (Tan and Wang, 2009a,b,c,d).

Cobalt (*Co*) is a constituent of B_{12}. Co was determined in *Daphnia* (Stepanova and Borshch, 1970). In natural waters (United States), the content of Co was indicated as c. 0/02−0.04 mg/m^3, but several mg/m^3 was reported for European water bodies (Hutchinson, 1957).

Iron (*Fe*). Iron is a component of Hb. Iron is present in water and more abundantly on the bottom of water bodies both in ferrous and ferric forms.

Magnesium (Mg). In *D. pulex*, 0.5−0.9% DW magnesium was found, and in *Leptodora*, 0.95% DW (as MgO in ash) (Birge and Juday, 1922).

Sodium (*Na*). Sodium plays an important part in osmotic relationships. Exposure of *D. magna* to silver nanoparticles (AgNPs coated with polyvinylpyrrolidone) and to $AgNO_3$ resulted in reduction of the Na body content in *D. magna*, but the Ca body content increased (Zhao and Wang, 2013) due to inhibited Na influx and elevated Na efflux. The ion-regulatory dysfunction was caused mainly by the soluble Ag released from nanoparticles.

Selenium (*Se*) is a constituent of glutathione peroxidase. It was shown with reference to *D. magna* that Se is a necessary element as the aforementioned enzyme protects its cells from peroxidation (Elendt, 1990). Elendt cultivated *D. magna* in the medium with selenium concentration 1 μg/L as Na_2SeO_3. Se is localized primarily in the cytoplasm (as shown for *D. pulex* cells). In mitochondrial matrices, excessive dense deposits were accumulated and, with time, the mitochondria degenerated (Schultz et al., 1980). Se is accumulated by *D. magna* more strongly from aqueous seleno-L-methionine than from inorganic sources (Besser et al., 1993). Se (as selenate) is toxic to *D. magna* at concentrations starting with 500 μg/L (Ogle and Knight, 1996).

It was reported that in algae Se may replace S in amino acids (Bodnar et al., 2014).

Sulfur (*S*) is a constituent of some amino acids (methionine, cysteine, cystine). The phosphorus cycle in lake waters is described by Hutchinson (1957).

Zinc (*Zn*) is a constituent of carbonic anhydrase, a metalloenzyme present in *Daphnia* (Culver and Morton, 2015).

3.4 ACCUMULATION OF XENOBIOTICS IN THE CLADOCERAN BODY

Xenobiotics affect aquatic invertebrates either by direct toxicity of dissolved toxicants or as contaminated food. The effects may be immediate or delayed, manifested as damage to metabolic links in the organism, or teratogenic, disrupting or discoordinating links in the natural biocenotic network. At low concentrations of xenobiotics, Cladocera continue to live and reproduce, but are less prolific. High concentrations of xenobiotics either cause death or decrease the life span. They also disturb natural adaptation processes. Generally, they have far-reaching effects at the species, population, and biocenotic levels.

Numerous experiments have shown a decrease in life span and fecundity in the presence of toxic substances. Beklemishev (1924) noted the necessity of taking into consideration the rate of penetration of toxicants through integuments. He observed that when cladocerans are placed in a toxic solution, at first they behave in their usual way ("as if nothing had happened"), then they manifest signs of anxiety, make desperate jerks, sink to the bottom, and make occasional movements there, then all movement stops, and finally the heart stops. Excess pollution may kill all animal life or, at lower concentrations, kill animal species selectively or inhibit certain vital functions or interrelations.

Here, the principal focus is on studies demonstrating how foreign chemicals become involved in their metabolism or are transformed by Cladocera, and how the chemicals or excessive factors impair particular functions or metabolic

links. Accordingly, measurements of the lethal dose, quantitative characteristics of fecundity, or life span are mostly not cited here.

Bioconcentration was shown to increase at higher temperatures with reference to dichlorodiphenyldichloroethylene (DDE): by 314% when the temperature increased from 5 to 25°C, obviously due to higher beating of thoracic limbs, and still more at fluctuating temperature (Nawaz and Kirk, 1996). Particular substances may not accumulate homogeneously throughout the cladoceran body but may preferentially accumulate in particular organs or tissues.

The following data have been obtained for particular elements.

Arsenic (*As*) is accumulated in the body of *D. magna* mainly from dietary sources. Arsenic from food algae is accumulated by *Moina* predominantly as nonmethylated arsenic and less so as dimethyl arsenic compounds (Maeda et al., 1990, 1992). In 7 days, *Moina* accumulated from 76 to 111 μg As/g DW (Maeda et al., 1990). Folt (2005) found that *Daphnia* accumulate As and Hg, with levels accumulated by *Daphnia* being 2−3 times higher than those in copepods. Bioaccumulation of arsenic is independent of P content in the body but was lower when food algae were P limited (and contained more As) (Miao et al., 2012). Efflux of As was mainly by excretion—51−60.6 of the total loss, less so via egestion (7.9−11.9%), via molting (3.6−4%). About 24.7−29.8% was transferred to embryos.

C*admium* (*Cd*) taken from solution is concentrated in the exoskeleton of *D. magna* (Carney et al., 1986). The internal distribution of ^{109}Cd (cadmium-109) has been determined by whole-animal autoradiography (Munger et al., 1999); it mainly accumulated in the diverticula of the gut, which are sites of uptake of nutrients and Ca. Cd accumulated by *Moina* (Yamamura et al., 1983) was mostly bound to low molecular weight proteins (a mixture of two isoproteins). It was also shown to be absorbed onto the surface of the carapaces of *D. magna* and *Ceriodaphnia dubia* (Robinson et al., 2003). Differences between *Daphnia, Ceriodaphnia*, and *Moina* were found in accumulation and efflux of Cd (Tan and Wang, 2011). The Cd content in *Holopedium* collected from natural lakes in Canada ranged from 0.9 to 31 μg/g (Yan et al., 1990). Bodar et al. (1990a) determined that *D. magna* can accumulate c. 115 ng Cd/mg DW/24 h and can develop resistance to Cd in a single generation. In addition, lethal concentrations of Cd differ for different clones of *D. magna*. Upon exposure to Cd, *D. magna* respond by reducing their Cd assimilation efficiency and ingestion; in addition, metallothioneins are formed in its body during detoxification (Guan and Wang, 2004a). *D. magna* juveniles obtain twice as much Cd from water than from algae (*Chlorella*) (Barata et al., 2002a,b). The gut ceca are the main sites of Cd and Ca intake. *D. magna* sensitivity to Cd increases following irradiation by a millimeter-range low-intensity electromagnetic field (Gapochka et al., 2012). *D. magna* may accumulate Cd over several generations and recover its biological parameters in Cd-free water over one or two generations (Guan and Wang, 2006); therefore, they have a high potential for recovery following Cd contamination. Cd has a cumulative effect in subsequent generations of *M. macrocopa* (Gama-Flores et al., 2014).

Cesium (*Cs*). In a solution of ^{137}Cs (cesium-137), *D. magna* accumulate radioactive Cs: the accumulation coefficient reaches 84 at day 5, but decreases at molting and with the liberation of juveniles (Nilov, 1983).

Fluorine (*F*). *D. magna* accumulates perfluoroalkyl substances, proteins present in the outer solution at 10−20 mg/L suppress bioaccumulation, but enhance bioaccumulation at 1 mg/L (Xia et al., 2013).

Lead (*Pb*) is thought accumulated in the shells, probably "mainly passively" (Holm-Jensen, 1948), in the shells of *D. magna* "probably by exchange" of Ca^{2+} for Pb^{2+}.

Manganese (*Mn*). The Mn concentration factor of *D. magna* is 65 and maximum uptake is reached after 8 h of exposure despite active excretion (Kwassnik et al., 1978).

Mercury (*Hg*). More Hg accumulated in *D. magna* from *Chlorella* it consumes in the form of methylmercury CH_3HgCl than as mercuric chloride $HgCl_2$ (Boudou and Ribeyre, 1981; Boudou et al., 1983). After 48 h exposure to methylmercury (in food), the Hg concentration in *D. magna* is c. 440 ng/g. The bioconcentration factor of $HgCl_2$ by *Ceriodaphnia affinis* reaches 2000 in the twentieth generation (7.2 μg/g DW) (Gremiachikh and Tomilina, 2010).

Neptunium (*Np*). Neptunium is concentrated by *D. magna* to 40 μg/g in 48 h when present in the medium at a concentration of 1.23 μg/mL (Poston et al., 1990).

Nickel (*Ni*) is mainly accumulated in *D. magna* in the carapace, gut, and hemolymph (Hall, 1982). *D. magna* exposed to Ni in solution accumulate it in the F_1 generation to a concentration exceeding 18 times that of untreated controls; at a Ni concentration of up to 42 μg/L, the glycogen content in the body is noticeably decreased (Pane et al., 2004). When fed with Ni-contaminated algae, *D. magna* can accumulate up to 72 μg Ni/g DW (Evens et al., 2009). Both Ca and Mg reduce Ni toxicity, but Na has no effect (Deleebeeck et al., 2008). According to Vandenbrouck et al. (2009, p. 18), in *D. magna* Ni affects functional gene classes that are "involved in different metabolic processes (mainly protein and chitin related processes), cuticular turnover, transport and signal transduction."

Silicon (*Si*). A total of 2.8% DW of silicon was reported in a sample of *D. pulex* (Birge and Juday, 1922).

Silver (*Ag*). Silver is known to be highly toxic for cladocerans (Rodgers et al., 1997), although the presence of organic matter alleviates this effect (Glover et al., 2005b). Ag is incorporated into *D. magna*, and not merely adsorbed onto its surface (Bianchini et al., 2002a; Bianchini and Wood, 2003a,b). Ag accumulation by neonates is higher in the presence of sulfide. Acute Ag toxicity in *D. magna* is proportional to the whole-body uptake of Na^+ (Bianchini et al., 2002b). Bianchini and Wood (2003a) determined that Ag causes ion-regulatory disturbance resulting in decreased levels of whole-body Na concentration due to the inhibition of Na^+ uptake; in contrast, whole-body Cl^- concentration is unaffected. Exposure to $AgNO_3$ also leads to the inhibition of Na^+, K^+-dependent adenosine triphosphatase (Na^+, K^+-ATPase), as a direct result of Ag accumulation in the body. Inhibition of Na^+ uptake by chronic Ag exposure is explained by the inhibition of Na^+ channels at the apical "gill" membrane (Bianchini and Wood, 2003b).

Diet-borne Ag or Cu (contained in algae grown in metal-contaminated media) contributes no more to *Ceriodaphnia dubia* survival or reproduction than do the equivalent waterborne metals (Kolts et al., 2009). In a solution of $AgNO_3$, *D. magna* accumulate Ag in their gills and digestive tract, but the accumulation is twice as high in the presence of sulfide (Bianchini et al., 2005). The latter fact was explained by the presence of sulfide-bound Ag in the digestive tract. Transfer to clean water for 5 h leads to a significant decrease in Ag concentration in all body compartments. In a solution of $AgNO_3$, *D. magna* can accumulate c. 11 ng Ag/mg WW in 24 h (Glover et al., 2005b).

CHAPTER

4

Nutrition

4.1 FEEDING

Nonpredatory Cladocera feed almost continuously. There is some selectivity and diurnal rhythmicity, however. Food rations are high, but the food does not stay long in the intestine (from c. 7 min). Amazingly, within so short a time Cladocera manage to extract the materials necessary for growth, reproduction, and current metabolism.

4.1.1 Anatomical Background

Cladocera obtain their food by means of their thoracic limbs, but there is a profound difference in the methods of food collection between littoral, planktonic, and predatory Cladocera species. There are two principal patterns: littoral species collect their food and remove extra water from the collected material, whereas planktonic species are filter feeders. Both methods may be combined: it was shown that *Ophryoxus* is able to use both scraping of food from substrata and filtration of food suspended in water (Sergeev, 1970a,b, 1971).

Feeding in Cladocera is inseparably linked to respiration, and it was shown with reference to *Daphnia magna* that the principal part of their oxygen supply is extracted from the feeding current (Pirow et al., 1999a).

Although generally bilaterally symmetrical, Cladocera have asymmetrical mandibles that are controlled by asymmetrical muscles. Fryer (1963, p. 347) observed that "the most important mandibular movements are those of rotation." Because mandibular muscles are asymmetrical, "this enables the two masticatory surfaces to move relative to each other and to exploit to the full their skeletal asymmetry" (Fryer, 1963, p. 351). To an extent, Cladocera crush their food clumps and algal cells with their mandibles but mostly the physical damage to food particles by the mandibles during ingestion is slight, as Hebert (1978a) remarked. For example, in the gut of *Picripleuroxus striatus*, none of the scraped off and ingested frustules of the *Cocconeis* diatom were broken (Smirnov, 1971, Fig. 233). Nevertheless, in the process of ingestion some diatoms are damaged, especially *Asterionella* (Infante, 1973; Lampert, 1978; Fryer, 1991), and *Eurycercus* spp. have been shown to crush large food clumps with their mandibles (Kotov, 1998).

Cladocera That Collect Food From Substrata

Cladocera that collect their food from substrata have formed various specializations of their food-collecting apparatus, as discovered and described for chydorids and macrothricids by Fryer (1963, 1968, 1974, 1991). In general, bottom-living Cladocera (e.g., Fig. 1.1), collect food from the surfaces of various substrata using strong, second thoracic limbs, push the collected mass forward toward the mouth, and remove

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excess water by undulating the exopodites of their posterior limbs. There are further specializations of this feeding activity in different genera and species. The basic (least modified) food-collecting system of the thoracic limbs in chydorids seems to be that present in *Eurycercus* and *Pleuroxus truncatus* (Fryer, 1963, 1968). The methods of food collection used by bottom-living cladocerans are diverse (Fryer, 1963, 1968, 1974); however, information that is more detailed on the physiological aspects of feeding is scarce, in contrast to the planktonic filter feeders.

The food particles collected by littoral Cladocera are glued together by the secretions of their salivary glands (Fig. 4.1) and of glands in the thoracic limbs (the latter are known in *Eurycercus* and *Alonopsis*; Fig. 4.2) and then forwarded to the intestine (Fryer, 1962, 1963, 1968). The salivary gland cells are supplied with nerve endings (Zeni and Zaffagnini, 1988). No attempts have yet been made to check whether the glands in the thoracic limbs are present in various other chydorids and macrothricids; nor is any information available as to whether these secretions contain digestive enzymes.

Littoral Cladocera, especially those which live on various substrata on the bottom of water bodies, exist within a seemingly unlimited food supply of organic debris at all stages of decomposition, as well of algae and bacteria. Fecal pellets containing variously digested organic matter also settle to the bottom and become a part of the detritus. A special place in the littoral zone belongs to some diatoms, mostly epiphytic and attached to substrata. Undoubtedly, therefore, among the bottom-living Cladocera are some types that are physiologically adapted to feeding on organic detritus at various stages of decomposition, from living algae to profoundly decomposed organic matter. Such a sequence of adaptations to different decomposition stages has never been specifically studied. It is likely that different decomposition levels of organic matter supply nutrition for different species of

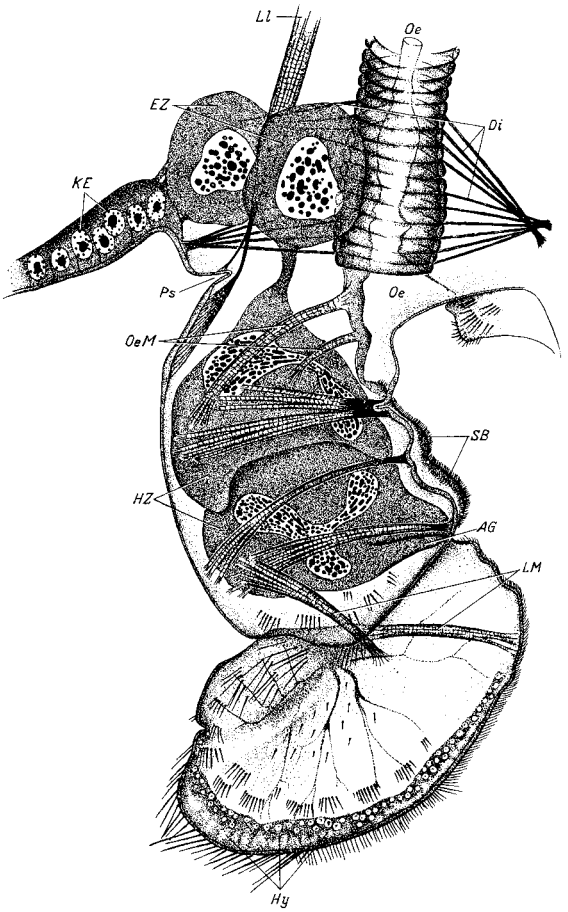


FIGURE 4.1 Labrum of *Daphnia* with salivary glands. *AG*, outlet duct; *Di*, dilators of esophagus; *EZ*, substituting cells; *Hy*, hypoderm of the lobe; *HZ*, principal cells; *KE*, epithelium of the head bottom; *Ll*, levator labra; *LM*, muscles of the lobe; *Oe*, esophagus; *OeM*, muscles of the esophagus; *Ps*, pseudosegment; *SB*, sensory setae. *From Sterba, G., 1957a. Riesenzellen der Daphnien-Oberlippe. Zeitschrift für Zellforschung 47, 198–213.*

Cladocera that are physiologically specialized for their consumption.

The sequence of decomposition in sediments of lakes was described by Rodina (1963, p. 388): "The processes of decay of protoplasm of animal and vegetative tissue and the processes of oxidation and reduction are caused by different physiologic groups of bacteria:

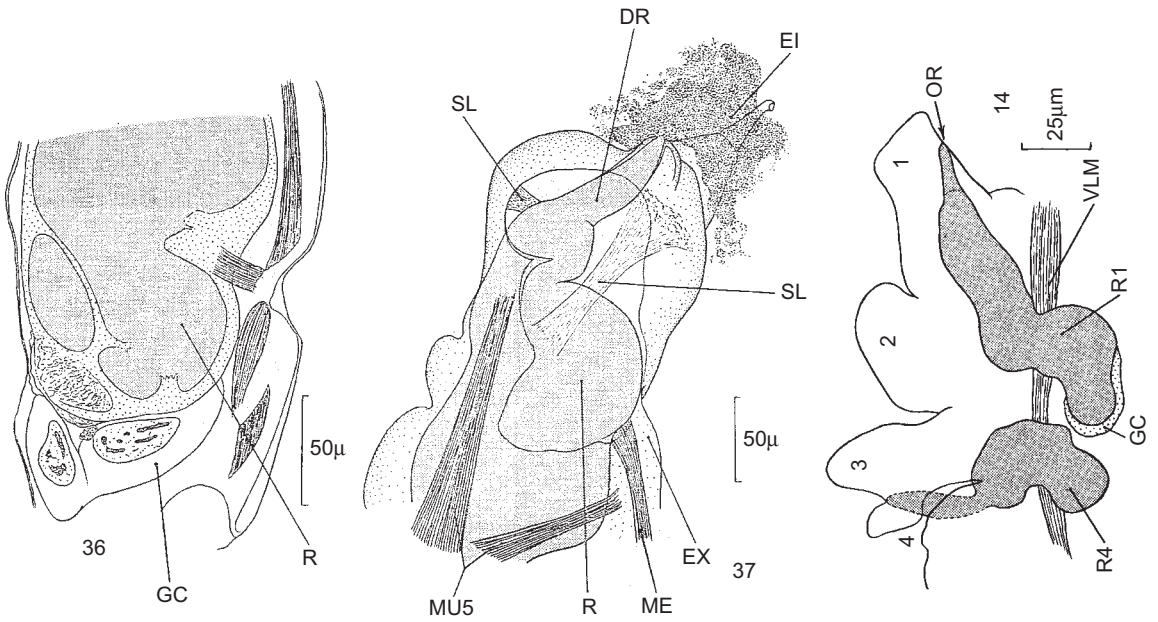


FIGURE 4.2 Slime glands in thoracic limb 4 of *Eurycercus* (*Fryer, 1953*), in thoracic limbs 1—4 of *Alonopsis elongata*. *DR*, duct of reservoir; *ET*, entangling secretion; *EX*, exopodite; *GC*, gland cells; *ME*, muscle to exopodite; *MUS*, muscles; *OR*, opening of reservoir; *R*, reservoir; *SL*, suspensory ligament; *VLM*, ventral longitudinal muscle. *Left and middle, from Fryer, G., 1963. The functional morphology and feeding mechanism of the chydorid cladoceran* Eurycercus lamellatus *(O.F. Müller). Transactions of the Royal Society of Edinburgh 65 (14), 335—381; right, from Fryer, G., 1968. Evolution and adaptive radiation in the Chydoridae (Crustacea: Cladocera): a study in comparative functional morphology and ecology. Philosophical Transactions of the Royal Society of London, B. Biology 254, 221—385; Fryer, G., 1953. An eyless* Daphnia *and a note on its progeny. Naturalist, 118.*

putrefactive, fat and carbohydrate decomposers, amylolytic, pectinous, cellulose decomposers, denitrifying, nitrogen fixers, phosphate liberators, sulfur bacteria, and yeasts."

Unfortunately, there are no relevant investigations, except for observations that in an assembly of collected littoral Cladocera kept in a jar with detritus its constituent species die off gradually and in a certain sequence, which might be related to decomposition of the food material. It has occasionally been observed that *Alonella* sp. remains alive in vessels with detritus for several months when all other companion species have perished.

There are rare but significant observations on feeding of chydorids preferably on fecal pellets of chironomid larvae. *Chydorus sphaericus* was observed attracted by feces of *Chironomus lugubris* and feeding on them (McLachlan et al., 1979). *Paralona pigra* (syn. *Chydorus piger)*, reproduces especially well when feeding on fecal pellets of *Chironomus riparius* ("a highly significant effect" was observed), as well as on natural detritus (Van de Bund, 2000). The latter author noted that in general feeding on chironomid fecal pellets (coprophagy) is important in reproduction of chydorids. *Chydorus sphaericus* are also collected on shells of dead Cladocera (Smirnov, 1971).

Although little is known on the subject, differences in food choice were made use of in combined cultures of *Daphnia* and *Chydorus*; production was improved by *Chydorus* feeding on the fecal remains of *Daphnia* (Bogatova, 1963, 1980).

Littoral *Scapholeberis* and *Dadaya* attach to the surface film of water and collect pollen, minor

particles, and, in all probability, the unimolecular film of organic matter. Filter feeding is performed by sedentary *Sida* and *Simocephalus* spp.

Chydorids and macrothricids can thrive for several months (e.g., Smirnov, 1964, 1965a) when fed periodically with fresh organic debris ("detritus"). Dekker et al. (2006) cultured *Ch. sphaericus* under similar conditions and obtained the best growth using artificial detritus prepared from *Urtica* and diatoms (*Nitzschia*).

Littoral *Lathonura* and *Drepanothrix* spp. may be observed to remain motionless for hours, lying on their backs and making no other movements than filter beating their thoracic limbs.

Cladocera That Collect Food Suspended in Open Water

In contrast to those living in the littoral zone, pelagic Cladocera periodically suffer food shortages or drastic seasonal changes in food quality. In filter-feeding (mostly planktonic) anomopods (Fryer, 1991) and in ctenopods (Storch, 1929), food particles suspended in the outer medium are filtered by filtering fans consisting of setae on thoracic limbs (e.g., Fig. 1.2). In anomopods, the filtering fans are formed by gnathobasic setae; in ctenopods, this is done by setae on endites in combination with a smaller area of gnathobasic setae. In daphnids, slime glands in thoracic limbs are not reported (Fryer, 1991) and are probably absent.

Planktonic Cladocera consume any algae that are present in the water, as well as organic particles and bacteria. The collected food is directed into the mouth opening, where it is formed into a conveniently narrow flow by rotation of the mandibles.

For pelagic Cladocera, there is obviously a gradation of food, from fresh or recently dead algae, as well as fecal pellets, to all stages of decomposition, potentially with specialized consumers. Pelagic Cladocera (*Daphnia*) reproduce successfully when feeding on algae (less efficiently when feeding on blue-green algae, bacteria (*Daphnia*, *Ceriodaphnia*, *Bosmina coregoni*, *Ch. sphaericus*, and *Polyphemus*), and yeast (*Daphnia*)) (Rodina, 1950).

Ostapenya and Pavlyutin (1968) observed feeding of *D. magna* on detritus prepared from algae or zooplankton at different stages of its decomposition. During 97 days, the content of organic matter and calorific value gradually decreased. It was found that *D. magna* used algal detritus at initial stages of its decomposition only.

Coprophagy in pelagic Cladocera was studied by Pilati et al. (2004). These authors obtained ^{14}C-labeled feces by feeding zooplankton with ^{14}C-labeled algae. A considerable clearance rate was found for such fecal matter: 0.084–0.089 mL/mg per h in *Holopedium gibberum* and 0.026 mL/mg per h in daphnids. These authors conclude that coprophagy is an important aspect of nutrient recycling in the pelagial zone.

Carnivorous Species

In contrast to most cladocerans, the littoral chydorid *Pseudochydorus globosus* is carnivorous and feeds on the dead bodies of cladocerans, whereas *Anchistropus* is specialized for feeding on living hydra (as recently observed by Van Damme and Dumont, 2007).

Predatory (carnivorous) Cladocera are also present in the pelagic zone. These include *Leptodora*, *Bythotrephes*, and *Polyphemus* spp., the latter being common in patches of open water in the littoral zone. These species successfully complete their life cycles when feeding on this food alone, but nothing is known of any special traits of their nutritional physiology.

4.1.2 Environmental Background and Food Resources

Extensive modern investigations are preceded by thorough studies of the food of planktonic Cladocera by Naumann (1918, 1921). He examined the filtering apparatus of various species, gut contents, and "filtrates ante os," i.e., food accumulated before swallowing.

Algal Food

In almost all waters, algae are available in quantities but the composition of their populations depends on season, latitude, and the type of water body. There are various sizes and types of algae, from those present as small cells to those forming clumps or filaments. Frequently, algae are covered with slime. In freshwaters, the dominant groups of algae are green algae (Chlorophyta), diatoms (Bacillariophyceae), Chrysophyceae, Xanthophyceae (Heterocontae), dinoflagellates (Pyrrophyta), cryptomonads, and blue-greens (the latter, with good reasons, are now considered cyanobacteria), differing in trophic qualities.

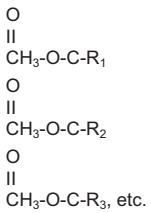
Generally, monosaccharide is produced by photosynthesis and is then is transformed, either into polysaccharides or into glycerol and saturated and unsaturated fatty acids. Glycerol combined with fatty acids yields fat. The schematic pathway from initial photosynthetic saccharides to lipids and amino acids in algae (Arts et al., 1997) is shown in Fig. 4.1.

The chemical composition of algal populations depends on the specific physiology of particular groups of algae.

The amino acid composition of Chlorophyceae (*Scenedesmus*, *Chlorella*, and *Chlamydomonas*), *Chromulina*, *Cryptomonas*, *Rhodomonas*, *Peridinium*, *Peridinopsis*, and various blue-greens was reported by Ahlgren et al. (1992). These authors (Ahlgren et al., 1992) also determined the fatty acid composition of these algae. The polyunsaturated fatty acid (PUFA) compositions of different organisms are shown in Tables 4.1–4.3.

ALGAL LIPIDS

Lipids are derived by Cladocera from various groups of algae, and different algae vary in their value as lipid sources for Cladocera (Fig. 3.3). Although diatoms supply the bulk of lipids for their consumers, their lipids are not necessarily the best types for Cladocera. The fatty acid composition of different algae is shown in Tables 3.9, 3.10, and in Fig. 4.3. A high content of lipids in freshwater diatoms, i.e., 13.6% dry weight (DW), has been determined by Birge and Juday (1922, p. 164): "The diatom sample contained a larger percentage of ether extract than any other sample of plant material." It may attain higher values (35% according to Barashkov, 1972), mostly consisting of unsaturated fat. According to Farkas (1970), these lipids are rich in 14:0 and relatively poor in 18:2 and 18:3 fatty acids.



Cyanobacteria (blue-green algae) are also a source of lipids for Cladocera. Based on extensive observations and analyses, Sushchik et al. (2002) concluded that the development of planktonic *Daphnia longispina* and *Daphnia cucullata* mostly depends on 18:3ω3 produced by cyanobacteria. However, at the same time, blue-greens contain compounds that are toxic for Cladocera (see Section 4.2.4).

Of the planktonic algae, the best food items for *D. longispina*, *Bosmina*, and *Ch. sphaericus* are cryptomonads, with *Cryptomonas* and *Rhodomonas* (class Cryptophyceae, division Pyrrophyta) containing high percentages of the PUFAs 20:5ω3 and 22:6ω3 (docosahexaenoic acid) (Ahlgren et al., 1990). These PUFAs were also found common in fish.

It was found that in *Daphnia obtusa* fed with the green alga *Scenedesmus* the content of lipid in the body does not positively correlate with growth rate (Sterner et al., 1992). *Daphnia pulex* fed with a blue-green *Aphanizomenon* did not accumulate oil drops, although this cyanobacterium is not directly toxic for them; on the other hand, when fed with the green protococcous alga *Ankistrodesmus*, *D. pulex* did accumulate oil drops [Lipid Index (LI): 0 and 3, respectively] (Holm and Shapiro, 1984). *D. pulex* fed on a

TABLE 4.1 The Fatty Acid Composition of Some Freshwater Algae, as Percentage of Total Fatty Acids

Fatty Acids	Green Algae	Diatom *Cyclotella*	Blue-Greens	Chrysophyceae *Chromulina*	Cryptophyceae	Dinoflagellates *Peridinium*
14:0	0.7–2.3	2.0	2.1–17.0	11.1	1.7–2	7.6
16:0	9.8–20.4	11.6	18.5–29.1	5.0	11.1–13	29.8
16:1ω7	0.3–1.1	5.2	1.8–22.6	2.1	0.3–0.6	2.1
18:0	0.6–4.2	3.3	1.6–1.9	1.5	0.7–1.3	0.4
18:1ω9	5.0–36.5	–	1.9–3.2	1.3	1–1.1	30.0
18:1ω7	0.2–1.8	–	0.5–2.5	2.3	0.6–2.1	0.4
18:2ω6	6.7–22.1	–	2.9–11.4	7.1	0.9–1.0	0.5
18:3ω6	02–0.4	3.7	0.3	3.4	–	–
18:3ω3	14.9–37.2	–	22.8–24.6	6.6	9.7–21.3	0.2
18:4ω3	2.5–3.4	–	2.5	26.6	21.8–24	4.3
20:0	0.1–0.2	–	–	–	–	–
20:1ω9	0.3	–	–	2.4	0.7	–
20:3ω6	–	–	–	0.4	–	–
20:4ω6	–	0.5	–	0.6	–	–
20:5ω3	–	35.1	0.6	0.6	15.8–20.5	6.9
22:0	–	–	–	–	–	–
22:4ω6	–	–	–	0.4	–	–
22:5ω3	–	3.1	–	–	–	–
22:5ω6	–	–	–	9.9	2.7–4.7	–
22:6ω3	–	6.5	–	2.5	4.3–7.2	12.2

–, none or <0.1%.

From Ahlgren, G., Lundstedt, L., Brett, M., Fosberg, C., 1990. Lipid composition and food quality of some freshwater phytoplankton for cladoceran zooplankters. Journal of Plankton Research 12 (4), 809–818; Cyclotella, from Desvilettes, C., Bourder, G., Amblard, C., Barth, B., 1997. Use of fatty acids for the assessment of zooplankton grazing on bacteria, protozoans and microalgae. Freshwater Biology 38, 629–637.

green alga *Scenedesmus* demonstrated better growth and reproduction than those fed on a blue-green (*Microcystis*) what was attributed to shortage of eicosapentaenoic acid (EPA) in the latter (Ravet et al., 2012).

Brett et al. (2006) studied the fatty acid composition of *D. pulex* fed on cryptomonads *Cryptomonas* and *Rhodomonas*, Chlorophyta, and cyanobacteria and found that it generally matched that of their algal food. However, there were some differences: in comparison with their food, the content of saturated fatty acids in *Daphnia* was substantially less and that of arachidonic acid was higher. According to these data, *Daphnia*, on the whole,transfers somewhat modified fatty acids up the food chain (Fig. 4.4).

The accumulation of arachidonic acid by *Daphnia* monitored in a European lake increased from early summer to autumn, even when the dietary supply decreased, thus indicating that its requirements are constant (Hartwitch et al., 2013).

Not all PUFAs have a positive role: γ-linolenic acid (a PUFA obtained commercially for

TABLE 4.2 Fatty Acid Composition of Some Algae, as μg per mg C

Fatty Acids	Blue-Greens (*Anabaena, Microcystis, Synechococcus*)	*Cryptomonas* sp.
16:0	25.7–60.0	22.1
16:1ω7	1.9–43.7	6.0
17:1ω7	0.7–1.3	0.9
18:0	1.7–4.8	4.7
18:1ω9	0.9–6.96	4.9
18:1ω7	0.6–3.3	8.0
18:2ω6	4.3–16.7	17.4
18:3ω6	30.6–33.1	0.9
18:3ω3	5.9–93.0	75.3
18:4ω3	1.6–11.9	42.7
20:1ω9	nd	1.2
20:4ω6	nd	0.6
20:3ω3	nd	0.8
20:5ω3	nd	32.2
20:6ω3	nd	3.96

*Modified after Martin-Creuzburg, D., von Elert, E., Hoffmann, K.H., 2008. Nutritional constrains at the cyanobacteria-*Daphnia magna *interface: the role of sterols. Limnology and Oceanography 53 (2), 456–468.*

experiments) is acutely toxic (!) for *D. magna* at a concentration of 9 μg/mL (Reinikainen et al., 2001).

THE PHYSIOLOGY OF DIATOM PHOTOSYNTHESIS

Of all the algal groups, diatoms (Bacillariophyceae) are the most prominent known producers of lipids. They have a very peculiar metabolism: they transform the bulk of the initial monosaccharide produced in photosynthesis into lipids (with the addition of some protein, pigments, and vitamins); they live in siliceous ("glass") frustules; and they do not synthesize much starch and cellulose from the primary products of photosynthesis. In contrast, they can exploit the reaction of fat formation probably of the kind generally characteristic of vascular plants and their metabolism is largely confined to this process.

From general plant physiology it is known that from the initial photosynthetic product fatty acids are synthesized by two enzyme systems; acetyl coenzyme-A (CoA) carboxylase and fatty acid synthase.

There are hints that in diatoms the controlling mechanism that channels photosynthesis to lipid formation must be the same, as acetyl CoA carboxylase correlates with lipid accumulation (Sheehan et al., 1998). Allen et al. (2011, p. 2)

TABLE 4.3 General Fatty Acid Composition of Some Algae, as a Percentage of Total Lipids

Fatty Acids	Diatoms: *Cyclotella*	Green Algae: *Pediastrum*	Dinoflagellates: *Peridinium*	Chrysophyceae: *Chromulina*	Cryptophytes: *Cryptomonas*	Blue-Greens: *Anabaena*
Saturated	17.7	21.5	36.4	17.6	15.1	40.5
Monoenes	10.7	12.0	30.6	8.1	3.0	18.6
Total PUFA	71.6	66.0	24.9	57.7	64.9	51.3
Total ω3	62.0	53.5	24.3	36.3	59.2	38.3
Total ω6	4.8	9.5	0.6	21.4	5.7	9.8

PUFA, polyunsaturated fatty acid.
From Desvilettes, C., Bourder, G., Amblard, C., Barth, B., 1997. Use of fatty acids for the assessment of zooplankton grazing on bacteria, protozoans and microalgae. Freshwater Biology 38, 629–637.

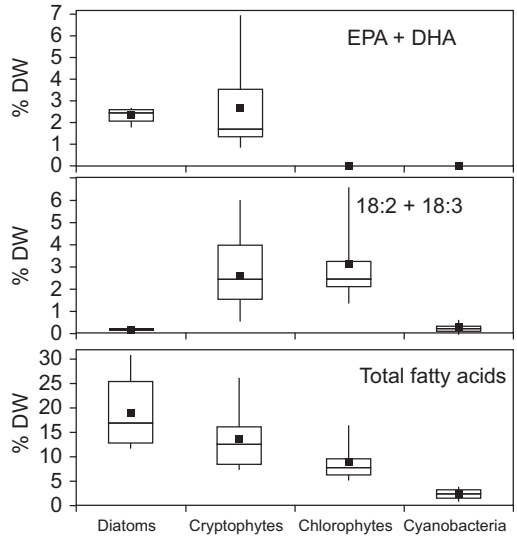


FIGURE 4.3 Content of highly unsaturated fatty acids in dominant groups of planktonic algae. *DHA*, docosahexaenoic acid; *EPA*, eicosapentaenoic acid. *From Brett, M.T., Müller-Navarra, D.C., 1997. The role of highly unsaturated fatty acids in aquatic foodweb processes. Freshwater Biology 38, 483–499.*

indicate that in diatoms fructose 1,6-biphosphate aldolases in glycolytic reactions "…split C_6 molecules into C_3 molecules, which are transformed into pyruvate. Pyruvate can then be transformed to acetyl CoA and oxidized to CO_2 through the Krebs cycle in the mitochondria or enter biosynthetic pathways such as fatty acid or amino acid biosynthesis." Writing specifically on photosynthesis of diatoms, Rabinovich (1945, 1951) commented that "the oil deposits of the diatoms … must be considered as products of comparatively slow secondary transformations" (1945, p. 43) of primary photosynthesis products; no other details are given. Some general considerations on the fate of initial products of photosynthesis in leaves of vascular plants are given by Sharapov (1959).

In the freshwater diatom *Navicula pelliculosa*, the total lipid content includes the following major components: sulfoquinosyl diglyceride, digalactosyl diglyceride, monogalactosyl diglyceride, phosphatidyl glycerol, lecithin, and phosphatidyl inositol (similar to green algae). The major fatty acid constituents are palmitoleic acid, palmitic acid, EPA, and eicosatetraenoic acid (Kates and Volcani, 1966). The concentration of linolenic acid (octadecatrienoic acid), on the other hand, is low. Opute (1974) confirmed that (1) the lipids of freshwater diatoms (*Nitzschia* and *Navicula*) consist mainly of triglycerides, monogalactosyl diglyceride, digalactosyl diglyceride, sulfoquinovosyl diglyceride, phosphatidyl glycerol, lecithin (phosphatidylcholine), and phosphatidylethanolamine; and (2) the major fatty acids are palmitoleic, palmitic, EPA, and eicosatetraenoic acids, with 16:0 and 16:1 prevailing. Volkova (1980) also studied fatty acids in the freshwater diatoms *Stephanodiscus* and *Melosira* and discovered a prevalence of palmitic acid (16:0; 12.4–31.8% of total fatty acids) and palmitoleic acid (16:1; 23.3–31.7%). The unsaturated acid content is 53.6% and 36%, respectively, and the saturated acid content is 21.2% and 35.1%, respectively.

Accumulation of the essential, unsaturated fatty acids 20:5ω3 and 22:6ω3 in benthic (periphytonic) diatoms from March to January was assessed by Gladyshev et al. (2005). They found that the maximum of both types, c. 40 $\mu g/m^3$, occurs in August. Sushchik (2006) noted that some diatoms (*Cyclotella*) are not rich in essential PUFAs and that some bluegreens (*Anabaena*) have a high content of these fatty acids, contrary to the generally accepted viewpoint, and thus are an important source.

Freshwater diatoms also produce polyunsaturated aldehydes when damaged, such cultures of diatoms generally had no negative effect on reproduction of *Daphnia pulicaria* (Carotenuto et al., 2005).

In some lakes (e.g., Myvatn and Kronotskoe), diatoms are highly dominant: as evidence, the ground surrounding the lakes consists almost entirely of diatom frustules (diatomite). Thus, the trophic chain in such lakes is based principally on the lipids produced by diatoms.

Some other groups of algae also synthesize lipids, but any sufficient information on their

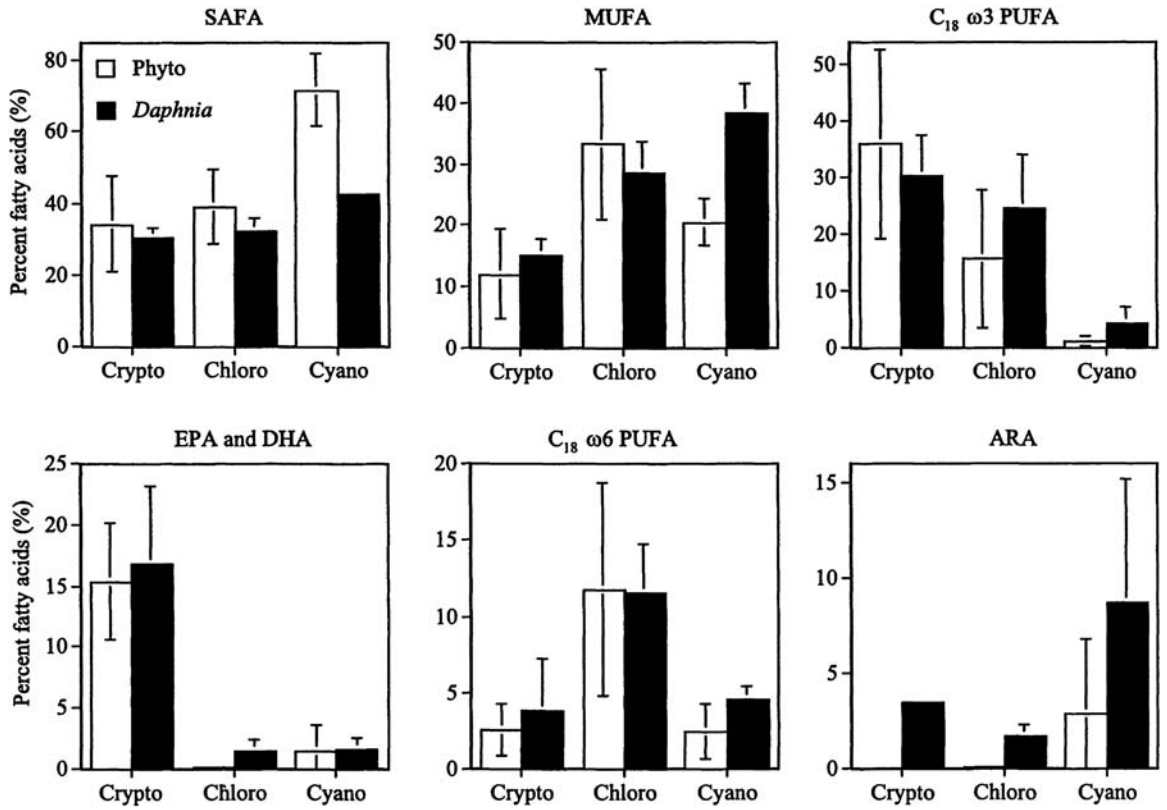


FIGURE 4.4 Fatty acid composition in *Daphnia* consuming various kinds of phytoplankton and in phytoplankton groups. *ARA*, arachidonic acid (20:4ω6); *Chloro*, Chlorophyceae; *Crypto*, Cryptophyceae; *Cyano*, blue-greens; *DHA*, docosahexaenoic acid (22:6ω3); *EPA*, eicosapentaenoic acid (20:5ω3). *From Brett, M.T., Müller-Navarra, D.C., Ballantyne, A.P., Ravet, J.L., Goldman, C.R., 2006.* Daphnia *fatty acid composition reflects that of their diet. Limnology and Oceanography 51 (5), 2428–2437.*

metabolism and accumulation of stock products is lacking. Green algae may be considered similar to green vascular plants as the monosaccharide produced by their photosynthesis is then transformed mainly into polysaccharides. They stock starch and contain little fat, the content of which increases in declining populations of green algae (von Dehn, 1950, 1955).

Algae from other divisions may be highly specific in their physiology and their metabolic products. However, little is known about physiology and metabolic products of algae from other divisions. Diatoms are not alone in having the peculiar trait of not producing and not accumulating starch. Fogg (1953, p. 105) noted that "starch evidently does not occur in the Xanthophyceae, Chrysophyceae or Bacillariophyceae, and the same three classes have a general tendency to store fats as reserve materials rather than polysaccharides." Sedova (1977) added that diatoms and dinoflagellates are especially rich in lipids.

Particular groups of algae are characterized by specific spectra of fatty acids (Sushchik, 2008). *Scenedesmus* (a green alga) is especially rich in 16:0, 18:1ω9, and 18:3ω3 (Masclaux et al., 2009),

whereas *Cryptomomas* is rich in 18:3ω3, 18:4ω3, and 20:5ω3 (Martin-Creuzburg et al., 2008; Maslaux et al., 2009) (Tables 4.1 and 4.2).

Dinoflagellates are known to contain starch grains, oil drops, and glycogen (Kiselev, 1950). Although there is rather extensive information on physiology of green algae and of diatoms, it seems that there are just occasional fragments on physiology of other major divisions (phyla!) of algae: dinoflagellates, cryptophyta, xanthophyta, etc. Meanwhile, physiology of Crustacea demands data on specific metabolic and stock products of particular groups of algae, some of which may travel along the trophic pathways as physiologically important compounds.

Blue-greens. Blue-greens are a major, almost ever-present component of freshwater life. Blue-greens are characterized by a low EPA (20:5ω3) content (Müller-Navarra et al., 2000; Martin-Creuzburg et al., 2008; Ravet et al., 2012) (Table 4.2) and a very low sterol content (von Elert and Franck, 1999; Martin-Creuzburg et al., 2008) which constrain the growth and reproduction of Cladocera. The nutritionally useful constituents of blue-greens are combined with toxins.

As demonstrated with reference to *D. pulex*, blue-greens are consumed as food but survival and reproduction are worse than in the case of feeding on green algae; in the absence of other food the blue-greens mostly did not support the population of *Daphnia* (Arnold, 1971) (Fig. 4.5).

In *Daphnia galeata* (Rohrlack et al., 2005), it was found that microcystins ingested with *Microcystis* from the gut were rapidly taken up into the blood, probably due to disruption of the gut epithelium. Then the beat rates of thoracic limbs, mandibles, second antennae, as well as the activity of the foregut decreased but the activity of muscles of the midgut was stimulated. Ingestion of 10.2–18.3 ng of microcystin per 1 mg wet weight (WW) of *D. galeata* killed them within 2 days.

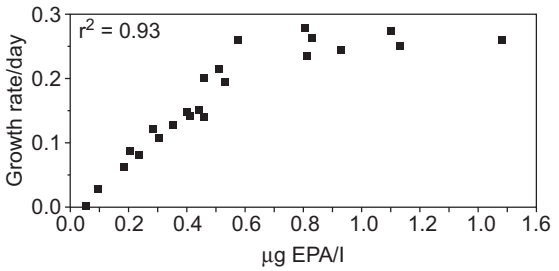


FIGURE 4.5 Relationship between available eicosapentaenoic acid and *Daphnia* growth (Schösee). *EPA*, eicosapentaenoic acid. *From Brett, M.T., Müller-Navarra, D.C., 1997. The role of highly unsaturated fatty acids in aquatic foodweb processes. Freshwater Biology 38, 483–499.*

In the course of decomposition, *Microcystis* becomes increasingly more suitable for growth of *Daphnia similoides* (Luo et al., 2015).

It was found that negative effects of microcystin in *D. magna* were connected with negative impact on lactate dehydrogenase leading to a low concentration of lactate; oxidative protection was more efficient in neonates (a higher catalase activity) (Ortiz-Rodríguez and Wiegand, 2010). Along with inhibition of growth and reproduction cyanobacterial toxins were shown to cause malformation of neonates of *D. magna* (Dao et al., 2010).

Experimenting with *D. magna*, von Elert et al. (2012) found that the blue-green *Microcystis aeruginosa* contains two chymotrypsin inhibitors and *D. magna* is able to develop increased tolerance to these inhibitors within its lifetime. Its chymotrypsins varied in mass depending on proportion between green algae and blue-green in the food. It is supposed that the mechanism of increased tolerance may be responsible for transfer of tolerance to the next generation. *D. magna* exposed to microcystin-LR produced young showing increased survival due to elevated activity of glutathione S transferase (GST), malate dehydrogenase, and catalase (suggesting maternal transfer) (Ortiz-Rodríguez et al., 2012).

It may be also mentioned that plastids from blue-greens were found in gut endocytes of *D. obtusa* (Chang and Jenkins, 2010).

Bacterial Food

Along with other food items, Cladocera consume bacteria. They are also internally populated by bacteria, including in their intestines. To solve the question as to whether bacteria are an obligatory food component, it was necessary to develop methods for sterilizing Cladocera. To achieve this aim, Gajewskaja (1938) maintained *Daphnia* and *Bosmina* in a 0.1% solution of rivanol (ethoxydiaminoacridine lactate) and obtained a high percentage of sterile cladocerans.

It was shown that feeding on bacteria, in combination with algae, supports a long series of parthenogenetic generations of *D. magna*; in contrast, cultures failed if fed either on bacteria or on sterile algae (Gajewskaja, 1940, 1948). The same conclusion was made by Taub and Dollar (1968) for *D. pulex* and Tezuka (1971) concluded that *Daphnia* could not survive when fed on bacteria alone. A considerable role of bacteria in feeding of *Simocephalus* and *Scapholeberis* is confirmed by Naberezhnyi and Kriventsova (1974).

Heterotrophic bacteria weakly support *D. magna*, but a culture developed well when supplemented with cholesterol or PUFAs (Martin-Creuzburg and Heilke, 2011). A pure bacterial diet (*Pseudomonas*, possessing a much lower PUFA content) did not support *D. galeata*; addition of 20−50% of *Rhodomonas* (a cryptomonad) provided for its successful reproduction (Wenzel et al., 2012). Martin-Creuzburg and Heilke (2011) believed that *Pseudomonas* and *Hydrogenophaga* are toxic for *D. magna*.

It has been shown that *D. magna* feed efficiently on suspended bacteria of size 0.1−1 μm, but for *D. galeata*, *Daphnia hyalina*, and *D. pulicaria*, feeding is less efficient (Gophen and Geller, 1984).

Daphnia consumes methanotrophic bacteria using methane-derived carbon (C) (Kankaala et al., 2006; Taipale et al., 2007; Deines and Fink, 2011).

Enteropathogenic nonagglutinating vibrios are consumed by *D. magna* but do not cause pathologies and are digested as food (Avtsyn and Petrova, 1986).

Overall it has been determined that bacteria may make up to 87% of the total carbon ingestion of *D. galeata* (Kamjunke et al., 1999). As well, it was shown that under conditions of mixed feeding (bacteria and algae), *D. magna* obtained 80% of its carbon from bacteria and in this situation they assimilated c. 46% of their fatty acids from bacteria (Taipale et al., 2012).

Favorable phosphorus-to-carbon ratio (P:C) in food was lower for *D. pulex* than for *Ceriodaphnia quadrangula*, i.e., *Ceriodaphnia* was disfavored by P-poor algae and showed a higher growth rate at addition of bacteria to the diet (Iwabuchi and Urabe, 2010).

Lampert (1987) demonstrated that *Daphnia* grow but do not reproduce when fed on bacteria. *D. magna* fed solely on bacterial diets die after 5−12 days (Taipale et al., 2012). Heterotrophic bacteria are scarce in sterols and thus limit growth; if fed on bacteria supplemented with cholesterol, *D. magna* manifested increased somatic growth Martin-Creuzburg et al. (2011).

Hydrogenophaga sp. and *Pseudomonas* sp. were toxic for *D. magna* (Martin-Creuzburg et al., 2011).

Organic Debris (Detritus)

Organic debris suspended in water and settling on the bottom of water bodies consists of decomposing algae, decomposing feces, and the bodies of dying animals, as well as all kinds of allochthonous material. Detritus is enriched with lipids from settling dead bodies of animals and algae. Detritus also harbors bacteria. Organic debris forms a major component of the food of littoral and pelagic Cladocera, as noted originally by Naumann (1918, 1921). Detritus with small living algae and animals suspended in water is termed seston.

The chemical composition of lake silt is shown in Table 4.4. Silt of Dubossary Reservoir (Moldova) contained vitamin B_{12} at a rate of 0.015–0.042 μg/g WW (Stepanova and Borshch, 1970). Proteins and carbohydrates in the detritus (debris) are decomposed by enzymes in the feces of various animals, as has been convincingly shown for fish by Kuzmina et al. (2010), as well as by bacteria. "Old," i.e., profoundly decomposed debris does not support *Daphnia* in culture (Lampert, 1987).

The seasonal periodicity of amino acid composition of organic bottom material may be a contributory factor in the periodicity of littoral Cladocera. Although not explored further, preliminary determinations by the author have demonstrated that various amino acids are present in organic bottom material, some of which are abundant and some present in low concentrations.

Some authors have attempted to investigate the food quality of littoral organic debris and its changes over time. Robertson (1990) estimated the content of organic matter, chlorophyll a, and phaeopigment in the River Thames mud in relation to the population dynamics of various cladocerans. The content of organic matter was higher (up to 16%) in February–April and lower in May–September (4–8%); chlorophyll a levels increased throughout the season, from c. 100 mg/L/m^2 in February to over 300 mg/L/m^2 in October. The phaeopigment content, a measure of algal decomposition, demonstrated no definite trend, fluctuating between 300 and 50–150 mg/L/m^2. The amino acid composition of seston (plankton + detritus) in small water bodies of the Yenissey Basin was studied by Kolmakova (2010) (see Table 4.4).

With reference to the eutrophic Lake Nesjøvatn (in Norway), it was shown that *D. galeata* obtain carbon principally from algae and detritus and phosphorus principally from algae (only a minor part of total phosphorus is obtained from bacteria) (Vadstein et al., 1995). In littoral *Eurycercus* and *Simocephalus* (Fig. 4.6), the prevailing fatty acids corresponded to those prevailing in the littoral particulate matter (Desvilettes et al., 1994). For planktonic *D. longispina*, it was shown that its phospholipid fatty acid profile and that of seston corresponded "rather well" in a dystrophic lake (Taipale et al., 2009) (Fig. 4.7).

Even when there is a considerable inflow of terrestrial organic matter, *Daphnia* derive most of their carbon from phytoplankton (Brett et al., 2009). However, this source of food may be important for littoral species. Taipale et al. (2015) demonstrated that the food of terrestrial origin is characterized by prevalence of saturated fatty acids (SAFA), especially long-chain ones (20:0, 22:0, 24:0, 26:0, 28:0), little assimilated by *D. magna* (Table 4.5). The food of terrestrial origin has a low ω-3:ω-6 ratio (0.3–1.6) retained by *Daphnia*. Low ω-3:ω-6 ratios in *Daphnia* indicate poor nutritional conditions.

Daphnia thrive when fed on organic debris prepared from algae or vascular plants. Good growth and reproduction of *D. magna* and *D. longispina* was obtained by using detritus prepared from phytoplankton treated with diluted

TABLE 4.4 The Chemical Composition of the Bottom Silt in Two Russian Lakes

Lake	Sugars, Hemicelluloses, Pentosans	Cellulose	Waxes and Resins	Fatty Acids	Organic Carbon	Total Nitrogen	Ash
Glubokoe	1.0	2.2	2.3	0.2	11.9	1.1	74.2
Beloe	7.1	6.4	6.5	1.0	27.0	2.5	46.4

Values represent % absolutely dry matter.
From Shcherbakov, A.P., 1967. Lake Glubokoe. Nauka, Moscow. 379 p. (in Russian).

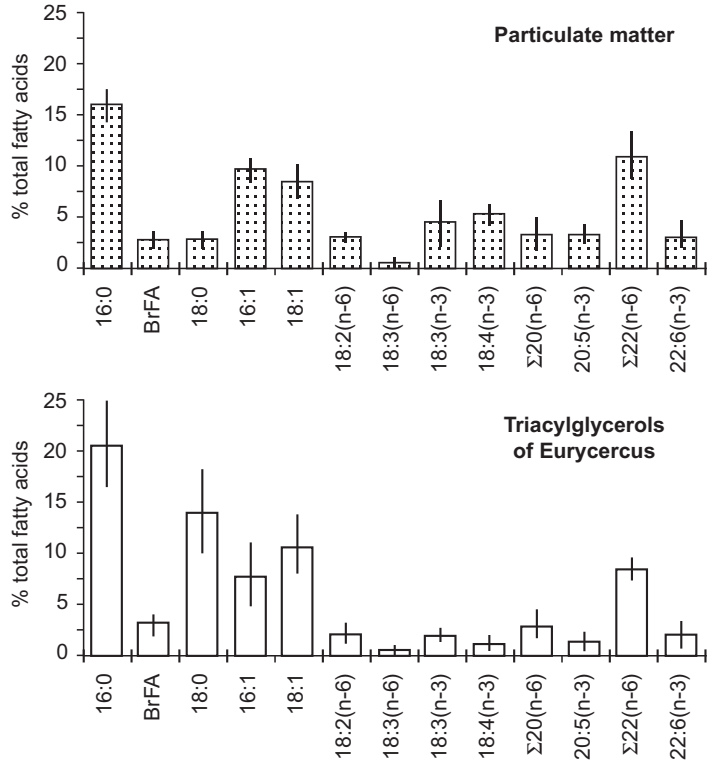


FIGURE 4.6 Fatty acid composition in *Eurycercus* and in its particulate food. *From Desvilettes, C., Bourder, G., Amblard, C., Barth, B., 1997. Use of fatty acids for the assessment of zooplankton grazing on bacteria, protozoans and microalgae. Freshwater Biology 38, 629–637.*

Lugol's solution to minimize the quantity of bacteria (Esipova, 1969).

It has also been shown experimentally that *Daphnia* assimilate some dissolved organic matter (e.g., amino acids), but this alone is insufficient for their normal lifestyle (Rodina, 1946, 1948, 1950).

4.1.3 Feeding Characteristics

Quantitative

RATE OF MANDIBLE ROLLING

Cladocera eat almost continuously. The rate of movement of the mandibles is c. 250 beats/min in *Leydigia leydigii* (Fryer, 1968), 177 beats/min in *Streblocerus serricaudata* (Fryer, 1974), and 110–190 beats/min in *Daphnia rosea* (in direct proportion to yeast concentration) (Burns, 1968a). In *P. globosus*, "rolling and swinging take place too rapidly to permit visual determination of the rate" (Fryer, 1968, p. 335).

In *D. pulex*, Starkweather (1978) found well-defined daily patterns in the rate of mandible rolling, with distinct nocturnal minima. In the daytime, the rate of mandibular beating in *D. pulex* was c. 140 beats/min, whereas at night (01:00–07:00) it was c. 80 beats/min. This rate decreased in larger specimens, from 180 beats/min for a body length of 2.0 mm to 80 beats/min for a body length of 3.4 mm. It increased in *D. magna* with algae concentration in the environment from c. 55 to c. 95 beats per min (Porter et al., 1982).

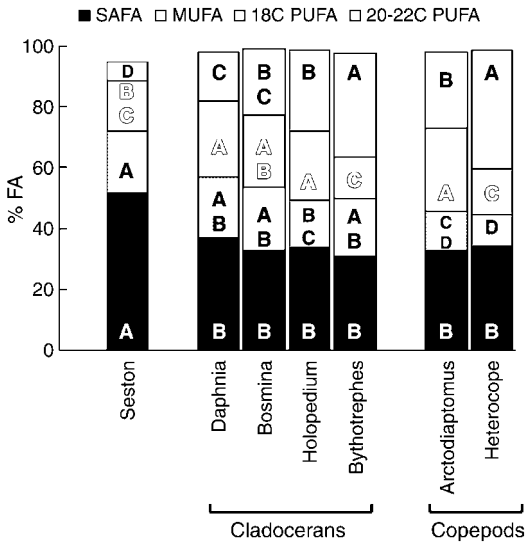


FIGURE 4.7 Fatty acid (FA) composition in planktonic cladocerans and in their seston food. A–D denote taxa that differ significantly in the content of the respective fatty acid group. SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. *From Persson, J., Vrede, T., 2006. Polyunsaturated fatty acids in zooplankton: variation due to taxonomy and trophic position. Freshwater Biology 51, 887–900.*

According to Murtaugh (1985), the rate of mandible movements in *Daphnia* may be taken as a measure of feeding intensity. In *D. pulicaria*, it ranges from a mean of 1.3 Hz (beats/sec) at a lower food concentration to 2.1 Hz at a higher food concentration.

WATER FLOWS CREATED BY THORACIC LIMBS

In Cladocera, water flows created by the thoracic limbs are important for both feeding and respiration. In Anomopoda and Ctenopoda the thoracic limbs move in a metachronal rhythm, i.e., with a certain phase lag for the movement of every subsequent pair (Cannon, 1933). Applying high-speed cinematography, Gophen (2014) determined in *D. magna* two alternatively operating internal water flows: (1) the lateral water flow directed from the second pair of thoracic limbs along the carapace edge and (2) the median one proceeding between thoracic limbs. Such situation is interpreted as a mechanism of alleviation of external water pulses making the animal less noticeable.

The stroke rate of the exopodites of thoracic limbs, which creates a feeding and respiratory current, is 170 cycles/min in *Ch. sphaericus*, over 300 cycles/min in *D. longispina*, 60–244 cycles/min in *Ceriodaphnia* (Harnisch, 1949), 150–470 cycles/min in *D. magna* (McMahon, 1968), and 290–300 cycles/min in chydorids (Smirnov, 1971). In different pelagic Cladocera, the rate of thoracic limbs generally decreases with size from c. 16 Hz (beats/sec) to c. 4 Hz (Porter et al., 1982).

TABLE 4.5 Percentage Fatty Acid Content of Freshwater Seston, Diatoms, *Daphnia* spp., and Fish

Fatty Acids	Seston (Persson and Vrede, 2006)	Diatoms: *Cyclotella* (Desvilettes et al., 1997)	*Daphnia* (Persson and Vrede, 2006)	Fish: *Coregonus* (Sushchik, 2008)
Total SAFAs	51.8	17.7	37.1	–
Total MUFAs	20.2	18.6	19.9	–
Total ω3 (PUFAs)	16.8	62.0	31.2	17.0
Total ω6 (PUFAs)	6.1	4.8	9.8	5.4
Total PUFAs	22.9	71.6	41.0	22.4

Values represent % of total fatty acid content. *MUFAs*, monounsaturated fatty acids; *PUFAs*, polyunsaturated fatty acids; *SAFAs*, saturated fatty acids.

The feeding current is maneuverable. At too high a food concentration, *D. magna* decreases the rate of strokes of its thoracic limbs and discards the food collected in the food groove if the intestine is full (Forsyth et al., 1992). Inhibiting the effect of too high food concentrations is pointed out by Sushchenya et al. (1990). According to Lampert and Bredelberger (1996), at low concentrations of food particles, *Daphnia* adapts to low food levels by slightly decreasing the appendage beat rate (from c. 300–550 to 300–450 beats/min in *D. pulicaria*); simultaneously, phenotypical enlargement of the area of its filtering screens occurs. The rate of strokes of the thoracic limbs varies both individually and under different environmental conditions (e.g., in *Daphnia*) (Peñalva-Arana et al., 2007).

The rate of strokes increases at decreased oxygen concentrations as shown in *D. pulex* (Wolvecamp and Waterman, 1960). In *Daphnia carinata*, the beat frequency of the thoracic limbs is 327 beats/min for a body length of 0.6 mm; this increases slightly to 360 beats/min for a body length of 2.15 mm, and then decreases to 310 beats/min for a body length of 2.6 mm.

The beat frequency decreases by c. 41% in response to natural water toxicity caused by phytoplankton (*Cyclotella* or *Anabaena*) (Forsyth et al., 1992).

WATER CLEARANCE RATE

LITTORAL CLADOCERA The water clearance rate in *Eurycercus* was determined to be 20.5 mL in 24 h (Marcolini, 1953) or up to 30.6 mL in 24 h for each specimen (Kryuchkova, 1969).

PELAGIC CLADOCERA Filtering intensity depends on quality and quantity of the available seston. Filtering rates are 390 mL/mg WW per 24 h in *Bosmina longirostris* and 398 in *Ceriodaphnia pulchella* according to Nikulina (1977). Filtering rates (in μL/μg DW/h) are 28.3 in *D. hyalina* and 49.5 in *D. cucullata* (Steiner and Kasprzak, 2000). In *Daphnia*, *Diaphanosoma*, and *Simocephalus*, the filtering rate is inversely proportional to the concentration of algae in the water (Sushchenya, 1958a,b). However, at a certain upper limit of food particle concentration (over 8 g/m^3 for *D. longispina*), regulation of the filtration rate is no longer possible (Monakov and Sorokin, 1960). In *D. magna*, food consumption is not increased further when the food concentration reaches 2×10^6 cell/mL of *Chlorella* or 1×10^6 cell/mL of yeast (Rigler, 1961b; McMahon and Rigler, 1963, 1965). The feeding rate increases with increasing *Chlorella* concentration up to a maximum and then does not increase further at higher concentrations (oxygen uptake behaves in a similar way) (Kersting, 1978). This concentration was termed the incipient limiting level (McMahon and Rigler, 1965).

While feeding on *Chlamydomonas*, filtering rates are 364 μL/ind./h in *Bosmina longirostris*, 399 μL/ind./h in *D. longispina*, 408 μL/ind./h in *Ceriodaphnia quadrangula*, and 403 μL/ind./h in *Ch. sphaericus* (Lair, 1991). Using a green alga *Chlorella* as food, Ivanova (1970) arrived at the conclusion that at a sufficient concentration of algae the filtration rate in Cladocera is a function of food concentration; at very high concentrations of algae the filtration rate decreases to a permanent minimal level, whereas at very low concentrations of algae the filtering structures work as intensively as they can. In the latter circumstances, cladocerans may starve. Similar results are obtained by Nikulina (1977). In the presence of blue-greens, the filtration rate of *D. pulex* was the highest (ca 220 μL/ind./min) at 75% of the green alga *Scenedesmus* in the food mixture (Pérez-Morales et al., 2014).

For Cladocera, filtering rate generally ranges from 0.9 to 135 mL in 24 h per specimen, depending on food abundance and other factors (Monakov, 1998). The filtration rate of *D. magna* ranges from 0.3 (and lower) to 1.8 mL/h, per specimen (Gulati, 1978). Clearance rates of 2.5–13.5 mL in 24 h per specimen were observed for *D. galeata* in Esch-sur–Sûre Reservoir (Luxembourg) (Darchambeau and Thys, 2005).

In the latter study, the clearance rate always correlated negatively with phosphorus concentration in the food.

As summarized by Sushchenya (1975), the filtering rates of different species (in mL per individual per 24 h/mL per mg DW per 24 h at 20°C) are 0.25–115/18–7780 for *Daphnia*, 1.2–15.6/500–3800 for *Diaphanosoma*, 1–40/125–5000 for *Bosmina*, 1/560 for *Ceriodaphnia*, 8.6–135/538–860 for 26/2760 for *Moina*, and 3–133/470–3021 for *Sida* and *Simocephalus*. The filtering rate (in mL/24 h, per specimen) was determined to be 0.9–5.15 for *D. pulex* 0.7–1.8 mm long, increasing with size in a curvilinear fashion (or 300–180 mL/mg DW/day) (Richman, 1958). For a length of 2 mm, it was about 5 mL in the daytime, increasing at night up to about 20 mL (Haney, 1985).

In ctenopods, as summarized by Korovchinsky (2004), it is 1–248 mL/ind./24 h in *Diaphanosoma brachyurum sensu lato*, 10–58 mL/ind./24 h in *Holopedium*, 2–657 mL/ind./24 h in *Sida*, and 32–252 mL/ind./24 h in *Penilia*.

A very wide scattering of the experimentally obtained values is observed. This may depend on environmental factors, particular experimental conditions, or the specific clone tested. Relationships for *Daphnia* species were suggested and discussed by various authors (e.g., Egloff and Palmer, 1971).

Nevertheless, Knoechel and Holtby (1986) estimated that filtering rate is mainly dependent on body length and comply with the following equation (Eq. 4.1)

$$F = 11.695L^{2.480}, \quad (4.1)$$

in which F is individual filtering rate in mL/day, and L is body length in mm.

The rate complies with this equation in morphologically different forms such as *Bosmina*, *Ceriodaphnia*, *Chydorus*, and *Daphnia* spp. In *Daphnia* species, the exponent is 2.48.

In hungry *D. magna*, filtering rates (in mL/ind./h) and feeding rates (in μm^3/ind./unit time) are higher than in well-fed specimens (Ringelberg and Royackers, 1985). *D. magna* quickly increase their respiratory rate with increasing concentration of food algae (Lampert, 1986).

Obtaining food particles from suspension has been shown, at least partly, to occur not only through mechanical screening. Gerritsen and Porter (1982) believe that collecting food particles also depends on viscosity and the electric charge of both the filtering limbs and the food particles. The filtering rate also depends on oxygen consumption, and this relationship seems species dependent. In contrast to *D. galeata*, oxygen consumption in *D. magna* rapidly declines at concentrations below c. 3 mg/L (Heisey and Porter, 1977).

In their analysis of food filtration by daphnids, Gerritsen and Porter (1982) measured the Reynolds number (Re): for *D. magna*, the Re for filtering combs of limbs III and IV was 0.4–2.0, for a single seta it was 10^{-2}–10^{-4}, and for a setule it was 10^{-3}–10^{-4}. The region of reduced flow around a setule extends far beyond the next setule. Thus, there is little flow between setules and, according to these authors, the appendage functions as a solid wall. As *Daphnia* collect smaller particles (i.e., smaller than the mesh size of the limbs) at a greater rate than larger particles, it was concluded that the mechanism of food collection does not involve direct sieving. It was assumed to be food capture by electrostatic attraction. In support of this, neutralization of the negative surface charge of small particles further increases the retention efficiency. However, subsequent authors, first of all Fryer (1991), confirmed the fundamental significance of direct filtration. It was shown in *Daphnia* that the strokes of the thoracic limbs are repetitive, with short delays between pumping actions; in the presence of food the delays between pumping sessions become longer (Peñalva-Arana et al., 2007).

Abrusán (2004) increased the viscosity of the medium using a sucrose polymer produced by

Leuconostoc or with Ficoll. Increasing the viscosity of the medium at a constant temperature decreased the growth of three *Daphnia* species (except for *D. galeata*) (Abrusán, 2004). It is likely that the decrease was due to interference with filtration. The latter is thought to occur at an Re of approximately 10^{-1}–10^{-4}.

QUANTITIES OF FOOD CONSUMED

LITTORAL CLADOCERA There is little information on littoral Cladocera. In chydorids, the daily ration was determined to be 80% of the body weight in *Ch. sphaericus* (Aksenova et al., 1969), 250% in *Acroperus* (Smirnov, 1971), and 2500% in *Eurycercus lamellatus* (Smirnov, 1962). *E. lamellatus* ingest periphyton within the range of 0.5–462 μg DW/specimen/24 h (Balayla and Moss, 2004).

PELAGIC CLADOCERA The food ration, i.e., the absolute quantity of food ingested (I) in the case of *D. pulicaria* fed on *Scenedesmus*, depends on the length (L) and is described by Eq. (4.2) (Pott, 1982):

$$\begin{aligned} \mathrm{I}(\mu\mathrm{gC/h} \times \mathrm{animal}) &= 0.214 \times \mathrm{L}^{2.105}[\mathrm{C}(\mu\mathrm{g} \times \mathrm{animal})] \\ &= 2.957 \times \mathrm{L}^{2.962} \end{aligned} \tag{4.2}$$

The daily ration of pelagic species (in % WW) was determined to be 17–140 in *Daphnia*, 16–101 in *Ceriodaphnia*, 129–285 in *Simocephalus*, 30–74 in *Moina brachiata* (Ostapenya et al., 1968; summarized in Smirnov, 1969, 1975), up to 750 in juvenile *Moina macrocopa* fed on *Chlorella* (Vorozhun, 2001), 4.9 in *Bosmina longirostris*, 7.7 in *Ceriodaphnia pulchella* (Nikulina, 1977), 5.7–21 in *Sida*, 29–49 in *D. cucullata*, and 3.5–24 in *D. longispina* (Kryuchkova, 1989). In *Bosmina*, the average daily ration is about 100% of its weight (Semenova, 1974).

Sushchenya (1975) reported the range of daily rations to be 67–129% WW, but also described the data of various authors as ranging from 12.5% to 246% WW. He further commented that his own previous data (Sushchenya, 1958a,b) was strongly underestimated. Monakov (1998) estimated the daily ration for pelagic Cladocera as ranging from 0.8% to 247% WW, depending on the food, and probably also on the methods used (Monakov, 1998). In juvenile *Moina macrocopa* fed on *Chlorella*, the ration reached 750% body weight (Vorozhun, 2001).

In the case of algal–bacterial food, the daily ration (% WW) of Cladocera (Aksenova et al., 1969) has been determined to be 17.2 in *D. magna*, 43.6 in *D. longispina*, 101% in *Ceriodaphnia reticulata*, 106 in *Moina rectirostris* (syn. *Moina brachiata*), of those feeding on detritus (Esipova, 1971)—69 in *D. magna*, 107 in *D. longispina*, 177 in *Simocephalus vetulus*, 28 in *C.quadrangula*, and 51 in *Moina rectirostris* (syn. *Moina brachiata*).

For predatory cladocerans, the daily ration at 16°–20°C of one *Leptodora* is 25 cladocerans (0.25 mg), of *Bythotrephes*—25–30 (0.25–30 mg) (Mordukhai-Boltovskaia, 1958). The average daily ration for *Leptodora* is estimated to be 30% of its WW (at 17°C) (Hillbricht-Ilkowska and Karabin, 1979).

Investigations into the diurnal rhythm of feeding rates have indicated that the feeding rate in *Holopedium* and *Daphnia* spp. is higher and less variable at night than during the day (Haney, 1985).

Ingestion rates of algae for *D. longispina* are different for different groups of algae (Schindler, 1971): highest values (μg/h) were determined for *Gloeocystis*, 110; *Coelastrum* (green algae), 168; *Asterionella* (diatom), 177; and *Oscillatoria* (blue-green), 227. It has been shown that algal cells may be ingested repeatedly by *D. magna* before being completely digested (Schindler, 1968; Kersting, 1978).

For planktonic cladocerans (especially with reference to *Daphnia* and *Moina*), Sushchenya et al. (1990) introduced the notion of trophic saturation, i.e., the food concentration at which the maximum ration is attained. At increasing concentrations of available food, the rate of oxygen consumption by *D. magna* increases: this

phenomenon is known as specific dynamic action of food (Barber et al., 1994). The latter parameter was also determined by Filippova and Postnov (1988) who compared oxygen consumption rate in *D. magna* and *Simocephalus vetulus* feeding on algae and on coal.

It should be noted that the values provided previously vary greatly depending on the dynamics of environmental factors.

Excessive and Insufficient Diets

LUXURY CONSUMPTION (SUPERFLUOUS FEEDING, SUPERALIMENTATION)

High concentrations of available food (above a certain level) may lead to excessive consumption of food particles (overcollection) (Porter et al., 1982). The term *luxury uptake* was initially used to describe the acquisition of a nutrient in excess of current demands (see Sterner and Schwalbach, 2001). It is now understood to mean the consumption of food in quantities exceeding physiological requirements. In this situation, the quantities of food material passing through the intestine increase but less of the food is digested, i.e., some food is discarded unused.

Redundancy of food consumed under conditions of excess available food (*Oocystis*) has been noted, e.g., in *D. magna* (Myrand and de la Noüe, 1983); it is thought that overcollection may lead to reduced assimilation rates (Schindler, 1968; Porter et al., 1982). Sterner and Schwalbach (2001) applied the term luxury consumption to the storage of phosphorus during favorable periods to be used during times of less food availability. A significant fact discovered by van Donk and Hessen (1993) is that P-saturated cells of green algae are efficiently assimilated by *Daphnia*, whereas P-limited cells passed through the gut largely intact.

Food requirements (or rations) may also be estimated using the oxycalorific coefficient.

If data on oxygen consumption are available, then the extent of redundant food consumption may be estimated using the oxycalorific coefficient (described, e.g., by Ivlev, 1939; Dediu, 1989): its value is 4.86 cal/mL O_2 or 3.38 cal/mg O_2, or 14.2 J/mg O_2 (1 mL O_2 = 1.6 mg O_2). Multiplying the values of oxygen consumption by the oxycalorific coefficient yields the minimum physiological energy demand (assuming that the food is physiologically balanced). The ratio of the ration value obtained by direct determination to that determined respirometrically is termed the excess index (Gajewskaja, 1948). The actual values of consumed food are higher, than those estimated by the oxycalorific coefficient, mainly by reason of incomplete digestion. For *E. lamellatus*, the daily relative ration determined directly was 2500%, and 220% when determined respirometrically (Smirnov, 1962). Thus, the excess index is c. 11 for adults; it is higher for juveniles.

As a result of staying within the intestine for only a short period, especially under conditions of "excessive" feeding, algal cells are digested incompletely or may even remain alive. The latter was demonstrated for algae ingested by *Daphnia* (Porter, 1975), especially for algae covered with gelatinous sheaths.

INSUFFICIENT FOOD

Daphnias that are undernourished throughout their life live about 40% longer than well-fed daphnias (Skadovskiy, 1955). If starving daphnias are given abundant food, they catch up with well-fed daphnias and finally produce the same quantity of eggs. However, if well-fed daphnias are given limited food, then their fecundity decreases by 2.5 times, their longevity decreases, and they manifest signs of senescence. Jacobs (1961) supplied cultures of *Daphnia* with a low (1/100 dilution) concentration of *Chlorella* versus standard cultures, and observed that under such conditions growth was slower, a much longer time was needed to reach the first instar, maturity was not reached at the fifth instar, and no eggs were deposited. *D. magna* receiving a

low quantity of food and low-P algae produce neonates with an increased lipid content (Boersma and Kreutzer, 2002). At low food concentrations, the starving mothers produce slow-feeding offspring whereas the offspring of well-fed mothers grow and reproduce better (Garbutt and Little, 2014).

In summer populations of *Daphnia* the N and C content were lower suggesting the food shortage in this season (Manca et al., 1914).

It has been shown experimentally that, under low-food conditions, *Daphnia* species develop larger filtering screens on their thoracic limbs (Lampert, 1994).

Qualitative Characteristics

SELECTIVITY

The presence of selectivity in filter-feeding daphnids was shown experimentally by Gajewskaja (1949), DeMott (1962). In the aspect of morphological prerequisites, the distance between filtering setae on the thoracic limbs may be one cause of selectivity. This distance was determined to be from 0.2 μm in *Diaphanosoma* to 4.7 μm in *Sida crystallina* (Geller and Mueller, 1981 a,b). In accordance, *Diaphanosoma*, *Ceriodaphnia quadrangula*, *D. cucullata*, *D. magna*, and juvenile *D. hyalina* were found efficient consumers of bacteria, and *D. hyalina* (adult), *D. galeata*, *D. pulicaria*, *Bosmina coregoni* to be low-efficiency consumers of bacteria. *Sida crystallina* and *H. gibberum* were found unable to consume suspended bacteria by filtration. In a suspension of latex beads, *Holopedium* selected the largest particles and *Diaphanosoma* was almost nonselective (Hessen, 1985).

Three kinds of food rejection were observed:

Postabdominal Rejection. Cleaning of the thoracic limbs by the postabdomen was observed in chydorids and *D. rosea* (Burns, 1968a,b). Rejection of too large or too abundant algae by *D. magna* was observed by Porter and Orcutt (1980). Suspended clay increases the frequency of postabdominal rejection and decreases the rate of thoracic limb beating (Kirk, 1991). In *Daphnia*, Burns (1968a,b) also noted that the action of the thoracic apparatus was interrupted during postabdominal rejection.

Labral Rejection. Labral rejection is the rejection of food intake by deflection of the labrum, thus covering the mouth. This has been shown in *D. rosea* (Burns, 1968a,b).

Regurgitation. Regurgitation has been observed by Rankin (1929) only in *Simocephalus* fed with *Palmella* algae stained with methyl violet.

Influence of Environmental Factors on Feeding

It was shown with reference to *D. pulex* and *Daphnia galeata mendotae* that about 85% of the daily filtering occurs during the dusk–dawn period; filtering rates are lowest in deep-water during the daytime, increased with reduced depth (ascent), decreased at midnight, and increased again before their descent in the morning (Haney and Hall, 1975). Other authors (Starkweather, 1975; Steiner and Kasprzak, 2000) noted that in daphnids individual filtering rates are higher at night.

Darkness does not prevent successful propagation of Cladocera. *Simocephalus* lived at least for 52 days in the dark (fed on *Scenedesmus*) (Smyly, 1967). *Ch. sphaericus* successfully propagated in the dark over a period of about 2 months and they had only slightly different characteristics (number of progeny and longevity) from controls kept in the light (Smirnov, 1971).

However, with reference to *Ceriodaphnia quadrangula*, it has been shown (Gladyshev et al., 2003, 2005) that feeding discontinues in the dark and that rhythmical changes of feeding intensity occur with a periodicity of 2–3 h. *Ceriodaphnia quadrangula* grown under conditions of 12 h light:12 h darkness and transferred to constant light manifested two maxima in the circadian rhythm of grazing; this does not occur

when they are maintained under constant light (Kolmakov et al., 2002).

Toxic *Microcystis* inhibits feeding of *Daphnia* and *Moina* (Zhu et al., 2013).

IMPACT OF SUSPENDED MINERALS

Skadovskiy (1955) noted that a water body is a suspension system in which various kinds of particles are present, ranging from larger than 0.1 μm to smaller than 0.1 μm. Cladocera live among particles of various sizes. Most of their food (algae and detrital fragments) are about 10 μm in size. The diameter of their midgut is c. 100 μm but the esophagus is much narrower (Fig. 1.2). Mineral suspensions, both natural and industrial, interfere with the filtration process (Rylov, 1940; Dubovskaya et al., 2003; Gladyshev et al., 2003) by decreasing the feeding efficiency, which leads to decreased fecundity and other biological parameters of *Daphnia*, *Moina*, and *Ceriodaphnia* (Gorbunova, 1993).

Turbidity becomes lethal at 6400 mg/L (at particle sizes <5 μm) (Gorbunova, 1993). Al_2O_3 nanoparticles cause partial mortality of *Ceriodaphnia dubia* at concentrations >200 mg/L, which is aggravated by the presence of other toxicants (Wang et al., 2011). Suspended clay decreases the rate of beating of thoracic limbs, decreases the algal ingestion rate by 60−70% at low algal concentrations, and increases the frequency of postabdominal rejection in *Daphnia* (Kirk, 1991). According to Kirk, the rejected boluses contained both clay and algae. At 50 mg/L suspended clay, the rate of beating of the thoracic appendages decreased from 246 to 93 beats/min, the rate of mandible movements from 83 to 34 beats/min. In addition, a negative impact on *D. hyalina* was observed by the presence of suspended particles at a concentration of 25 mg/L (Rellstab and Spaak, 2007).

Suspended material decreases the sensitivity of the investigated Cladocera species to phenol (Gorbunova, 1993); in contrast, the toxicity of copper was reduced by turbidity (if the concentration of copper ions was not above 0.1 mg/L).

IMPACT OF NANOPARTICLES

To various natural organic and mineral suspensions, industrially produced particles of a smaller size class, nanoparticles (1−100 nm in size), are now added (values for calculations: 1 μm = 0.001 mm, 1 μm = 1000 nm). Silver nanoparticles studied by Zhao and Wang (2010) were c. 20 nm (those with cysteine addition up to 50 nm). This is the realm of colloid chemistry.

Negative effect. Various nanoparticles are ingested by *D. magna* and cause immobilization and mortality increasing with increasing concentrations (Lovern and Klaper, 2006; Zhu et al., 2009). Exposure of *D. magna* to nano-C_{60} and $C_{60}HxC_{70}Hx$ increased hopping frequency, appendage movement rate (Lovern et al., 2007). Nanosilver and nanocopper are toxic to *D. magna*, but no toxicity of titanium dioxide was observed.

More than 70% of silver nanoparticles (AgNP) were accumulated by *D. magna* through ingestion of algae; the efflux rate constants of silver nanoparticles were lower than those for Ag; most AgNP was removed with feces (Zhao and Wang, 2010). Silver nanoparticles tend to aggregate and inhibit growth and reproduction rates of *Ceriodaphnia affinis* (Gremyachikh and Tomilina, 2014). As shown for *Daphnia* sp. and *Bosmina*, free silver is the main cause of toxicity of silver nanoparticles (Sakamoto et al., 2014).

Tomilina et al. (2011) studied action on fecundity of *Ceriodaphnia affinis* of nanoparticles of TiO_2 (10−50 nm × 8−10 nm), ZnO (15−350 nm) and of microparticles of TiO_2 (25−35 μm), ZnO (15−20 μm), CeO_2 (1−2 μm). Nanoparticles of TiO_2 and CeO_2 turned out comparatively more toxic and microparticles of ZnO—vice versa. Nanoparticles are accumulated in the gut but depuration is slow as was shown with reference to *D. magna* and TiO_2 nanoparticles (Zhu et al., 2010) and to *Ceriodaphnia dubia* and nanoparticles of Fe_2O_3 (Hu et al., 2012b).

ZnO-nanoparticles impair feeding of *D. magna* (Lopes et al., 2014). Over 70% of silver nanoparticles (AgNP) were accumulated by *D. magna* through ingestion of algae; the efflux rate constants of silver nanoparticles were lower than those for Ag; most AgNP was removed with feces (Zhao and Wang, 2010). ZnO-nanoparticles impair feeding of *D. magna* (Lopes et al., 2014).

Investigation of the effects of nano-Cu_2O crystals of different shape demonstrated that the highest mortality of *D. magna* was caused by octahedrons, the lowest by cubes; the effect was related to solubility of crystals in the gut (Wang et al., 2014).

Nanotubes make another group of such contaminants. Lysophosphatidylcholine-coated nanotubes were ingested by *D. magna* and the lysophosphatidylcholine coating was used as food, thus modifying this nanomaterial (Roberts et al., 2007). Carbon nanotubes ingested by *D. magna* were accumulated in the intestine, in the presence of algae 50−85% of them were released (Petersen et al., 2009).

Toxicity of lead in the presence of nano-CeO_2 or TiO_2 was greater than that of lead alone due to ingestion of Pb-loaded nanoparticles (Hu et al., 2012a).

Having studied 12 kinds of carbon nanomaterials of different chemistry a conclusion was obtained that they render different effects on *D. magna* (Arndt et al., 2013). It was shown that quantum dots can cross the intestinal−epithelial barrier of *Daphnia* (Feswick et al., 2013). The quantum dots used were "10−20 nm in diameter, comprised of[sic] a cadmium selenide core, with a zinc sulfide shell core with a functionalized amphiphilic polymer coating." Exposure of *D. magna* during 48 h to Zn nanoparticles smaller than 100 nm and smaller than 50 nm was followed by their penetration within midgut cells, in microvilli, in spaces between adjacent cells, in basal membrane, and in gut muscles, suggesting that they cross epithelial barriers (Santo et al., 2014).

However, gold nanoparticles ingested by *D. magna* were observed in close proximity to the peritrophic membrane, but did not penetrate to the gut epithelium (Khan et al., 2014).

Positive effect. A positive effect was found in case of polyhydroxy fullerenes (PHFs) (a kind of carbon nanomaterial) supplied to *Ceriodaphnia dubia.* PHFs were found accumulated in the gut and at the background of feeding with green algae and yeast extract to extend the life span and stimulate the reproduction of *C. dubia* (Gao et al., 2011). At 20 mg/L of PHF the life span increased by 38%.

4.1.4 Impact of Xenobiotics on Feeding

Movements of the thoracic limbs are reduced by nicotine, epinephrine, cyanide, strychnine, metrazol, and carbon dioxide (i.e., the corresponding striated muscles are affected) in *D. magna* (Sollman and Webb, 1941).

The rate of filtration in *D. magna* decreased in the presence of the insecticide methylparathion (Fernández-Casalderrey et al., 1993), pesticides endosulfan and diazinon (the control rate was 1000−1200 μL/ind./h) (Fernández-Casalderrey et al., 1993), the acaricide tetradifon (4-chlorophenyl 2,4,5-trichlorophenyl sulfone) (Villarroel et al., 1998), and tebuconazole (Sancho et al., 2009). In the case of tetradifon, the reduction was 50% in comparison with the control, which occurred at 0.02 mg/L for filtration and 0.24 mg/L for ingestion. In *D. magna* exposed to the insecticide cypermethrin (at 0.1 μg/L) or the fungicide azoxystrobin (at 0.5 mg/L), the limb-filtering rate, the mandibles-rolling rate, and the heart rate decreased (Friberg-Jensen and Nachman, 2010); in contrast, all of these parameters increased in the presence of 1 μg/L cypermethrin. Sublethal concentrations of the surfactant dodecyl sulfate decrease the filtration rate as shown with reference to *Daphnia catawba* (Jones et al., 1991) and *D. magna* (Vorozhun and Ostroumov, 2009). At low levels of available food the sensitivity of

D. magna to carbaryl increased greatly (Takahashi and Hanazato, 2007). The filtration and ingestion rates of *D. magna* decreased proportionally to the alkyl chain length of pollutants (Luo et al., 2008). The filtration rate of *D. pulex* (feeding on a green alga *Scenedesmus*) is decreased by 40% following preexposure to glyphosate and by 30% following preexposure to *p,p′*-dichlorodiphenyldichloroethylene (Bengtsson et al., 2004).

Presence of crude-oil emulsion decreases activity of thoracic limbs, mandibles, and swallowing but increases postabdominal and labral rejection, accompanied by toxicity (Wong et al., 1983). As to presence of petroleum (and of sunflower seed oil as its imitation) Borodulina et al. (2011) arrived at the conclusion that the presence of oil complicates life activities of *D. magna* rather by physical impact than due to chemical factors.

In the presence of 0.15 mg Cd/L, the filtration rate of *D. magna* is decreased by 52% (of the control) (Domal-Kwiatkowska et al., 1994). The filtration rate of *D. magna* is inhibited by copper (Cu^{2+}, as copper acetate) already at 0.03 mg/L, contrary to Ni^{2+}, Zn^{2+}, and Co^{2+}, the effect for which was observed at higher concentrations (Lobkova et al., 2009; Shilova, 2010; Shilova et al., 2010; Shilova and Rogachyova, 2011a,b,c; Konyukhov and Vorobieva, 2013). The preliminary high-frequency low-intensity electromagnetic irradiation of *D. magna* moderates the toxic effect, in that the filtration rates approach and even exceed the control values (Shilova and Rogacheva, 2013a,b).

Although most xenobiotics depressed the feeding of *D. magna*, not all of them caused postexposure feeding depression (McWilliam and Baird, 2002).

The decreased filtration rate in *D. magna* consuming fluorescing microbeads was used to estimate the acute toxicity of metals (Ginatullina and Kamaya, 2012). *D. magna* ingested Au nanoparticles of average size 10 nm, both directly or with *Euglena* and *Chlamydomonas* loaded with Au nanoparticles (Lee et al., 2015). At the concentration 0.05 mg/L immobilization started.

The filtering activity of *D. magna* increased in the presence of NaCl (by c. 38% in the presence of 2.5 g NaCl/L) (Shilova and Rogacheva, 2013b).

4.2 DIGESTION

4.2.1 Anatomical Background

The cladoceran intestine consists of a stomodaeum (foregut or esophagus) passing into the mesenteron (midgut) and proctodaeum (hindgut) (Hardy and McDougall, 1894). The labrum comprises paired salivary glands. In *Daphnia*, the salivary gland on each side consists of a defined number of cells: two main cells, two substitutional cells, and 32 basal cells (Fig. 4.1) (Sterba, 1957a). The labral gland of *Simocephalus* was described by Cannon (1922). Slime is stored in paired reservoirs letting the secretion into the lumen of the esophagus. In some species these reservoirs are “enormous” (Fryer, 1974), as in *Onchobunops tuberculatus* and *Macrothrix triserialis*, partly protruding in the latter into the head.

The inner epithelium of the foregut and the hindgut is of ectodermal origin (embryologically), whereas that of the midgut is of endodermal origin (Chatton, 1920). The stomodeum and hindgut (proctodeum) have a cuticular lining.

In many littoral species the intestine is convoluted, thus its relative length is enlarged. In chydorids, on the ventral side, at the boundary between the midgut and the hindgut a blind cecum is present, variously developed in different species (Fig. 4.8). In planktonic species the intestine makes no convolutions. The ventral blind cecum and its function were first described by Claus (1876). Food is transferred from the midgut to the blind cecum and accumulates there before being finally evacuated (Fryer,

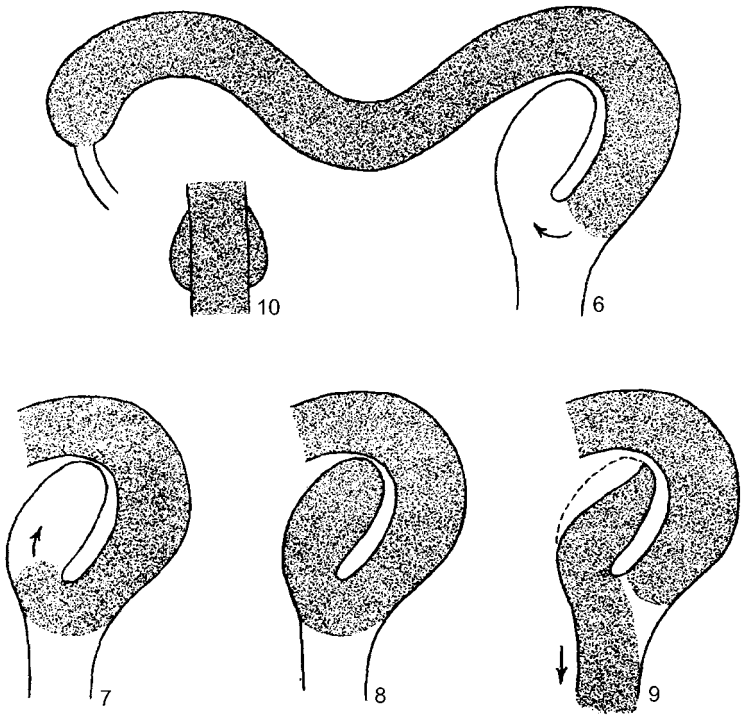


FIGURE 4.8 The ventral blind cecum of *Acantholeberis curvirostris*, showing its filling and evacuation. *From Fryer, G., 1970. Defecation in some macrothricid and chydorid cladocerans and some problems of water intake and digestion in the Anomopoda. Zoological Journal of the Linnean Society 49 (4), 255–270.*

1970; Smirnov, 1969, 1971). In many species of chydorids, the blind cecum on the ventral side of the intestine is rather long, as in *Alona intermedia* (Tollinger, 1909) and species of the genera *Pleuroxus*, *Chydorus*, *Graptoleberis*, *Acroperus*, and *Camptocercus* (Smirnov, 1971). The equivalent cecum is short and wide in *Eurycercus* and *Leydigia*. In *Ilyocryptus*, Sergeev (1973) reported a peculiar vascular widening of hindgut.

On the dorsal side of the anterior part of midgut, a pair of blind diverticula (digestive ceca) is present in Daphniidae, *Ophryoxus* (Macrothricidae), and in some *Eurycercus* species. As reviewed by Korovchinsky (2004), in ctenopods a pair of short dorsal ceca is present in *Holopedium* and *Latona parviremis*; *Sida* and *Limnosida* have one dorsal cecum; *Diaphanosoma* has no dorsal ceca. Chydorids also have no dorsal ceca. As shown with reference to *Daphnia pulex*, the dorsal ceca are just evaginations of the midgut, their wall consists of an epithelial layer, a basement lamina, and a peripheral muscle network (Schultz and Kennedy, 1976a).

The inner surface of the gut is folded (Fryer, 1969) and densely covered with numerous microvilli (Fig. 4.9) (Schultz and Kennedy, 1976a; Quaglia et al., 1976; Avtsyn and Petrova, 1986; Peter and Sommaruga, 2008; Feswick et al., 2013). The microvilli are long, occasionally branching, and situated close to each other, as shown in *Daphnia* (Fig. 4.10) (Quaglia et al., 1976). Microvilli also line the inside of the digestive diverticula.

The fine structure of the *Daphnia* gut is described by Schultz and Kennedy (1976a). They conclude that enzyme secretion is

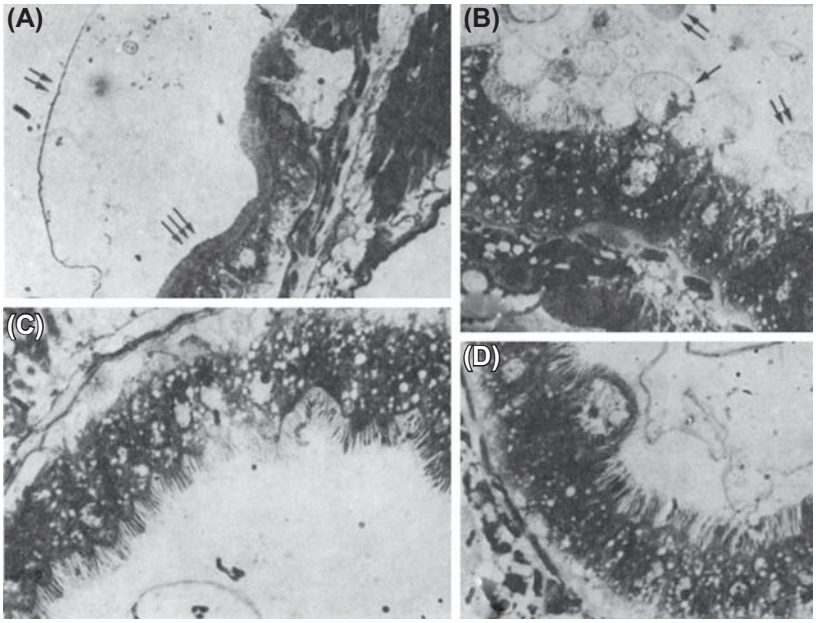


FIGURE 4.9 Cross section of the gut of *Daphnia* showing plications and villi. (A) Anterior part, *one arrow*, secreting epitheliocytes (holocrine type of secretion); *two arrows*, perithrophic membrane; *three arrows*, chitinous membrane; (B) middle part, secreting epitheliocyte, *one arrow*, macrolemmocrine type of secretion; *two arrows*, secretion granules; (C) middle part, epitheliocytes with villi; (D) protruding secreting epitheliocyte (macroapocrine type of secretion). *From Avtsyn, A.P., Petrova, T.P. 1986. Resistance of the alimentary canal to enteropathogenic NAG- vibrios. Byulleten eksperimentalnoi biologii i meditsyny 102 (9), 342–345.*

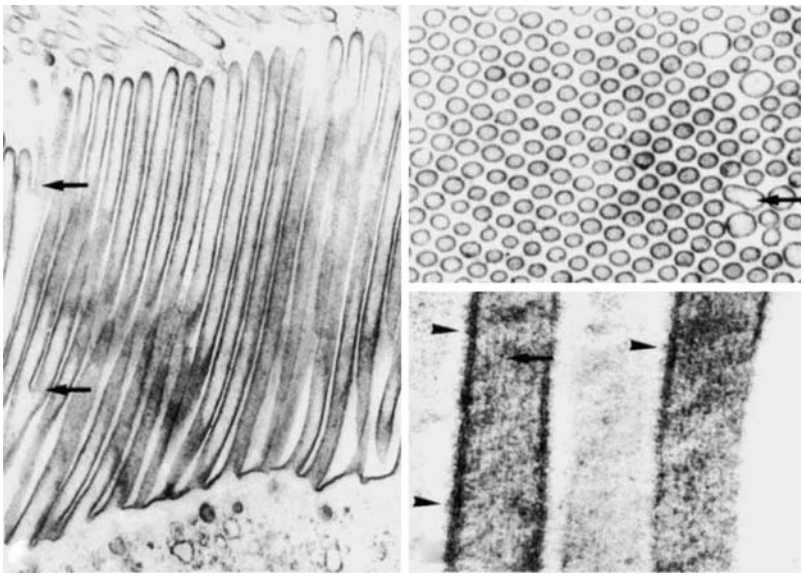


FIGURE 4.10 Villi in the gut of *Daphnia* in longitudinal and transverse section. *From Quaglia, A., Sabelli, B., Villani, L., 1976. Studies on the intestine of Daphniidae (Crustacea, Cladocera). Journal of Morphology 150 (3), 711–726.*

holocrinous. Digestion takes place in the midmeseterial region, absorption—principally in the anterior region—and feces are formed in the posterior part.

The gut lumen is populated by bacteria as was shown for *Daphnia* sp. sp. (Peter and Sommaruga, 2008). and parasites (in the gut wall) (see Chapter 17). As observed by Metchnikoff [1892 (1947)], spores of *Metschnikowiella bicuspidata* (syn. *Monospora bicuspidata*, Ascomycetes) that are consumed by *D. magna* are liberated from their coat and penetrate the body cavity through the wall of the intestine, where they are surrounded, ingested, and destroyed by leukocytes (phagocytosis).

Beim and Lavrentieva (1981) investigated the pH in the intestinal canal of *Daphnia* by staining it with eosin, Congo red, methyl red, neutral red, and uranine. Within the gut lumen of *Daphnia*, the pH is 5.6−6.2.

The stained material passed through the gut in 20 min. During this time, these fluorochromes did not stain hemolymph, oil drops, or the brood pouch. Therefore, Lavrentjeva and Beim (1978) suggested the presence of a histohemolymphatic intestinal barrier to these stains. They also stated the possibility that organic toxicants may stay within the intestine and not penetrate the gut wall. See also the data of Fonviller and Itkin (1938) on the permeability of integuments in Section 8.3.

Peritrophic Membrane

The food collected in the intestine is surrounded by a peritrophic membrane; this membrane has been shown in *Daphnia* (Chatton, 1920; Fox, 1952; Quaglia et al., 1976) and *Acantholeberis* (Fryer, 1970). The tubular peritrophic membrane surrounds the food and extends through the midgut and hindgut. Chatton (1920) investigated the peritrophic membrane in *Daphnia* in detail and demonstrated that two peritrophic membranes are present. The posterior peritrophic membrane is formed, according to Chatton (1920), by delamination of the proctodeal cuticle and is attached to the posterior circular furrow between the midgut and the hindgut. It surrounds the posterior part of the anterior peritrophic membrane (Fig. 4.11).

The inner ectodermal lining of the esophagus and the entodermal lining of the midgut are also separated by a circular furrow. Both linings are in contact but are not fused. The circular furrow seems to be the site of formation of the anterior peritrophic membrane (Fig. 4.12). Chatton, however, believes that the anterior peritrophic membrane is a lining of the esophagus that is disconnected at the mouth but connected in the area of the furrow between the esophagus and

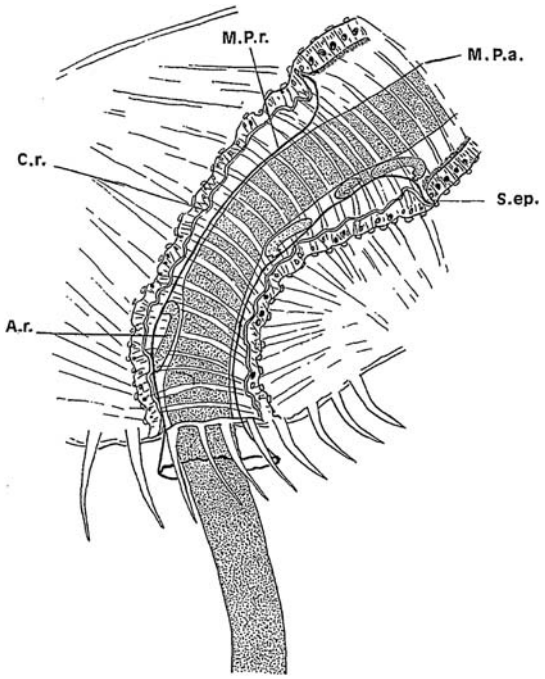


FIGURE 4.11 The posterior peritrophic membrane of *Daphnia magna* surrounding the anterior peritrophic membrane. *A.r.*, amoebidium on the inner side of the rectal peritrophic membrane; *c.r.*, cuticle; *p.a.*, part away from anus; *p.r.*, rectal peritrophic membrane; *s.ep.*, emargination in the gut tissue. *From Chatton, E., 1920. Les membranes péritrophique des Drosophiles (Diptères) et des Daphnies (Cladocères) leur genese et leur role a l'égard des parasites intestinaux. Bulletin de la Société Zoologique de France 25 (2), 265—280.*

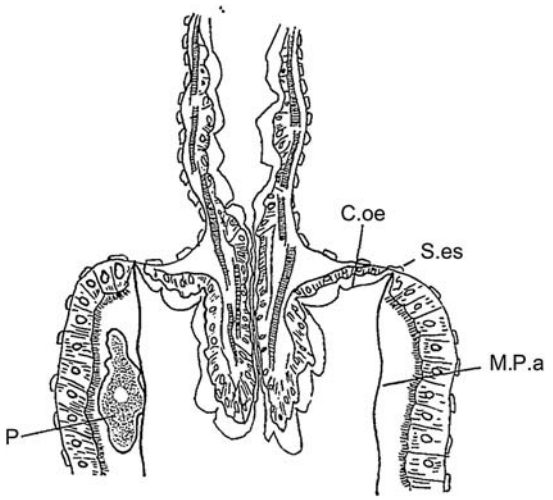


FIGURE 4.12 The anterior peritrophic membrane (p.a.) of *Daphnia magna* in the place of transition of the esophagus to the midgut, in cross section. *c.oe.*, separating ectodermal cuticle of esophagus; *P*, *Pansporella* in space between the peritrophic membrane and the gut wall; *p.a.*, peritrophic membrane; *s. es.*, enterostomodeal emargination. *From Chatton, E., 1920. Les membranes péritrophique des Drosophiles (Diptères) et des Daphnies (Cladocères) leur genese et leur role a l'égard des parasites intestinaux. Bulletin de la Société Zoologique de France 25 (2), 265–280.*

the midgut, turned inside out, and extended by secretions of the midgut. This membrane fully surrounds the flow of food and feces to the anus. It isolates the midgut mucosa from direct contact with ingested food and acts as a kind of dialyzer. The food within the peritrophic membrane of *Daphnia* is moved to and fro by antiperistalsis of the intestinal wall (Chatton, 1920; Fox, 1952). Chatton (1920) noted that at strong contractions of the rectal opening, the posterior part of the anterior peritrophic membrane may be torn off.

It is not quite clear whether peritrophic membranes are discarded with each act of defecation or at each molting. This could easily be observed in representatives of various genera, but has not yet been done.

According to Hansen and Peters (1997), the peritrophic membranes are formed by secretion of the midgut epithelium. They consist of a network of chitin-containing microfibrils embedded in a matrix of proteins, glycoproteins, and proteoglycans. The thickness of the peritrophic envelope is 280 nm. The lumen of a peritrophic membrane is termed the *endoperitrophic space* and the space outside the peritrophic membrane is called the *ectoperitrophic space*. Hansen and Peters (1997) experimentally determined that the peritrophic membrane of *D. magna* is permeable to dextrans with a molecular weight of up to 2000 kDa (Einstein–Stokes radius of 31 nm) and to latex beads with a diameter 139 nm.

The Fat Body

The fat body is a special organ involved in fat metabolism, as well as having other functions. It has a variable structure. In addition to their role in fat metabolism, fat cells play a role in glycogen metabolism. Fat cells are the sites of hemoglobin formation (Goldmann et al., 1999).

The fat body, formed from special cells containing oil drops, was first observed in the Cladocera by Leydig (1860, pp. 51–52). It may be slightly yellowish or reddish. Leydig also noted that the appearance of these cells depends on the season and the developmental stage. In addition, Hardy (1892) believed that blood cells, which absorb fat, may attach to the inner substrata and form fat tissue.

Special investigations into the fat body of Cladocera were initiated by Jaeger (1935) and continued by Sterba (1956a). Jaeger noted that oil drops in Cladocera are present not only inside the body but they are also included in special cells. According to Jaeger (1935), the fat body is connected to the ovaries and is situated ventrally along the gut (Fig. 4.13). The sequence of development of the fat body and the ovaries is shown in Fig. 4.14.

The structure of the fat body is rather irregular and complex. Some of its cells are scattered and attached to various organs and membranes. According to Jaeger (1935), the cells of the fat

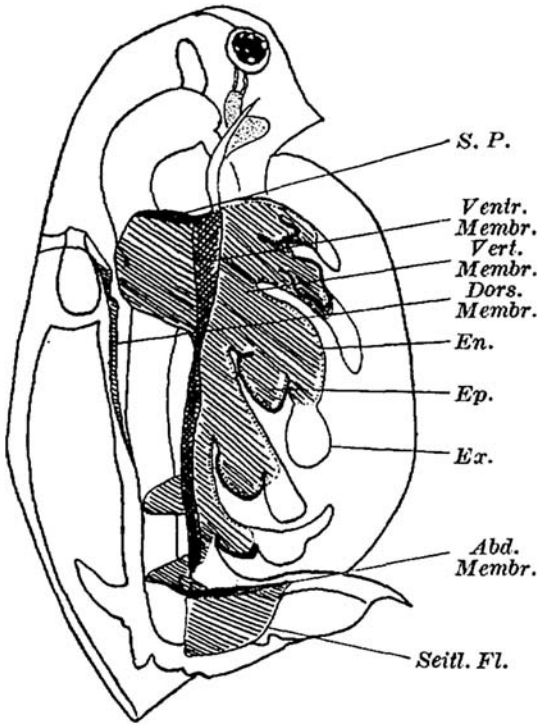


FIGURE 4.13 Position of fat body in *Daphnia*. Fat body is shaded. *En.*, endopodite; *Ep.*, epipodite; *Ex.*, exopodite; *Seitl.Fl.*, lateral lobe. *From Jaeger, G., 1935. Über den Fettkoerper von* Daphnia magna. *Zeitschrift für Zellforschung und Mikroskopische Anatomie. 22 (2), 98—181.*

body are situated near to muscles at sites where they contact the membranes that direct blood flow, on intestinal muscles, and in the abdomen. In the abdomen, they comprise the plate between adductors of the abdomen and two plates on both sides of the gut, joining at the abdominal membrane. In thoracic limbs, the fat body extends to the base of epipodites and into endopodites, but not into exopodites. Plates of the fat body are present in valves. The head does not contain fat cells. Fat cells are present on both sides of the membranes that separate blood flows, except for the dorsal membrane (Jaeger, 1935). They are clearly separated from the membranes. In Cladocera, fat is concentrated in the cells of the fat body. As long as germinal groups are present in the ovary, the fat content of the fat body increases. After the start of yolk formation in the ovary, the fat body decreases in size (Jaeger, 1935; Sterba, 1956a). The cells of the fat body are basophilic. Their only inclusions are fat drops. There is no intercellular substance. Due to the accumulation and expenditure of fat, the cells of the fat body are in either a fat-free or a fat phase.

With fat expenditure, the fat cells are completely reduced. Jaeger (1935) observed that during this process part of the cell may be reduced. At the same time, substituting cells are formed. Restoration of the fat body occurs through the action of blood cells (Jaeger, 1935): blood cells loaded with fat may settle onto tissues and turn into cells of the fat body. Jaeger (1935) did not observe any division stages in the substituting cells or the fat cells, but assumed that division stages are initially present in both. Sterba (1956a) presented data on "the life cycles"

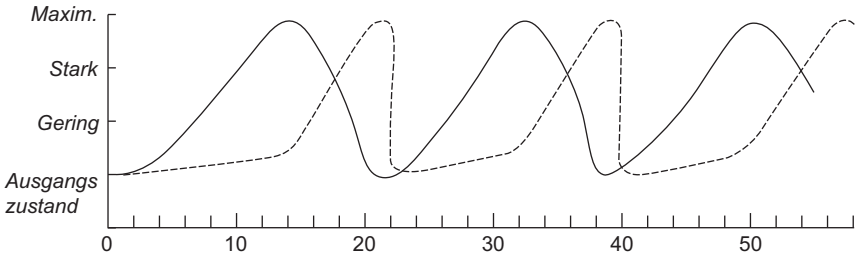


FIGURE 4.14 Sequence of fat body (*continuous line*) and of ovaries (*interrupted line*) development. Abscissa, days. *From Sterba, G., 1956a. Zytologische Untersuchungen an Grosskernigen Fettzellen von* Daphnia pulex *unter besonderer Beruecksichtigung des Mitochondrien-Formwechsels. Zeitschrift für Zellforschung 44, 456—487.*

of fat cells in response to feeding on material rich in fat or rich in carbohydrate (Fig. 4.15).

In *Daphnia*, fat globules and other particles are carried from the gut lumen through the gut wall by blood corpuscles (Hardy, 1892) and via cells of the anterior region of the midgut (Hardy and McDougall, 1894). According to Jaeger (1935), osmic acid stains both fat in *Daphnia* blood cells and minute fat particles (hemokonia) in hemolymph, thus making the hemolymph turbid. This indicates a distribution of fat from the alimentary canal throughout the body both in blood cells and as minute drops.

Despite much information on lipids in Cladocera now being available, no further investigations have been made into the fat distribution.

4.2.2 Peristalsis

Peristalsis and antiperistalsis of the gut can be easily observed and were reported for *Daphnia* as early as 1894 by Hardy and McDougall. Action of purgatives on peristalsis was observed in *Sida* by MacCallum (1905), who placed five or more *Sida* in a watch glass in various solutions. Sollman and Webb (1941) observed *Daphnia* either on a hanging-drop slide or in test tubes with solutions of drugs.

MacCallum (1905) saw that peristalsis in *Sida* was stimulated by pilocarpine (0.1−1% solution), barium chloride, sodium citrate, sodium sulfate, and sodium fluoride by a solution of calomel in sodium bicarbonate. Dilution of pilocarpine delayed the action, which remained the same. Presence of calcium chloride did not delay the action of pilocarpine. Alkaline solutions of cascara sagrada, aloin, or calomel in diluted $NaHCO_3$ caused active peristalsis (not caused by $NaHCO_3$ alone). Colchicin had the same effect.

Viehoever (1936) applied various purgatives to *Daphnia* and observed evacuation of the food canal within 19 min or less than 1 h. His conclusions may be put to doubt, as this is normal evacuation time in daphnids. Stimulation of peristalsis in *D. magna* was shown by addition of pilocarpine, mecholyl, and guanidine (Sollman and Webb, 1941), of physostigmine (eserine) (Sollman and Webb, 1941; Obreshkove, 1941a), of acetylcholine (Obreshkove, 1941a); in *Simocephalus*—by addition of acetylcholine, carbaminoylcholine, and prostigmine (Mooney and Obreshkove, 1948).

Atropine and calcium chloride blocked the action of pilocarpine in experiments of MacCallum (1905) on *Sida*. Atropine blocked the actions of acetylcholine and physostigmine in experiments of Obreshkove (1941a) on *D. magna*. Peristalsis was also inhibited by epinephrine, quinine, quinidine, metrazol, and cocaine in *D. magna* (Sollman and Webb, 1941).

Rankin (1929) observed strong reverse peristalsis in *D. magna* and *Simocephalus serrulatus* fed on stained food.

In *Daphnia*, calcium ions (Ca^{2+}) cause contracture of the intestine, whereas potassium ions (K^+) cause relaxation of intestinal muscles (Jermakoff and Ermakov, 1936). Intestinal evacuation in *D. magna* is accelerated by incubation in 100−250 μM Na_2CO_3, 100 μM $NaNO_3$, or 1 μM KCl (Gophen and Gold, 1981).

Hardy and McDougall (1894, p. 45) note especially that peristaltic movements of the proctodaeum (hindgut) "undoubtedly lead to the entrance and exit of water."

The ceca situated on the dorsal side of the anterior gut also make rhythmic contractions, 6−10 times/min in *D. magna* and much less frequently in *Simocephalus serrulatus* (Rankin, 1929).

4.2.3 Defecation

The formation of feces is confined to the posterior region of the midgut and to the proctodaeum (Hardy and McDougall, 1894). These authors noted that peristalsis in the proctodaeum is independent of the central nervous system as it takes place in the isolated proctodaeum.

Defecation occurs very frequently in Cladocera. Defecation was initially described in

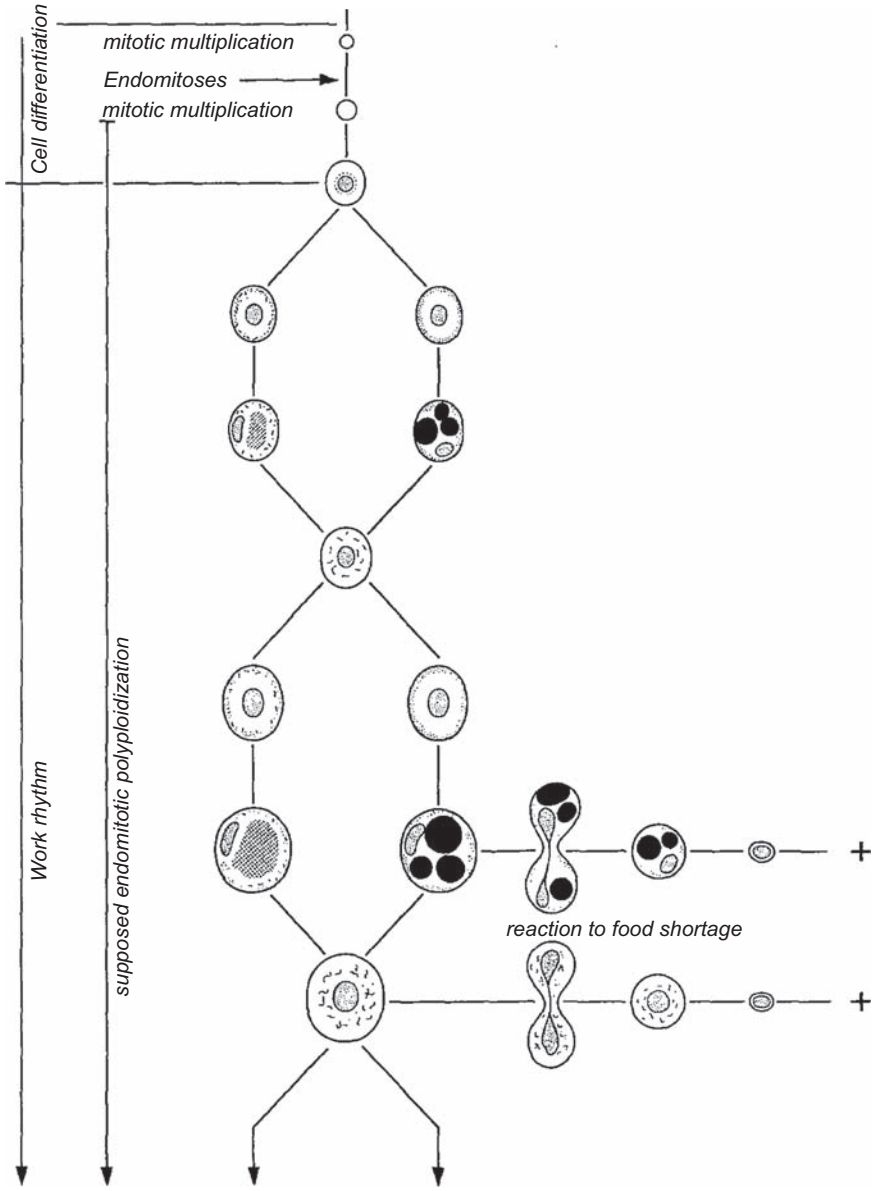


FIGURE 4.15 Cycle of fat cells in *Daphnia*. Left, with food rich in carbohydrate; right, with food rich in fat. *Black drops* designate fat. *Lower arrow* to the right — reaction to food shortage. *From Sterba, G., 1956a. Zytologische Untersuchungen an Grosskernigen Fettzellen von* Daphnia pulex *unter besonderer Beruecksichtigung des Mitochondrien-Formwechsels. Zeitschrift für Zellforschung 44, 456–487.*

Daphnia by Hardy and McDougall (1894), who noted the presence of a sphincter muscle at the junction of the midgut and the hindgut.

In chydorids and *Acantholeberis*, food is passed through midgut, transferred to the blind cecum, and evacuated when the cecum is filled and dilated (Smirnov, 1969, 1971; Fryer, 1970). The discharge of feces is followed by anal water intake. Feces form cylindrical columns that do not immediately dissolve in water; they settle to the bottom.

With regard to littoral species, the intestine is evacuated every 7 min in *E. lamellatus* (Smirnov, 1962), every 11–19 min in *Acantholeberis* (Fryer, 1970), and every 10–15 min in *Lathonura* (Sergeev, 1971). Fryer (1969, p. 371) reported that *L. leydigii* "was watched for 2 min 5 s as it fed, during which time it discharged 15 fecal ribbons. Rough calculations indicate that at such a rate of ejection the entire alimentary canal could be evacuated in less than 6 min, and possibly considerably less." In periods of food scarcity, the intestine remains full for longer periods (Fryer, 1968). Such a short retention of food in the intestine is somewhat compensated anatomically in chydorids and in many macrothricids by elongation of the intestine, through making convolutions.

For planktonic species, the intestine is evacuated frequently, but at somewhat longer intervals: every 31–35 min in *D. magna*, *D. longispina*, and *Simocephalus vetulus*, and 20–24 min in *Ceriodaphnia quadrangula* and *Moina brachiata* (Esipova, 1971). In *Bosmina*, it takes 10 min for the intestine to fill with food and a portion of food is digested in 30–35 min (Semenova, 1974). In *Moina macrocopa*, the intestine is filled in about 20 min and fully evacuated in less than 50 min (Morales Ventura et al., 2011). Gut clearance in *D. magna* is more efficient when green algae are available than in clear water.

For *D. magna*, it was noted that sudden transfer to low-quality food increased egestion of feces, to discharge excess carbon (Lukas and Wacker, 2014a,b).

Further comparative studies of living Cladocera are desirable.

Despite food being retained in the intestine only for short periods, cladocerans grow normally and produce young.

4.2.4 Digestion of Particular Substances

Cladocera feed on various algae, bacteria, and organic detritus at various stages of decomposition. These food sources contain protein, carbohydrates, lipids, and indigestible materials. Protein, lipids, and soluble carbohydrates are definitely digested. A diagram of the intake and transformation of food substances in an adult daphnid is shown in Fig. 4.16 (based on Hallam et al., 1990). Digestion and assimilation of food occurs in the midgut (mesenteron). Its tissue is not differentiated, but Hardy and McDougall (1894) drew attention to the unique and remarkable fact that digestion takes place in the middle region and absorption in the anterior region. Antiperistalsis and peristalsis are involved in this process. A fundamental fact that in Cladocera fat globules and other particles are carried from the gut lumen through the gut wall by blood cells was noted by Hardy (1892). Hardy and McDougall (1894) also noted that fat globules are ingested by columnar cells of the anterior region of the midgut, including the liver diverticula. However, these observations do not seem to be followed up.

The enzymes that take part in cladoceran digestion have been investigated since 1929 (Rankin, 1929). An amylolytic enzyme has been identified in *D. magna* and *Simocephalus serrulatus* (Rankin, 1929), and proteinase and polypeptidases were reported in *D. pulex* (Hasler, 1937). In *Polyphemus* (a mixed herbivore and carnivore), proteinases, amylases, and lipases were identified (von Dehn, 1930; Hasler, 1937). The major proteinases in *D. magna* are trypsins and chymotrypsins (Agrawal et al., 2005b); these were identified in a gut homogenate. Agrawal et al. (2005b) also demonstrated that a blue-

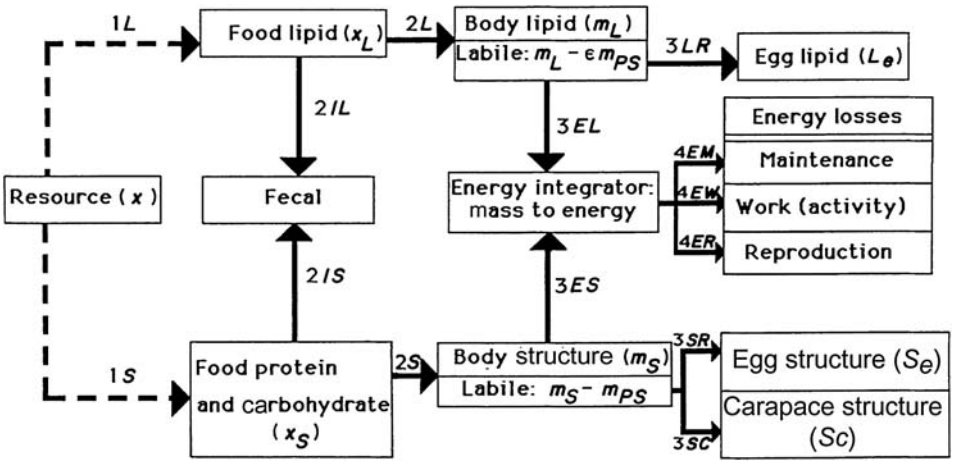


FIGURE 4.16 Metabolic flow diagram of an individual adult daphnid. *From Hallam, T.G., Lassiter, R.R., Li, J., Suarez, L.A., 1990. Modeling individuals employing an integrated energy response: application to* Daphnia. *Ecology 71 (3), 938–954.*

green *Microcystis* produces several inhibitors that differ in their specificity for these enzymes.

Much later (Schoenberg et al., 1984), endogenous cellulase was detected in the gut of *Daphnia*, *Acantholeberis*, and *Pseudosida*; as no microbial gut flora was found, this enzyme enables cellulose to be digested. Cellulose digestion has also been reported in *Daphnia* by Lampert (1987) and De Coen and Janssen (1997).

In *Simocephalus*, the pH of the anterior part of the midgut is acidic and it changes to alkaline nearer to the anus (Rankin, 1929). In *Daphnia*, the pH of the anterior part of the midgut is 6.0–6.2 (Rodina, 1950; Beim and Lavrentieva, 1981; Beim et al., 1994), i.e., lower than in the external medium, and increases to 7.4 in the hindgut (Rodina, 1950). Secretions of the hepatic ceca of *Daphnia* and *Simocephalus* are acidic (Rankin, 1929).

Protein Metabolism

Protein and, accordingly, amino acids are obtained by Cladocera from algae and seston. Indeed, it was shown with reference to *D. obtusa* that the amounts of released nitrogen and phosphorus are generally proportional to the amounts ingested with food algae (Sterner and Smith, 1993).

The principal final product of protein metabolism in Cladocera is ammonia (Parry, 1960); of the secondary products, the most abundant is urea.

After Hasler (1937), the proteolytic enzymes (trypsin, β-galactogenase, and esterase) were found and measured in homogenates of *D. magna* by De Coen et al. (1998). The activities of these enzymes decreased during 90-min incubations with various inorganic and organic toxicants. Von Elert et al. (2004) also identified proteases in homogenates of *D. magna*: two major ones (trypsin and chymotrypsin) and nine others. The two major proteases account for up to 83% of the proteolytic activity of the gut contents. Trypsin is strongly inhibited by *N-p*-tosyl-lysine chloroketone and 4-amidinophenylmethanesulfonyl fluoride; chymotrypsin is strongly inhibited by chymostatin. Both activities have alkaline optima. Proteases (trypsin, chymotrypsin, elastase, and cysteine protease) were also found in whole body homogenates of *Moina macrocopa* (Agrawal et al., 2005a). Nine digestive proteases were

found in *D. magna* (Schwarzenberger et al., 2010).

Their activity was significantly inhibited by an extract of a blue-green *Microcystis aeruginosa*. In *D. carinata*, proteinase activity was on average, 0.42 U/mg protein/min, that of chymotrypsin was 0.49 U/mg protein/min, and that of trypsin was 0.21 U/mg protein/min (Kumar et al., 2005b). Peptide metabolites of *Microcystis* that inhibit the trypsin-like activity in *Daphnia* were identified by Czarnecki et al. (2006). It was shown that *D. magna* responds physiologically to cyanobacterial protease inhibitors by phenotypic plasticity of gut proteases (Schwarzenberger et al., 2010).

For *D. magna*, algae are a better source of nitrogen than bacteria (Schmidt, 1968). According to this author, an adult *D. magna* consumes 0.37−0.47 μg N from green algae per 24 h per μg body N and excretes 0.17−0.19 μg/24 h of N per μg body N. Release rates in *Daphnia* were measured: nitrogen was 3−3.42 and phosphorus was 1−1.24 μg/mg C/h. No interspecies differences were found (Pérez-Martínez and Gulati, 1998/1999).

The amino acid composition of various Cladocera was already noted in Table 3.3. It is also reported for *D. pulicaria* by Ventura and Catalan (2010): the total amino acid concentration in females was determined as 25−29% of DW, in eggs − 34%, free amino acids making a small part (1.9−3.3%) of total amino acids. Essential amino acids made 45−46% of total amino acids.

Supplementation of algal food with amino acids demonstrated that some of them (in particular, arginine and histidine) enhanced subitaneous reproduction and prevented production of resting eggs in *D. pulex* (Fink et al., 2011; Koch et al., 2011).

It has been suggested, with reference to *Daphnia*, that hemoglobin (Hb) functions as both a respiratory protein and a protein store (Rudiger and Zeis, 2011).

Under conditions of acute hypoxia (i.e., a decrease in O_2 concentration to c. 0.5 mg/L), the typical protein−lipid catabolism of *Moina micrura* changes completely to the anaerobic protein catabolism (Hubareva, 2000a,b; Svetlichny and Hubareva, 2002a,b,2004). Further, the excretion of NH_3-N drastically decreases from 0.079 μg N/L/h to 0.019 μg N/L/h, in proportion to the O_2 concentration.

Phosphorus Metabolism

Cladocera are commonly deficient in phosphorus. Inorganic phosphate is taken from solution, as shown for *Daphnia schødleri*, mostly through the epipodites (Parker and Olson, 1966). However, this intake is low in the presence of abundant algal food. Tan and Wang (2009c) demonstrated that *D. magna* obtain most of P from food, only 1−2% is incorporated from water; at higher concentration of the dissolved Ca (within 0.5−200 mg/L) more P is stored in exuvia and less in released in the dissolved phase. The addition of 3 μM $AgNO_3$ to the surrounding water reduced the uptake of phosphorus as a result of silver impregnating the surface of epipodites. The growth of *D. magna* is limited in the case of feeding on P-deficient green algae (Urabe, 1991).

In Cladocera, phosphorus is used for protein synthesis and forms part of nucleic acids and phospholipids. Other potential pools of phosphorus in Cladocera are minor metabolites (adenosine triphosphate and adenosine diphosphate), reduced nicotinamide adenine dinucleotide, reduced nicotinamide adenine dinucleotide phosphate, and calcium-associated phosphorus (hydroxyapatite) in integuments (Vrede et al., 1999). The daily renewal rate of phosphorus in the body (*D. pulex*) ranges from 35% to 60% (i.e., 35−60% of P is turned over each day) (Lehman, 1980).

Having determined that *D. magna* can extract phosphorus from food both rich and scarce in phosphorus, Sterner and Schwalbach (2001, p. 415) asked the following further questions: "In what chemical form is phosphorus stored? Where is it stored? How does it relate to the

entire phosphorus budget? Are some species better able to exploit temporal variation due to enhanced storage capabilities? And, perhaps most fundamentally, how should we relate growth studies on simplified, constant foods to in situ conditions where these parameters vary in time and space?"

According to Vrede et al. (1998, 1999), about 67% of phosphorus is located in the body of *Daphnia*, 24% in eggs, and 14% in the carapace. With a longer exposure, phosphorus accumulates in muscles, a cerebral ganglion, and the ovaries. Phosphorus present in the carapace of *Daphnia* is discarded during molting and is thought inorganic (Vrede et al., 1998).

Moina macrocopa need choline and its efficient source is lecithin (phosphatidylcholine), preferably particulated (D'Abramo and Baum, 1981). The requirement is 750−850 mg/100 g of the diet. *Moina* may synthesize choline by methylation of ethanolamine.

It was shown with reference to *D. magna* that shortage of P in the food is entailed by consequences in the next generation: neonates were smaller and contained less P, when fed P-rich food they grew and reproduced faster, but when fed P-poor food they were only able to grow slowly and were delayed in reproduction (Frost et al., 2010). Lukas et al. (2011) found that somatic growth rates of *D. magna* increased with increasing P and with increasing cholesterol availability; the body content of P increased with increasing P availability in food and then stabilized (having reached a homeostatic level).

Macroergic phosphorus compounds [adenine nucleotides: adenosine triphosphate (ATP), adenosine diphosphate (ADP), and adenosine monophosphate (AMP)] were found (Romanenko et al., 2004) to decrease considerably (by 2−3 times) during the growth of an enrichment culture of *Moina macrocopa*.

With P-deficient food (C:P of c. 700), *D. magna* had a higher alkaline phosphatase (AP) activity in their bodies compared with the amount present following phosphorus-rich food (C:P of c. 100); poor phosphorus nutrition also lowers the activity of AP in released materials (containing dissolved enzymes) (McCarthy et al., 2010). This was also shown for *Holopedium* (Elser et al., 2010). Alkaline phosphatase is supposed to be a part of a mechanism to increase acquisition and retention of P. Alkaline phosphatase is a potential biomarker of phosphorus limitation.

Acid phosphatase maintains the level of inorganic phosphorus in cells. The highest AP activity in *D. magna* was found at lower temperatures (10°C), and for every 10°C reduction in body temperature from 25°C, AP activity increases by a factor of 1.67 (Wojewodzic et al., 2011). It was shown that phosphatases liberated into the environment are produced by *D. magna* and not derived from its algal food (Boavida and Heath, 1984).

Most of the liberated phosphorus is from freshly assimilated material, although a fraction is from phosphorus incorporated into tissues. Phosphorus content in *D. pulex* was found to be higher when it is fed with green algae rich in phosphorus (Lehman and Naumoski, 1985).

At higher temperatures (25°C) a greater risk of P limitation was recorded in *D. magna* in comparison with 10°C (Persson et al., 2011).

Phosphorus is liberated from glycerophosphate at the rate (γ/ind. per h): by *Ch. sphaericus*—0.0154, *Daphnia*—0.6 (Margalef, 1951). The phosphorus release rate in *D. galeata* varies from 0.16 to 1.18 μg P/mg C per h in juveniles and 0.06−0.96 μg P/mg C per h in adults (Vadstein et al., 1995).

Lipid Metabolism

According to early observations made by Hardy (1892, p. 187) in daphnias fed with egg yolk "In the course of a few hours the gut walls are found studded with fat globules which are imbedded in the columnar cells; in about 10 to 12 h every blood corpuscle in the body is found to contain a fat globule or globules. *Daphnia* possesses a special fat-holding tissue which is composed essentially of rounded cells anchored

by fine processes, Under the circumstances just mentioned these become charged with fat particles. I further convinced myself that this tissue may, when a great quantity of fat is absorbed, be recruited from the blood cells which fix themselves as now stationary fat-holfing[sic] cells."

There is an extensive record of investigations into the place of lipids in Cladocera digestion and metabolism. Some publications report the chemical composition of lipids in Cladocera, whereas other studies investigate the sources of lipids in Cladocera and try to answer the question of whether Cladocera transform the ingested lipids.

The lipid composition of Cladocera is shown in Tables 3.4−3.8. Total fatty acid concentrations in herbivorous cladocerans is 117−147 mg/g C, and 104 mg/g C in the predatory *Bythotrephes* (Persson and Vrede, 2006). Lipid composition has been determined in total lipid extracts from whole dried Cladocera (Arts et al., 1992, 1993; Sushchik et al., 2002).

Fat droplets are commonly seen in the body of cladocerans and are supposed to be used as a stock material. However, obviously in case of disbalance of nutrients, *Daphnia* that consume food deficient in a vitally necessary material may accumulate pink fat droplets and die soon thereafter (Flückiger, 1951).

Cladocera almost do not synthesize lipids de novo. Taking into consideration that the fatty acids may be either derived from food or synthesized de novo in the body and that acetate (derived from the breakdown of carbohydrates or amino acids) is necessary for their synthesis, Goulden and Place (1993) used [^{14}C]acetate or $^{3}H_2O$ precursors and determined that the lipids synthesized by well-fed *Daphnia* (following incubation of up to 4 h) make up no more than 1.6% of the accumulated fatty acids. Food-restricted *Daphnia* synthesize 0.16% of their accumulated lipids; the overwhelming remainder is derived from algal food. According to D'Abramo (1979), *Moina macrocopa* are entirely dependent upon the sources of fatty acids in their diet. Farkas et al. (1981) found that *D. magna* cannot form docosapolyenoic acids (C22).

VARIATION OF LIPID COMPOSITION

Pelagic Cladocera show seasonal changes in the lipid content that are clearly dependent on the available algal food. In Humboldt Lake (Canada), triacylglycerol was found to be the principal component in *D. pulex*, especially during September−May, after the decline of blue-greens (Arts et al., 1992). An abundance of bacteria following the collapse of a blue-green bloom resulted in increased lipid content in *Ch. sphaericus* and *Diaphanosoma leuchtenbergiana*. The authors concluded that there is an outstanding role for cryptophytes (*Cryptomonas* and *Rhodomonas*) as lipid sources, while *Daphnia* were at near starvation during the blue-green bloom.

Significant seasonal changes in the relative content of fatty acids were measured in *D. galeata*, *Leptodora*, and *Bythotrephes* in the Middle Volga (Fig. 4.17) (Bychek and Guschina, 2001). For example, the content of linolenic acid in *Daphnia* was especially high in July. Further, the content of C16:0 (palmitic acid) was especially high from June to August, similar to that in seston, in both herbivorous *Daphnia* and the predators *Leptodora* and *Bythotrephes*.

As shown in *Daphnia*, PUFAs are components of cell membranes and precursors of eicosanoids (hormone-like mediators) (Schlotz and Martin-Creuzburg, 2011).

Concentration of PUFA in cladocerans was higher at lower temperature (14 vs 20°C) (Masclaux et al., 2012). At increasing temperature (from 20 to 30°C) the percentage of linoleic and linolenic acids decreased in *Moina macrocopa* (Gama-Flores et al., 2015).

Following feeding with EPA-free or EPA-enriched *Scenedesmus* (a green alga), 18:3ω3, 18:4ω3, 20:3ω3, and 20:4ω3 tissue concentrations were higher in *D. galeata* than in *D. hyalina*, indicating that assimilation and biosynthesis of PUFAs is higher in *D galeata* (von Elert, 2004).

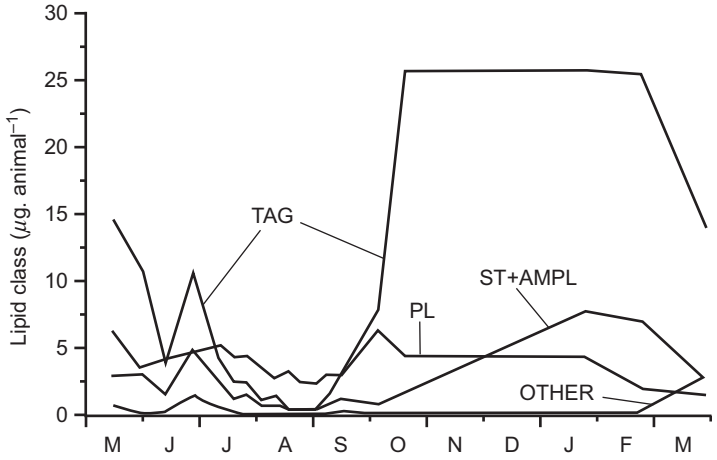


FIGURE 4.17 Seasonal changes in the content of lipid classes in *Daphnia pulex*. *AMPL*, acetone-mobile polar lipids; *PL*, phospholipids; *ST*, sterols; *TAG*, triacylglycerols. Abscissa, months. *From Arts, M.T., Evans, M.S., Robarts, R.D., 1992. Seasonal patterns of total energy reserve lipids of dominant zooplankton crustaceans from a hyper-eutrophic lake. Oecologia 90, 560–571.*

Copper may induce lipid accumulation, especially at high concentrations (such as 12 μg Cu/L), in later generations (e.g., the fourteenth) of *D. magna* (Bossuyt and Janssen, 2004).

Bouchnak and Steinberg (2014) fed *Moina macrocopa* and *Moina micrura* with green algae of known chemical composition and arrived to a conclusion that P-content triggers reproduction and α-linolenic acid triggers longevity.

TO WHAT EXTENT DO CLADOCERA TRANSFORM FATTY ACIDS?

Cladocera possess limited ability to modify the fatty acids obtained from food. According to von Elert (von Elert, 2002; von Elert et al., 2002), *D. galeata* can convert docosahexaenoic acid (C22:5) and C18 PUFAs into EPA (C20:5n-3); α-linolenic acid (C18:3n-3) is the limiting PUFA, as EPA cannot be converted into α-linolenic acid. Thus, these algal sources (i.e., either α-linolenic or EPA) are usable and nonsubstitutable for *Daphnia*, and α-linolenic acid may be a source of EPA. *D. galeata* and *D. magna* are able to convert α-linolenic acid (18:3n-3) into EPA (20:5n-3) (Ahlgren et al., 2009).

As Schlechtriem et al. (2006) summarized: *Daphnia* feeding on highly unsaturated fatty acids [HUFAs; e.g., EPA (20:5n3) or arachidonic acid (ARA) (20:4n6)] become enriched with these fatty acids; and α-linolenic acid (18:3n3) and linoleic acid (18:2n6) are used as precursors of EPA and arachidonic acid. Docosahexaenoic acid (DHA) (22:6n3) is not accumulated but is converted to EPA (Schlechtriem et al., 2006; Martin-Creuzburg et al., 2010).

Later, Masclaux et al. (2012) determined that daphnids (*Daphnia, Ceriodaphnia, Simocephalus, Scapholeberis*) fed on HUFA-deficient diet showed some ability to convert C18 PUFAs into arachidonic acid (C20:4ω6) and EPA (C20:5ω3); the process is more pronounced at lower temperatures.

LITTORAL CLADOCERA

The sources of lipids in littoral *Eurycercus* were determined by Desvilettes et al. (1994, 1997). These authors found a striking similarity between the lipid composition of *Eurycercus* and its seston food (Fig. 4.6). The food of *Eurycercus* consisted of *Cryptomonas*, diatoms, dinoflagellates, ciliated

strombiids, and bacteria. Cladoceran triacylglycerols comprise odd-branched fatty acids, a high percentage of ω6 PUFAs, and significant levels of 18:4ω3, 20:5ω3, and 22:6ω3. The latter three were thought derived from *Cryptomonas*, whereas the odd-branched fatty acids came from consumed bacteria. *Eurycercus* flourished when fed on periphyton (Masclaux et al., 2012) but died in a few days if fed on *Scenedesmus*. The latter was also the case in cultures of *Eurycercus* staged by the author of tne present text.

In *Eurycercus*, the isotopic signatures corresponded to $\delta^{13}C$ and $\delta^{15}N$ values in epiphyton and seston (Masclaux et al., 2014).

No similar investigations have been done in any other littoral Cladocera.

PELAGIC CLADOCERA

There is good evidence that PUFAs are highly important components of Cladocera food. The lipid content of some planktonic cladocerans, and its fractional components, was determined (Lizenko et al., 1977; Bychek and Gushchina, 1999); it was found that fatty acid percentages change with age (Tables 3.4–3.8). Similarities between phytoplankton fatty acids and the neutral lipids of *Daphnia* were noted by Bourdier and Bauchart (1986/1987), namely an abundance of 14:0, 16:0, 16.1, and 18:0 PUFAs.

In *D. cucullata*, the level of PUFAs 22:5ω6 and 22:6ω3 was determined to be 1.76% by weight of total fat (lipids), 23.9% of monosaturated fatty acids (18:0, 9.3%), and 28.4% of SAFA (16:0, 12.6%) (Farkas, 1970). In *D. pulex*, triacylglycerols are the dominant lipid class, followed by phospholipids and sterols (Arts et al., 1993). Weers et al. (1997) found that C16:4ω3 and C22:5 were in lower abundance in *D. galeata* than in their algal food. For various different amounts in their food, *Daphnia* tends to contain high levels of EPA (20:5ω3). *Daphnia* demonstrates a low rate of [^{14}C]linoleic acid (C18:2ω6) and [^{14}C]linolenic acid (C18:3) conversion into C20 PUFAs. Thus, the latter are trophically important.

It was found that the efficiency of cladoceran growth is proportional, first of all, to the availability of fatty acids (HUFAs), and much less so to phosphorus, nitrogen, or carbon (Brett and Müller-Navarra, 1997; DeMott and Müller-Navarra, 1997) (Fig. 4.4); EPA is the most significant food component. The total lipid content of *D. magna* depends on the composition of its food (Cowgill et al., 1984). The quantity of fat in *D. magna* was found directly proportional to the fat in their food algae (green algae, *Ankistrodesmus* and *Selenastrum*; Fig. 4.18) (Cowgill et al., 1984); this effect lasted for five generations. Only C14:0, C14:1, and C18:0 were more concentrated in *Daphnia* than in their algal food (Cowgill et al., 1984). In *Daphnia* fed with yeast, the monounsaturated fatty acid (MUFA) concentration was the highest, compared with those fed with other foods; the MUFAs were mainly C16:1ω7 and C18:1ω9. If the food was *Chlorella* (from seawater), the PUFA concentration was comparatively high (Huang et al., 2001).

The general fatty acid composition of *D. magna* reflected that of their food (Martin-Creuzburg et al., 2010).

Zooplankton growth and egg production strongly correlates with the C20:5ω3 to carbon ratio (Müller-Navarra et al., 2000). *Daphnia* growth is favored by HUFAs from lipid reserves of the diet (DeMott and Müller-Navarra, 1997; Park et al., 2003). A very high correlation was found between *D. galeata* growth and the seston content of EPA, a HUFA (Müller-Navarra, 1995a). Sixty-nine percent of the variation in *D. magna* growth was explained by the algal ω3 PUFA content (Park et al., 2002).

PUFAs present in phytoplankton, EPA especially, are important food ingredients for somatic growth and egg formation in *Daphnia* (Ravet et al., 2003). *Daphnia* growth is also favored by EPA at a threshold concentration of 13 mg/L (Makhutova et al., 2009); EPA is an ω3-type PUFA.

There is a differential response of *D. magna* to different fatty acids consumed in food, as shown by Becker and Boersma (2005, 2007). PUFAs are

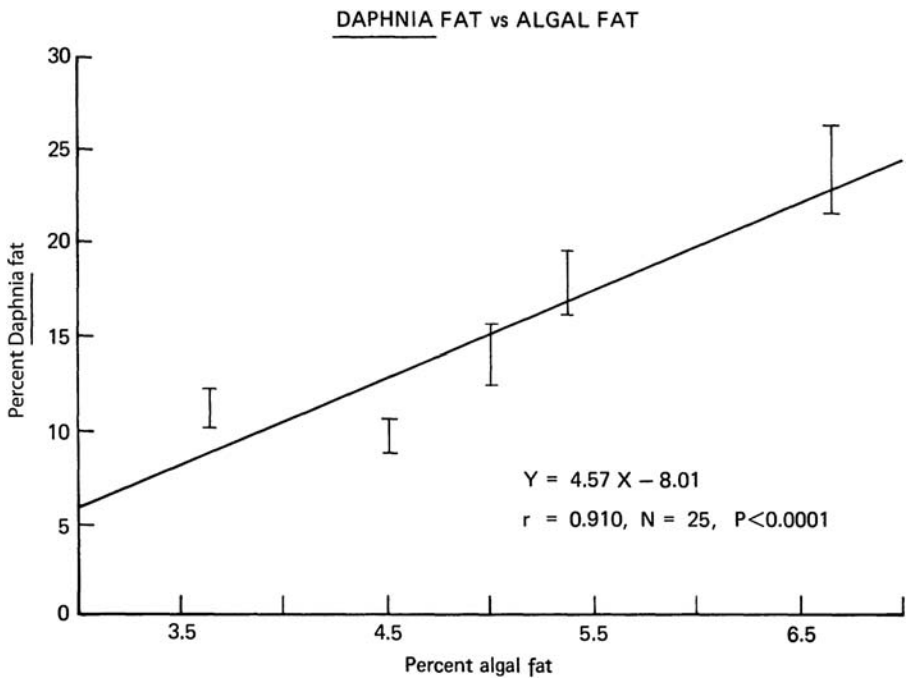


FIGURE 4.18 Fat content in *Daphnia* versus fat content in the consumed algae. *From Cowgill, U.M., Williams, D.M., Esquivel, J.B., 1984. Effect of maternal nutrition on fat content and longevity of neonates of* Daphnia magna. *Journal of Crustacean Biology 4 (2), 173–190.*

predominantly stored, whereas SAFA (20:0; EPA) are metabolized. In eggs, EPA is present in higher concentrations than arachidonic acid. The accumulated SAFA are used during periods of inadequate food supply, and are also passed into the eggs. Egg production is a major drain on *Daphnia* fatty acids. Changes in fatty acid concentrations are smaller compared with phosphorus changes. EPA in seston was shown necessary for the *D. longispina* group, the threshold level being c. 13 μg/L (Gladyshev et al., 2008).

In *Daphnia* spp., the EPA content varies least among the ω3 PUFAs; less so than in the food available to them (Müller-Navarra, 2006). Müller-Navarra fed *Daphnia* with a diatom *Nitzschia* culture and published the fatty acid profiles of this alga and of *Daphnia*: in *Daphnia*, a higher content is reported of the fatty acids that are present in high concentrations in the diatom. *D. magna* and *Simocephalus vetulus* vary in their requirements of eicosapentaenoic acid (EPA), arachidonic acid, and stearidonic acid, the difference disappearing at 20–25°C in comparison with lower temperatures (12–15°C) (Masclaux et al., 2009).

On a PUFA-rich diet, *D. magna* accumulate more ω3 PUFA at 15°C than at higher temperatures (Sperfeld and Wacker, 2011).

For elucidation of the oxidation pathways, *D. magna* were fed with pure carotenoids with yeast and the presence of various pigments in the body was determined (Herring, 1968).

Steroids. Cladocera do not synthesize sterols de novo but obtain them from food. Enriching a poor-in-sterols blue-green *Synechococcus* with various sterols, Martin-Creuzburg and von Elert (2004) found that Δ^5 sterols and $\Delta^{5,7}$ sterols better

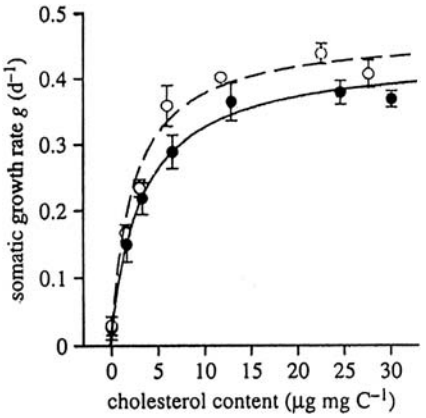


FIGURE 4.19 Growth of *Daphnia magna* in response to sterol content in food. *Filled circles*, cholesterol; *open circles*, cholesterol + EPA. *From Martin-Creuzburg, D., von Elert, E., Hoffmann, K.H., 2008. Nutritional constrains at the cyanobacteria*-Daphnia magna *interface: the role of sterols. Limnology and Oceanography 53 (2), 456–468, Fig. 2a.*

support growth of *D. galeata* than Δ^7 sterol (lathosterol), and Δ^4 sterol (alloholesterol) even adversely affected growth. Cholesterol was the main sterol in *D. galeata*, its content reached 55 ng/ind. $\Delta^{5,7}$ sterols may be converted to Δ^5 sterols (cholesterol) by *D. galeata*.

Growth of *D. magna* was also favored by tetrahymanol and hopanoids (triterpenoid alcohols present in ciliates) (Martin-Creuzburg et al., 2005a,b).

Relative quantity of cholesterol modifies percentages of carbon flows in metabolism (Fig. 4.19).

Carbohydrate Metabolism

The general scheme of carbohydrate metabolism of Cladocera conforms to that shown in Fig. 3.6 (Hohnke and Scheer, 1970). The diets of Cladocera mostly contain an excess of carbon. In *D. magna* and *Simocephalus serrulatus*, the presence of an amylolytic enzyme was demonstrated by Rankin (1929). The ingested starch, stained blue with iodine, changed color to pinkish as it passed down the gut, thus indicating a change from starch to dextrin.

LITTORAL CLADOCERA

The only available data on carbon assimilation by littoral Cladocera are likely those obtained by Infante (1973). *E. lamellatus* fed with *Scenedesmus* incorporated 12 µg C/100 µg C animal/24 h; much less (2.5 µg C/100 µg C animal/24 h) was incorporated when it was fed with *Staurastrum.*

PELAGIC CLADOCERA

Carbon assimilation in filter feeders *(Daphnia* and *Sida)* is 1.7–4 µg C/100 µg C animal/24 h, depending on the type of algal food (Infante, 1973). Stable isotope analysis showed that *D. hyalina* derives almost all of its body carbon from algal sources (Grey and Jones, 2007).

Urabe (1991) determined the distribution of carbon use in subsequent instars of *Bosmina longirostris* and showed that the proportion of assimilated carbon used for respiration increases in subsequent instars and that used for growth decreases (Fig. 4.20). Carbon assimilation in *D. magna* was estimated to be approximately 1.8 µg C/ind./h (Myrand and de la Noüe, 1983). Carbon release per single *Daphnia* containing one γC was calculated to be c. 0.26 γC/day (von Metz, 1973). Cellulose is also digested and assimilated with an efficiency of <11.5%, as found for *Acantholeberis curvirostris*, *D. magna*, and *Pseudosida bidentata* (Schoenberg et al., 1984).

Lampert (1978) determined that 10–17% of the carbon contained in algae ingested by *D. pulex* is transformed and liberated as dissolved organic carbon. Most of the carbon is liberated into the environment from algal cells: 4% from algal cells swallowed whole; and the rest resulting from *Daphnia* secretions and leaching from their feces. Thus, *D. magna* must dispose of excess ingested carbon by means of respiration and a higher excretion rate of dissolved organic carbon to maintain their carbon balance and support their homeostatic carbon content. Juvenile *D. magna* liberate 55–72% of their carbon as dissolved organic carbon and 9–37% of the total carbon loss as carbon dioxide (CO_2).

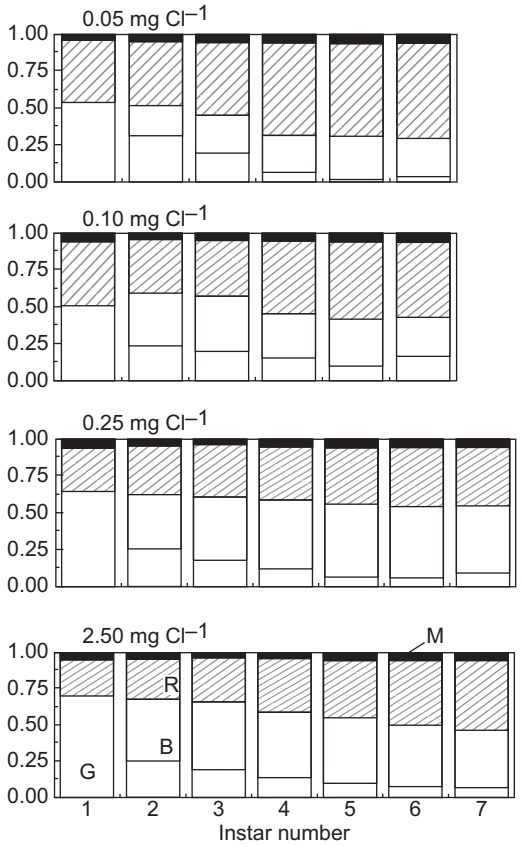


FIGURE 4.20 Carbon allocation in *Bosmina longirostris*. Proportion of carbon allocated in body growth (*G*), reproduction (*B*), respiration (*R*), and molting (*M*) in at four food concentrations. *From Urabe, J., 1991. Effect of food concentration on the carbon balance of* Bosmina longirostris *(Crustacea: Cladocera). Freshwater Biology 26, 57–68.*

The obvious excess of carbon in the food of *D. magna* and its fate during digestion were investigated by Darchambeau et al. (2003). *Daphnia* were fed cultures of the green alga *Selenastrum* that differed with respect to their C:P ratios (400 and 80). The respiration rate of *Daphnia* fed with high C:P algae was significantly higher than that of *Daphnia* fed with low C:P algae. The rate of excretion of dissolved organic carbon was higher in *Daphnia* fed on the high C:P algae (13.4% of body C/day), in comparison with *Daphnia* fed on low C:P algae (5.7% of body C/day).

Carbon lost by *D. magna* as dissolved organic carbon consists mainly of the high molecular weight organic fraction (He and Wang, 2006a). For adults, total loss of organic carbon and of high-molecular organic compounds were 44–64% and 20–47%, respectively. The release of some excess carbon as CO_2 by *D. magna* was also noted by Jensen and Hessen (2007).

Metabolic flows of carbon are labile and are much influenced, inter alia, by the level of available cholesterol in food (Fig. 4.21) (Lukas and Wacker, 2014a,b).

Metabolism of Various Elements

In Cladocera, the metabolic significance of some elements has been investigated. It has been shown for *D. magna* that Ca, Fe, Na, Cu, Se, and Zn are the essential elements, whereas Cd and U are not (Barata et al., 1998).

Calcium (Ca). Calcium takes part in various basic biological processes, e.g., in cell proliferation, signal transduction, muscle activity. Although Cladocera do not possess a strongly calcified carapace, some, e.g., *Daphnia*, still retain some ability to accumulate calcium (Ca) and strontium (Sr) in their shells (Porcella et al., 1967, 1969). In both littoral and pelagic species (*Alona*, *Chydorus*, *Pleuroxus*, *Ceriodaphnia*, *Simocephalus*, and *Daphnia*), the integuments may sometimes be reinforced by $CaCO_3$ (Leydig, 1860, plate II; Gicklhorn, 1925; Schmidt, 1943; Taub and Dollar, 1968; Porcella et al., 1969). This is similar to the deposition of calcium in the exoskeletons of ostracods or of large crustaceans. In *D. magna*, calcification was found to occur by the deposition of fine grains beginning just prior to molting (Porcella et al., 1969).

CLADOCERA DERIVE CALCIUM FROM FOOD AND WATER

The calcium content of *D. magna* decreased from 4.2% to 1% DW over a range of 0.25–0.013 mM Ca in the culture medium;

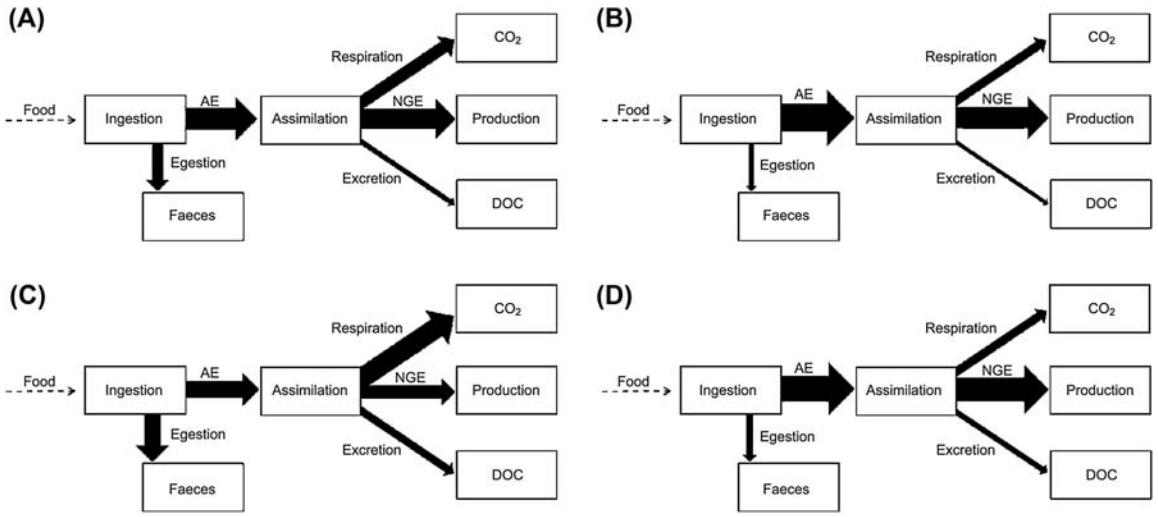


FIGURE 4.21 Intensity of carbon pathways under different cholesterol and food abundance in *Daphnia magna*. (A) High cholesterol − high quality; (B) high cholesterol − low quality; (C) low cholesterol − high quality; (D) low cholesterol − low quality. *AE*, assimilation efficiency; *DOC*, dissolved organic carbon; *NGE*, net growth efficiency. *From Lukas, M., Wacker, A., 2014a. Acclimation to dietary shifts impacts the carbon shifts of* Daphnia magna. *Journal of Plankton Research 36 (3) 848−858, from Lucas, et al., 2014. Journal of Experimental Biology 217, Fig. 4.5, 1082. http://dx.doi.org/10.1242/jeb.094151.*

saturated calcification was reached at a calcium concentration of >0.13 mM; about 40% of calcium is lost with an old exuvium (Alstad et al., 1999). The calcium content in soft tissues of *D. magna* was 8−26% of the total Ca concentration in the body (Muyssen et al., 2009). The latter authors supposed that Ca homeostasis is supported by the rate of its absorption from food.

For *D. magna*, He et al. (2009) determined the Ca content as 1.9−6.5% DW, with molting 20−47% is removed, via excretion, 50−60% of the body Ca; the Ca balance depends on available Ca, C, and P. Tan and Wang (2009a,b,c,d) confirm that most Ca is lost by *Daphnia* via excretion—60−85%, a lesser part—15−40% at molts.

In *Daphnia* and *Ceriodaphnia* with a high-Ca content the dietary assimilation rates of Cd and Zn decreased (Tan and Wang, 2009a); they had higher accumulation rates of Cd than low-Ca *Moina* (Tan and Wang, 2011).

Strontium. Strontium is concentrated by Cladocera to a much lower degree than is calcium (Marshall et al., 1964). Similar to calcium, 95% of strontium is accumulated in the exoskeleton and is eliminated at each molting, thus dropping to minimum; thereafter, the content of Sr gradually increases, as shown in *D. magna* by Marshall et al. (1964) (Fig. 3.12).

Copper (Cu). An optimum copper concentration range for *D. magna* was determined to be 1−35 μg Cu/L (Bossuyt and Janssen, 2004), and active copper regulation between 0.5 and 35 μg Cu/L was observed. For the metabolic role of Cu, see also Fig. 9.1.

Iron (Fe). It is a part of hemoglobin. The content of Fe in *D. pulex* was determined as 95.4 mg% DW (i.e., 95.4 mg per 100 g DW) (Malikova, 1953). Iron is discussed in Chapter 5. See also Table 3.11.

Selenium (Se). The minimum essential concentration of Se to support a culture of *D. pulex* and *D. magna* was determined to be 0.1 ppb (Keating

and Dagbusan, 1984). Below this concentration in the environment, *Daphnia* progressively lost distal antennal segments at molting, manifested cuticle deterioration, and had a shortened life span. Elendt (1990) demonstrated that in *Daphnia* necrotic bands are formed in basal segments of antennal rami in the absence of selenium, and that distal parts of antennal branches are rejected and not restored in subsequent molting. Selenium deprivation causes alterations in mitochondria and the sarcoplasmic reticulum, and complete lysis of muscle fibrils in antennal muscle cells. This damage periodically occurs in the absence of Se, a constituent of glutathione peroxidase, which protects cells and their membrane polyunsaturated lipids against peroxidation. Uptake of selenium by unfed *D. pulex* reaches a maximum after 12 h, and depuration is slow (Schultz et al., 1980).

Sodium (Na). The normal concentration of sodium in *D. magna* is 26.3 mM/kg WW (Stobbart et al., 1977). Excessive Na is not accumulated. Na uptake and release is considered further in Chapter 8, "Osmotic regulation."

Zinc (Zn). For *D. magna*, the optimum range of Zn is 300−600 μg/L; at these concentrations, *Daphnia* contain 212−254 μg Zn/g DW (Muyssen and Janssen, 2002). Decreased or increased Zn levels within the body occur within 1 day. After 24 h of exposure of *Daphnia* to ^{65}Zn (zinc-65), the bioconcentration factor was 1110; after 30 days in a solution of 250 μg Zn/L, *D. magna* accumulated c. 800 μg/g DW (from both solution and particles containing Zn) (Nilov, 1980). Zn bioaccumulation is enhanced by preexposure to Cd (Guan and Wang, 2004a,b).

In laboratory cultures, in the author's experience, the green alga *Scenedesmus* flourishes in Zn-coated metal trays in a mineral culture medium. However, *Daphnia* perish when fed with these algae, which indicates high levels of Zn accumulation by the algae. Nevertheless, toxicity may depend on Zn concentration. For example, the green alga *Pseudokirchneriella* was grown at different Zn levels and accumulated different Zn concentrations suitable for *D. magna* feeding (Canli, 2005). When Zn concentrations in the algae increased from 100 to 220 ng/mg DW, the protein content of *D. magna* increased from 220 to 300 μg/mg DW.

4.2.5 Assimilation

By averaging data from various authors, Sushchenya (1975) calculated the average assimilation of food consumed by freshwater planktonic Cladocera to be 58.4% of that ingested. In addition, the percentage assimilation of consumed food (green algae, bacteria, pink yeast, and bacteria) was determined radiometrically to be 40−56.7% in *Daphnia* and *Simocephalus* (Fedorov and Sorokin, 1967). In *Daphnia*, assimilation is highest for yeast and assimilation is highest in *Simocephalus*-fed bacteria. The assimilation efficiency of *D. pulex* is 35% for *Ankistrodesmus falcatus* (a green alga) and 11% for *Aphanizomenon flos-aquae* (a cyanobacterium) (Holm et al., 1983). Assimilation efficiencies reach c. 50% in *D. longispina, Ceriodaphnia quadrangula, Bosmina longirostris*, and *Ch. sphaericus* (Lair, 1991), 85% in the predatory *Bythotrephes* (Lehman, 1993).

Anderson et al. (2005) highlighted the fact that the food available for herbivorous Cladocera is frequently nutritionally unbalanced and its composition fluctuates. Therefore, to support overall homeostasis it is necessary to release excess substances, especially carbon, which may be done either as selective absorption in the gut or the excretion of excess substances in an organic form. With reference to carbon, regulation of homeostasis of the chemical composition has been investigated in *D. magna* by Darchambeau et al. (2003).

However, the assimilation values also depend on environmental conditions. According to experimental data obtained by Buikema (1975), the percentage assimilation [i.e., the total energy accumulated during growth (for young specimens) and used in respiration] in *Daphnia* is

highest at illumination above 7 foot-candles (fc; approximately 75.3 lx), but is higher with polarized light than with nonpolarized light of the same intensity.

The feeding ratio (i.e., food units spent per unit of weight increment) of algal food for *D. magna* is about 4 but has been shown experimentally to be 6 (Gajewskaja, 1945). In *Daphnia* and *Moina* fed on green algae, the percentage assimilation was 48–60% of the consumed food; the weight gain was 16% in *Daphnia* and 7.6% in *Moina* (Ostapenya et al., 1968). $K_1 = P/R$ and $K_2 = P/R + T$ were found to be 22.5% and 49.9% in *Daphnia*, respectively, and 11% and 23% in *Moina*, respectively (Ostapenya et al., 1968), in which P is weight gain, R is the ration, and T is the energy expenditure for metabolism.

Food items are of varying composition and assimilation by Cladocera is dependent on the composition of the consumed food. In experiments on feeding of *D. magna* with green algae (*Scenedesmus*), the phosphorus content per DW of *Daphnia* decreased when the algal content was low (DeMott et al., 1998). When the ratio of C:P in the food increased from 120 to 900, carbon assimilation declined and the phosphorus content in *D. magna* decreased from 1.47% to 1.08%. The assimilation by Cladocera differs for different groups of algae (Schindler, 1971): high values (in μg/h) were determined for *Asterionella* (diatom) 68, *Ankistrodesmus* (green alga) 72, *Cryptomonas* (Cryptophyceae) 77, and *Oscillatoria* (blue-green) 58.

At higher food concentrations, the assimilation efficiency decreases, as has been shown for *D. pulex* (assimilation 31.1% at low concentration of algae, 13.7% in dense suspension of algae) (Richman, 1958) and later for *D. magna* (Schindler, 1968). The assimilation efficiency by *D. galeata* becomes higher with decreasing food concentration, which affects the carbon balance (Urabe and Watanabe, 1991). In addition, the clearance rate decreases with increasing food concentration.

Hallam et al. (1990) proposed a model summarizing the flow of assimilated resources in the adult daphnid, with special reference to lipids as a labile energy source (Fig. 4.16). This model has greatly contributed to the organization of various relevant data available in the literature.

Digestion Efficiency

GUT PASSAGE TIME

Food stays in the cladoceran intestine for only a few minutes. In littoral species, food passes through the intestine in about 5–7 min (Fryer, 1974; Smirnov, 1969, 1971, 1974), in planktonic *Daphnia* spp., in 25–54 min (Geller, 1975; Pavlutin, 1983; Haney et al., 1986; Christofersen, 1988). Murtaugh (1985) reported a retention time of 4–106 min in *D. pulicaria*. Cauchie et al. (2000) summarized the data of various authors on gut passage time in *Daphnia* and *Bosmina* species to be 4–6 min in *D. galeata*, 15–25 min in *D. longispina*, 2–55 min in *D. magna*, <2–60 min in *D. pulex*, 19–40 min in *D. pulicaria*, 5–6 min in *D. rosea*, and 5–10 min in *Bosmina longirostris*.

Pavlutin (1983) observed the rate of food evacuation to increase in larger animals and at higher food concentrations. Stained material passes through the intestine of *Daphnia* in 20 min (Beim and Lavrentieva, 1981). The only report of a longer retention time was for *D. schoedleri*, which has a gut passage time of 135 min. See also Section 4.7.

Digestion in Cladocera is surprisingly efficient. With this in mind, Fryer (1970, p. 266) noted: "A puzzling feature of the Anomopoda is the rapidity with which the food passes through the gut. A further, related, problem … is the effect of the tremendous dilution of the gut contents brought about by the great intake of water."

This high efficiency may depend on the following facts:

1. The dense cover of microvilli in the intestinal lumen (Figs. 4.1 and 4.4) greatly increases the gut surface. Quaglia et al. (1976) propose that food is absorbed from the lumen of the

midgut in a digested form and liquids are reabsorbed in the proctodeum (hindgut);
2. The inner surface of the intestine is plicate (or folded), which further increases the inner surface;
3. In addition to the huge surface area created by microvilli, the efficiency of digestion is increased through intensive mixing of the food by peristalsis;
4. Food in the intestine is intensively mixed by anal water intake;
5. Powerful enzymes are present in the intestine; and
6. Dialysis occurs through the peritrophic membrane.

In *Daphnia*, the collected food particles are glued together by salivary gland secretions and then sucked into the gut by periodic dilations of the esophagus (Sterba, 1957a). In *Daphnia* and *Simocephalus*, the food also receives secretions from the hepatic ceca. The principal function of the latter is thought to be secretion of digestive enzymes. However, hepatic ceca are completely absent in many cladocerans (e.g., in chydorids). The hepatic ceca contract 6–10 times/min in *D. magna*, but much less frequently in *Simocephalus serrulatus* (Rankin, 1929). It was not shown whether enzymes are present in these secretions.

The tonus (or contraction) of the circular muscles of the intestine of *Daphnia* was found by Flückiger (1952) increased by the sympathomimetics L-adrenaline bitartrate, D-adrenaline bitartrate, L-noradrenaline bitartrate, L-ephedrine hydrochloride, and oxyphenylethanomethylamine tartrate.

Schwerin et al. (2009) observed compensatory control of physiological processes in the cold (10° versus 20°C): in *D. pulex*, cold-repressed proteins comprise enzymes involved in protein digestion (trypsins, chymotrypsins, astacin, and carboxypeptidases); cold-induced proteins include several vitellogenin and actin isoforms.

A schematic representation of digestion and food transformation in Cladocera (Hallam et al., 1990) is shown in Fig. 4.16.

Incomplete Digestion

It is clear that food is incompletely digested by Cladocera. Some of the substances expelled into the environment by *Daphnia* have been identified as unsaturated fatty acids (von Elert, 2000) and are considered infochemicals, i.e., biologically active substances that influence the companion species of algae and other animals. The release of such substances may be a result of incomplete resorption.

In a culture of *D. magna*, the following dissolved substances were identified as influencing cenobium formation in *Scenedesmus* (von Elert, 2000): linoleic acid, α-linolenic acid, myristic acid, palmitic acid, palmitoleic acid, stearic acid, oleic acid, and stearidonic acid. Considering the great abundance of Cladocera, such large amounts of released infochemicals may have a considerable influence on the metabolism and behavior of hydrobionts.

Unbalanced Diet

The available food always lacks some components: nitrogen or phosphorus deficiencies are especially common. This fact partly explains excessive food consumption by Cladocera. Characteristics used to assess food quality for planktonic Cladocera (e.g., *Daphnia*) are the C:P ratio (Schulz and Sterner, 2000) and the C:N ratio. Factors influencing the former ratio have been better investigated than those affecting the latter. It was noted that *Daphnia* species grow fastest under conditions of phosphorus-rich food (Acharya et al., 2004) and that the phosphorus content of *D. magna* directly depends on the phosphorus level of their food (Sterner, 1993; Urabe et al., 1997; Becker and Boersma, 2005). According to the latter authors, the phosphorus content in *D. magna* decreased from c. 16.7 to c. 10 mg/g DW as the C:P ratio increased, and enrichment of food with inorganic phosphorus (PO_4)

significantly stimulated *Daphnia dentifera* growth (Elser et al., 2001).

The phosphorus content of *Daphnia* species is directly proportional to the phosphorus content of their food (i.e., it decreases with an increasing C:P ratio in the seston) (DeMott et al., 2004) in both natural and experimental conditions. Interestingly, phenotypic variation prevails over genetic differences among species. Müller-Navarra (1995b) fed *D. galeata* on algae saturated with phosphorus or on P-limited diet: *Cyclotella* (a diatom) or *Scenedesmus* (a green alga). *Cyclotella* was a better food, as estimated by *Daphnia* growth, owing to its substantially higher content of 20:5ω3 (both phosphorus saturated and phosphorus limited) than *Scenedesmus* under both conditions. *D. galeata* growth, survival, and reproduction were all higher when fed on non P-limited *Scenedesmus*, compared with P-limited algae (Sundbom and Vrede, 1997). The impact of phosphorus on these biological outcomes was higher than that of ω3 HUFA.

In *Bosmina*, phosphorus content is thought to be lower, at c. 0.7−1% DW. Growth and fecundity were unaffected in *Bosmina* fed on low-phosphorus algae, whereas in *Daphnia* these parameters declined (Schulz and Sterner, 1999). Thus, under natural conditions *Bosmina* are better able to survive a low-phosphorus diet.

The phosphorus content in Cladocera has been studied when C:P ratios ranging from 140 to 1000 are present in their food (Ferrão-Filho et al., 2007). The phosphorus content changed substantially over this C:P range in food in *Daphnia ambigua* and a hybrid clone of *Daphnia pulex−pulicaria*, whereas *Ceriodaphnia richardi* and *Moina micrura* showed tight phosphorus homeostasis. The critical algal food quality required for the propagation of *Daphnia* spp. was determined as a C:P ratio of 225−375 (Brett et al., 2000); Anderson and Hessen (2005) indicate that for *Daphnia* carbon is limiting at low levels of available food and phosphorus becomes limiting with abundant food. At a C:P ratio above 230 in scarce seston, phosphorus becomes limiting.

Exposed to imidacloprid, *D. magna* demonstrated the best reproduction at C:P in its food (green algae) and the lowest reproduction at C:P 1300, i.e., at deficiency of P (Ieronima et al., 2014).

The consumption of unbalanced food may lead to the failure of Cladocera cultures provided with abundant food. When fed exclusively on green protococcaceous algae, *D. magna* accumulated oil drops and stopped reproducing (Flückiger, 1951). The daphnias became filled with pink fat droplets, which were grouped abundantly around the intestine (from the first third to the anus), in thoracic limbs, and around nephridia. In such animals, the absence of a certain vitally necessary food ingredient caused reproduction to stop; further, ovaries could not be discerned, molting occurred very rarely, and they died within 2 days. These daphnias did not use their accumulated fat; in contrast, those fed normally use their fat reserves in the absence of food. The addition of egg yolk or yeast improved conditions but the essential component remains unknown.

Sterner et al. (1993) fed *D. obtusa* with *Scenedesmus* containing different levels of nitrogen and phosphorus (moderately N limited, severely N limited, and severely P limited) and found that "no amount of low-quality food would support rapid *Daphnia* growth." The nitrogen content and N:P ratio in *Daphnia* were essentially constant despite varying in the *Scenedesmus*. Feeding rates were lower with P-limited than with N-limited *Scenedesmus*. These authors concluded that the mineral nutritional value of algae may influence the demographics of herbivorous Cladocera more than is commonly estimated. In *D. pulicaria* fed on N- or P-limited green algae, fecundity was reduced compared with controls fed on nonlimited algae (Kilham et al., 1997).

The activity of AP in *D. magna* and *D. pulex* was investigated as an indicator of dietary P-deficiency stress (Wagner and Frost, 2012). P-poor food (green algae) decreased P content in the body followed by increased activity of AP in both species. However, it also increased in

P-sufficient *D. magna* and *D. pulex* fed on blue-greens.

In Cladocera, HUFAs are necessary to support the normal condition of cell membranes. Schlechtriem et al. (2006) cultivated *D. pulex* at 11°C and 22°C for 1 month on a HUFA-free diet. The conversion of C18 fatty acid precursors to EPA (20:5ω3) and ARA (20:4ω6) was observed, and it was concluded that "HUFA such as ARA and EPA are highly conserved during starvation" (Schlechtriem et al., 2006, p. 397).

Fed on food with a low content of PUFAs (blue-green *Synechoccus*) *D. magna* reduced the number of eggs and concentrations of EPA and cholesterol in both eggs and somatic tissues, the concentrations of α-linolenic acid and cholesterol were more homeostatic in eggs than in somatic tissues (Wacker and Martin-Creuzburg, 2007). On food with a high content of PUFAs (heterocont alga *Nannochloropsis*) *D. magna* recovered the concentrations of fatty acids in the eggs and somatic tissues. Cholesterol was supposedly more important for somatic growth than for reproduction.

If fed solely with starch, in *D. longispina* the fat resources were depleted during one to two ovarial cycles, the fat body was not seen any more, the functioning of muscles of the antennae and of thoracic limbs is depressed, work of different heart fibers is uncoordinated, and *Daphnia* died in about 6 days (Flückiger and Flück, 1952). Thus, *Daphnia* is not able to produce fat directly from starch. Addition of vitamin B_1 (aneurin) to the culture medium of *Daphnia* fed solely on starch restored a normal heart rate (Flückiger and Flück, 1952), and the addition of pantothenic acid to a culture of *Daphnia* fed solely on *Chlamydomonas* increased the duration of their life by 3 times (Fritsch, 1953).

The Fate of Ingested Chlorophyll

Cladocera consume great quantities of chlorophyll with algae. Chlorophylls are a group of green pigments, they are a Mg-porphyrin complex bound with protein. "Chemically, chlorophylls are a group of magnesium-metallated tetrapyrroles related to porphyrins but containing a characteristic fifth isocyclic ring and a long-chain isoprenoid alcohol group" (Ma and Dolphin, 1999, p. 196). The algal food, collected in nature by Cladocera, contains a lot of chlorophyll: green algae contain 40−300 mg% WW (i.e., 40−300 mg per 100 g WW), cryptomonads—236−420 mg% WW, and blue-greens—68−483 mg% WW (Lavrovskaya, 1965). The chlorophyll content in lakes may average 5−14 mg/m^3 and reach even higher concentrations (Westlake, 1980; Schulz and Sterner, 2000; Brandl et al., 2010a,b).

This question of the fate of ingested chlorophyll arose rather a long time ago, but generally it remains unanswered. Hardy and McDougall (1894, p. 5) noted that "in the chlorophyll of the algae which form so large a portion of the diet of Daphniidae, we have a substance whose fate we can to a certain extent trace, and we can find that as digestion proceeds the food mass in the middle region loses its green tint, whereas the fluid contents of the anterior become colored a vivid green. Further[sic] there is evidence that this dissolved chlorophyll is absorbed, for the striated border of the epithelium becomes colored an intense green and the cells charge themselves with yellow pigment masses." This is, however, the exterior view.

As a protein, chlorophyll (tetrapyrrole) should undergo the general process of digestion.

Of course, some chlorophyll is discarded undigested. But what happens with its molecule which is degraded in the course of digestion? It would be wasteful if chlorophyll, or partly decomposed chlorophyll, is simply discarded from the organism. Surprisingly, information on the fate of chlorophyll during digestion and its further metabolism is scarce, both in the Cladocera literature and from experts on photosynthesis and animal physiology. For insects, Kuznetsov (1948, p. 111) noted: "Very interesting theoretically processes of digestion of

chlorophyll and of other food pigments are almost not traced in insects"; "The question on the origin of chlorophyll in hemolymph of insects is very interesting," (p.12); and "information is yet insufficient for final judgment on so important biological issue" (p. 13). Kuznetsov (1948) reviewed the opinions of various authors that in herbivorous insects, which consume a lot of chlorophyll, the hemolymph becomes green due to the permeation of chlorophyll or to the creation of green pigment formed from chlorophyll derivatives.

Recent studies on aquatic and terrestrial animals discovered that some metabolic derivatives of chlorophyll are natural antioxidants and cellular signaling mediators (Ma and Dolphin, 1999). For similar functions, "the dietary source of chlorophylls would appear to provide a seemingly inexhaustible treasury for herbivorous feeders" (Ma and Dolphin, 1999, p. 201). Such data convince that further special investigations of chlorophyll transformations in the course of digestion with reference to Cladocera will yield revealing results.

Daphnids fed on the algae *Scenedesmus* and *Rhodomonas* release most of the consumed chlorophyll in their feces; some of it is degraded to chlorophyllide a, pheophytin a, and pheophorbide a. It is assumed that either the pigment or the chloroplasts themselves are poorly assimilated. It has also been shown that Daphniidae convert ingested algal chlorophyll into pheophorbide (Fundel et al., 1998; Pandolfini et al., 2000). At low concentrations of food algae, less pheophorbide a and more digestion products that were colorless were liberated, thus indicating that more pheophorbide a was digested (Fundel et al., 1998). It was shown (Kashiyama et al., 2012) that heterotrophic protists (*Cryptomonas*) consume chlorophyll, degrade it, and take part in detoxification of the resulting products; *Daphnia* fed on *Cryptomonas* is involved in this process.

The levels of pheopigments in the gut of filter-feeding Cladocera were used by Gorbunova (pers. comm.) to indicate decreased consumption of algae in the presence of mineral turbidity. It may be added that the decreased chlorophyll content in the gut of *D. magna*, determined chromatographically, has also been used to indicate reduced feeding in the presence of cypermethrin (Christensen et al., 2006).

Fox et al. (1949) believed that Hb formation in Cladocera is not influenced by the presence of chlorophyll in their food.

It seems that little is actually known about the process of chlorophyll digestion. At best, handbooks on animal husbandry note that ruminants need magnesium and obtain it from plants, in which chlorophyll contains magnesium. Further studies on the fate of chlorophyll in the process of Cladocera digestion are therefore highly desirable.

Chemical Composition of Feces

Little is known about the chemical composition of cladoceran feces. Steryl chlorin esters (SCEs) are products of the transformation of chlorophyll. The composition of sterols in SCEs within *D. magna* fecal pellets was studied by Soma et al. (2005), who showed that they contain both sterols formed by metabolism and unaltered sterols from dietary algae. C_{27} sterols (except for cholesterol and C_{28} sterols, major sterols in diatoms) were scarce in these SCEs. Cholesterol, probably a product of crustacean metabolism, was relatively abundant in these SCEs.

4.2.6 Starvation

Culturing *Simocephalus vetulus* and *Moina* under starvation conditions Papanicolau (1910) observed that the growth was retarded, maturation was attained at a later instar, the number of broods and the number and size of eggs decreased, and time between liberation of broods increased. The life span under starvation conditions was, according to Threlkeld (1976): 4–6 days for *D. magna*, 11 days for *D. pulex*, 5 days for *Daphnia pulex* var. *pulicaria* Forbes, and 4 days for *Moina macrocopa*. The mean

survival time of starved *D. magna* was 7.6 days (Elendt, 1989), with a maximum of 10 days (Porter and Orcutt, 1980). Fully starving adult *D. magna* survived for 160–246 h, *D. galeata* for 105–140 h, neonates of *D. magna* for 66–134 h (longer with a higher maternal lipid content), and neonates of *D. galeata* for 60 h (Tessier et al., 1983). Predatory *Leptodora* and *Bythotrephes* may starve up to 7 days.

It is clear that these organisms use their intrinsic resources in the complete absence of food. When maintained in autoclaved tap water filtered through a 0.22 μm pore filter, *Ceriodaphnia cornuta*, *Moina macrocopa*, *Diaphanosoma sarsi*, and *Scapholeberis kingi* could reproduce for three generations, although they matured later than did females fed with *Chlorella* (Kumar et al., 2005a). In contrast, *Macrothrix* spp. were barely able to undergo a single generation. Over 48 h of starvation the DW of *D. magna* reduced from 279–299 μg to 219–239 μg (Glazier and Calow, 1992), a similar decrease over 3 days of starvation was recorded in *D. pulicaria* (DeMott and Müller-Navarra, 1997). In starving *D. magna*, the greatest losses were those of DW proteins and lipids (including those due to the release of young formed before starvation), and reductions in total carbohydrate and glycogen occurred during the first day of starvation (Elendt, 1989). The triglyceride:total lipid ratio decreased during 5–6 days of starvation from 0.52 to c. 0.15.

The changes in the chemical composition of *D. pulex* caused by starvation are shown in Fig. 3.1 (Lemcke and Lampert, 1975). The chemical composition of starved *D. magna* was analyzed in detail by Elendt (1989) and Hessen (1990). Hessen (1990) found that the mean phosphorus content of starved *Daphnia* was 1.38% DW; this was unaffected by the quantity of available food. With reference to *Simocephalus* (Green, 1966b), starvation resulted in carotenoids being depleted from all organs.

The highest priority in starving *D. magna* was carapace formation, comparing with respiration, growth, and reproduction (however, varying with age) (Glazier and Calow, 1992).

In *D. magna* starved for 1–3 days, the beating rate of the thoracic appendages decreased; it increased immediately (from c. 6 Hz to c. 8 Hz) when food was added (Plath, 1998). Respiration was low in fasting *Daphnia* in comparison with animals provided with high- or low-food levels (Jensen and Hessen, 2007). Addition of food was followed by a three-to fourfold increase in the respiration rate and increased swimming activity (Schmoker and Henández-León, 2003). By the fourth day of starvation, the oxygen consumption of *D. obtusa* had decreased to 50% of the level of well-nourished specimens (Vollenweider and Ravera, 1968).

The respiratory quotient (RQ) in *D. pulex* decreased from 1.13 to 0.71 over 5 days of starvation (Richman, 1958). This decrease in RQ demonstrates a change from predominant carbohydrate utilization in the metabolism to protein and fat metabolism.

In starved female *D. magna*, the following events occur in midgut enterocytes (Elendt and Storch, 1990): depletion of lipid and glycogen reserves, swelling of mitochondria, reduction of the endoplasmic reticulum and of dictyosomes, and a decrease in cell height. Prolonged starvation of *D. magna* also results in a loss of lactate dehydrogenase activity (anaerobic metabolic activity) in *Daphnia* homogenates (Hebert, 1973). In starving *Daphnia*, there is also a drop in blood concentration (Fritsche, 1917). Belayev (1950) interpreted the decrease in blood hypertony relative to the external water as an indication that food is the source of substances that support hypertony. Carotenoids in all tissues are depleted during starvation in *Simocephalus* (Green, 1966a) and the heart rate in starving *D. longispina* decreases (Ingle et al., 1937).

Hungry daphnias actively filter the food particles; it was noted that a period of about 30 min is sufficient to overcome the starvation effect (Lampert, 1987). At starvation, daphnid growth continues for a time as a result of internal stores,

and refeeding results in "catch-up" growth (Bradley et al., 1991a,b). Under conditions of minimum food, *D. longispina* produce fewer young (Ingle et al., 1937); when they are again fed abundantly, they "promptly produce many more young in each brood." In *D. magna*, temporary starvation has been demonstrated experimentally to be followed by immediate cessation of energy allocation to reproduction (by ceasing egg production) (Bradley et al., 1991a,b). This energy allocation is confined to the first half of the instar. Refeeding with *Chlorella* results in a recovery of fecundity. Trubetskova and Lampert (1995) also found that with starvation, the eggs of *D. magna* are fewer and smaller. Of course, the reaction to food shortage (i.e., a decrease in egg number) is delayed. See also Chapter 11 for the lag effect.

4.2.7 Lipid Pathway From Algae via Cladocera to Fish

Müller-Navarra et al. (2004) advocate, with good reasons and reference to *Daphnia* data, that the HUFAs EPA (C20:5ω3) and docosahexaenoic acid (C22:6ω3) levels provide a strong predictor of zooplankton growth. These fatty acids are conservatively transferred up the food web to fish and to man.

In temperate latitudes, diatoms (with their stock of lipids) dominate in the spring, followed by blue-green and green algae, the latter of which mostly contain a stock of starch. This change has profound alimentary significance, as algal lipids are assimilated with little change. Following extensive analytical measurements, Sushchik (2006) found that the growth of planktonic Cladocera *(Daphnia)* is controlled by EPA and nitrogen (N) availability; a general conclusion was also made that EPA and N levels indicate the quality of their food within a water body. The seasonal distribution of EPA in different links of the trophic chain was determined from May till October by Sushchik et al. (2008) in phytoplankton (in May the diatom *Stephanodiscus* was dominant), zooplankton, and fish (*Carassius*) in a reservoir near Krasnoyarsk (Russia). The quantitative distribution of EPA in zooplankton generally resembled that in phytoplankton (with a peak of c. 120 g/L in phytoplankton in May). In general, the quantity of EPA in a cyprinid fish *(Carassius)* followed that in zooplankton. The predominant fatty acids in the fish were 16:0, 18:1, and 22:6ω3. Sushchik (2001) had previously observed that *Daphnia* development is controlled by PUFAs derived from diatoms and blue-green algae. Sometimes, the diatoms were lacking in 20:5ω3 or other PUFAs, and the blue-green algae contained most of the essential 18:3ω3.

Koussoroplis et al. (2013, p. 1017) found that at low availability, if PUFAs 20:5ω3/20:4ω6 were supplied in proportion 3.6:1, their proportion in *D. magna* increased to 3.8:1. These authors concluded: "*D. magna* is an efficient gatherer, accumulator, and repository of PUFA under low/fragmentary dietary availability," thus qualifying the role of daphnids in the transfer of PUFA in food webs. However, Kainz et al. (2004) remarked that the concentration of an essential fatty acid (docosahexaenoic acid) in the food chain decreased in the cladoceran link in all of the lakes studied.

It should be noted that in lakes in which diatoms are the dominant primary producers, as indicated by a sediment consisting of diatomite (e.g., in Kamchatka or in Iceland), the trophic chain is based principally on lipids.

Lipids in Freshwater Fish. Fats are known to be incompletely transformed by their consumers; the composition of their fat is similar to that of their food items. For their normal development, fish mainly require three long-chain PUFAs (Sargent et al., 1999): docosahexaenoic acid (22:6ω3), EPA (20:5ω3), and arachidonic acid (20:4ω6). Following consideration of the experimental data, Herodek and Farkas (1967) suggested that most of the fatty acids in fish originate in the crustaceans they consume.

The fatty acids are just slightly modified along their pathway from algae via crustaceans to fish. The extent of fatty acid modification at the algae—crustacea and crustacea—fish links should be the focus of further investigations. With good reason, Arts et al. (2001, p. 122) indicate that fatty acids "are important 'drivers' of ecosystem's health/stability," and may determine humanity's past and future health. In fish, man consumes the algal (slightly modified) fat. The fish smell is not actually of fish, rather, of algal fat.

Importance of the discussed issue is much wider. Arts et al. (2009) draw attention that human brain tissue consists by about 60% DW of lipid, most of which is docosahexaenoic acid. Principal de novo producers of it and other fatty acids are algae, from which they reach humans via crustaceans and fish.

It may be added that physiology of organic synthesis of particular groups of algae is insufficently known.

4.2.8 Natural Toxicity

Cladocera encounter many toxic agents under natural conditions. Periodically developing in great quantities, blue-green either unfavorably modify the environment or are directly toxic. Uptake of the toxic microcystin by *Daphnia* and *Moina* occurs from both the dissolved fraction (Ferrão-Filho et al., 2014a,b) and the ingested food. Toxins of blue-greens may be directly toxic or decrease the feeding rate in *Daphnia* (e.g., Rohrlack et al., 2001). Extracts from *Microcystis* can inhibit protease activity in *Moina macrocopa* (Agrawal et al., 2001). Further, Rohrlack et al. (2004) found that *Daphnia* feeding on *Microcystis* are unable to shed their old integument, despite a new integument being produced. These authors also found that blue-green algae contain the protease inhibitor microviridin J. From the gut of *Daphnia*, it penetrates into the blood and disrupts normal metabolism, which leads to disturbances in molting and interference with normal swimming and filtration, which ends fatally. Derived from blue-greens neurotoxin B-N-methylamino-L-alanine (BMAA) causes in *D. magna* decrease in clutch size and in mobility (Lűrling et al., 2011). *Daphnia* accumulate BMAA and convey it via food webs.

Paralytic shellfish toxins are obtained by *Moina mongolica* following ingestion of the dinoflagellate *Alexandrium* and then are transferred to fish that predate on the *Moina* (Jiang et al., 2007).

4.2.9 Impact of Xenobiotics on Digestion

Cadmium. Following exposure to Cd (at 20 μg/L) large fat cells (i.e., storage cells) in *D. magna*, which are especially numerous at the posterior curve of the digestive tract, became smaller, contained less glycogen, and their mitochondria became more spherical (Bodar et al., 1990b). Cadmium is accumulated by *Ceriodaphnia dubia* from their diet (from green algae loaded with cadmium) and from water, but uptake from the diet is slower (Sofyan et al., 2007). Exposure of *D. magna* for 48 h to sublethal solutions of $CdCl_2$ or $HgCl_2$ inhibits cellulase, amylase, galactosidase, trypsin, and esterase; in contrast, exposure to $K_2Cr_2O_7$ increases activity of these enzymes (De Coen and Janssen, 1997). Assimilation of the green alga *Selenastrum* by *D. magna* decreased several fold upon exposure to 100 μM Cd^{2+} (Baillieul and Blust, 1999). The overall activity of the acid phosphatase complex increases following exposure to $CdCl_2$, as found in *D. magna* (Zarubin et al., 1997) and *Ceriodaphnia affinis* (Tsvetkov et al., 2012).

Tin. Tributyltin disrupts the lipid homeostasis, interferes with the transfer of glycerophospholipids and triacylglycerols to eggs and thus is an obesogen increasing their accumulation in females (obesogenic effect) as shown in *D. magna* (Jordão et al., 2015). Survival of the progeny hatched from such eggs is lower than that of the control.

Uranium. Exposure of *D. magna* to depleted uranium resulted in reduction in food

assimilation due to damage to intestinal epithelium and presence of uranium precipitates in the epithelium (Massarin et al., 2011).

Zinc. Zinc effect in Zn-contaminated diets on reproduction of *D. magna* was ascribed (Evens et al., 2012) not to Zn itself but to Zn-induced changes in the content of dietary P.

Makrushin (1995) observed that pieces of the gut epithelium separated from the basal membrane in the zone of industrial pollution in *Bythotrephes*.

Chlorophos in concentrations 0.0004−1 mg/L caused pathological changes in the midgut epithelium of *D. longispina* (Makrushin, 1974). The insecticide tanrec retards the body growth in *D. magna*, causes erosion of the midgut epithelium, leads to disappearance of vacuoles in the fat body, blocks the growth of oocytes, and causes their destruction (Papchenkova and Makrushin, 2013).

In solutions of phenol and aluminum sulfate, pH in the intestine of *Daphnia* increases from the normal value of 6 up to 8 (Beim et al., 1994).

Histologically, it is shown that bromine, a constituent of deltamethrin, is deposited on the gut wall, microvilli, and peritrophic membrane of *D. magna* (Eybe et al., 2009).

Nanosized particles, such as C_{60} fullerenes, cause damage of cells in the alimentary canal of *D. magna* but in short-term exposures are protective against polycyclic aromatic hydrocarbons (Yang et al., 2010).

CHAPTER

5

Respiration

5.1 ANATOMICAL BACKGROUND

In contrast to the former opinion that respiration of Cladocera is realized by means of the epipodites of thoracic limbs, it is shown now that most gas exchange in Cladocera occurs through the body surface (Peters, 1987; Pirow et al., 1999a,b). Most of the oxygen necessary is extracted from the feeding current (Pirow et al., 1999a; Seidl et al., 2002). Thus, feeding and gas exchange are closely connected. Oxygen is then distributed throughout the body and a branch of hemolymph reaches the brood chamber (Fig. 5.1) (Seidl et al., 2002; Pirow and Buchen, 2004). Another area of active respiration is the rectum, during the frequent anal water intake.

Gas exchange is also favored by the small size of cladocerans. The smaller the specimen, the greater is its relative surface (surface:volume ratio). As well, this ratio is minimal in globular forms and is much greater in commensurate lenticular cladocerans (e.g., extremely compressed *Acroperus* spp.). Hebert (1978b, p. 318) also noted that development of cephalic crests "suggests a role in gas exchange."

Due to the dual function of movement of the appendages, the rate of their movement is labile and depends on both the concentration of food particles and the oxygen concentration (Pirow and Buchen, 2004). According to these authors, hypoxic exposure results in tachycardia under food-free conditions and ventilatory compensation under food-rich conditions.

It was previously thought that the sites of active gas exchange were the gills (epipodites) of thoracic limbs because it is easily observed that the gill surface is the area of active silver reduction in an $AgNO_3$ solution. Accordingly, $AgNO_3$ is extremely toxic as silver precipitates on gills. For example, *Chydorus sphaericus* is killed by 0.00001% solution of $AgNO_3$, whereas most of compounds of K and Na are lethal at 0.05−2% (Smirnov, 1971).

5.2 ENVIRONMENTAL BACKGROUND

Littoral Cladocera frequently live under conditions of oxygen deficiency, especially those that bury themselves in the bottom sediment. In general, pelagic Cladocera suffer less often from oxygen deficiency, except under conditions such as the mass decomposition of algae or the morning oxygen deficit due to algal respiration. They are also exposed to diurnal and seasonal changes in the distribution of oxygen concentration, as well as the decreasing vertical oxygen gradient, which has been well described in the context of limnology (see, e.g., Hutchinson, 1957, Chapter 9; Macan, 1963; Dodson, 2005; Kitaev, 2007). It should also be remembered that the oxygen concentration in water decreases with increasing temperature.

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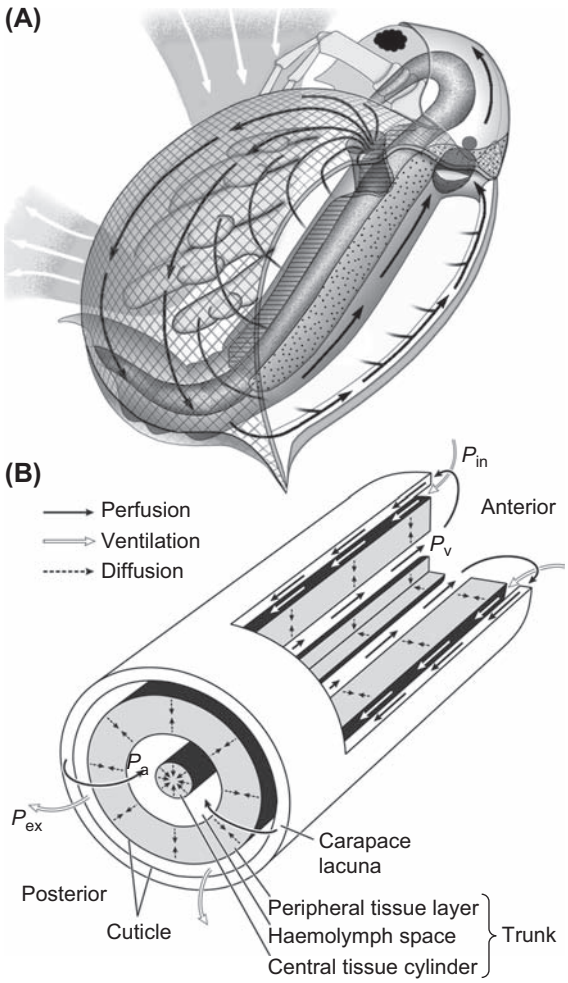

FIGURE 5.1 (A) Blood flows in *Daphnia magna*. (B) P_a, oxygen partial pressure of the hemolymph entering the trunk; P_{ex}, expiratory oxygen partial pressure; P_{in}, inspiratory oxygen partial pressure; P_v, oxygen partial pressure of the hemolymph leaving the trunk. *From Pirow, R., Buchen, I., 2004. The dichotomous oxyregulatory behaviour of the planktonic crustacean* Daphnia magna. *Journal of Experimental Biology 207, 683–696.*

5.3 OXYGEN CONSUMPTION

5.3.1 Methods

The quantity of oxygen used as the difference of the oxygen content before and after a certain period of breathing was determined by Winkler's titration method (e.g., for *Daphnia*, by Skadovskiy, 1937; Rechkalov, 1994). Thunberg's microrespirometer was applied for determination of the volume of oxygen consumption in early developmental stages (Obreshkove, 1930). In principle, it consists of two bottles connected by an index tube. The differential microrespirometer of Drastich type was used for determination of respiration of *Eurycercus* (Smirnov, 1962). This apparatus was also commented by Polyakov (1937). (Note for recalculation: 1 mL O_2 = 1.6 mg O_2).

The Cartesian diver was also used (O'Connor, 1950; Ivanova and Klekowski, 1972).

A polarographic oxygen electrode system was used by Porter et al. (1982).

Paul et al. (1997) and Pirow et al. (2001, 2004) devised special computerized recording techniques and have achieved remarkable results in the field of *Daphnia* respiration. A special setup for optophysiological recording with a video recorder and personal computer, as well as computerized respirometry, was devised by Paul et al. (1997). For this, *Daphnia* were placed in a glass vessel of 0.45 mL volume. Pirow et al. (2001) measured tissue oxygenation using an apparatus consisting of a reversed microscope equipped with systems for measuring hemoglobin oxygenation, reduced nicotinamide adenine dinucleotide (NADH) fluorescence (as an indicator of tissue oxygenation state), and the movements of organs. Remarkable results were obtained by Pirow et al. (2004) using injection of the oxygen-sensitive phosphorescence probe Oxyphor R2 into the circulatory system followed by phosphorescence imaging. In experiments made by Pirow et al. (2004), fasting animals were immobilized by gluing their posterior apical spine with histoacryl to a bristle that was then fixed to a coverslip with plastilin. The dye was then microinjected into the circulatory system from the dorsal side into the space "directly downstream of the heart." A single daphnid was fixed onto a coverslip and then placed onto the bottom of a thermostatted perfusion chamber; in this

apparatus, the individual daphnid was able to move its antennae freely. Measurements of the distribution of oxygen partial pressure (P) in the hemolymph were followed by phosphorescence imaging of the P range (0–6 kPa).

5.3.2 Respiration Intensity

The oxygen affinity of blood is numerically expressed by the partial pressure of oxygen that induces half saturation [semisaturation (P_{50}), i.e., the formation of 50% of oxyhemoglobin at a certain partial pressure of oxygen (mmHg)]. Ability of cladocerans to exist at higher or lower concentrations of oxygen gas (O_2) is characterized by the property of their blood to reach semisaturation at a certain oxygen pressure. For example, at 17°C in *Ceriodaphnia laticaudata*, semisaturation occurs at 0.8 mmHg of O_2, i.e., much lower than that in *Daphnia magna* (3.1 mmHg) (Fox, 1945). Different species of *Ceriodaphnia* can withstand different oxygen limits. *C. laticaudata* is an example of a species able to live at lower oxygen concentrations (Burgis, 1967). The hemolymph of cladocerans becomes oxygenated while it flows through the ventral part of the carapace and the rostral region via direct diffusion (Pirow et al., 1999b).

Winberg (1950) summarized all quantitative data on oxygen consumption by crustaceans available at that time. These data included, *inter alia*, freshwater planktonic cladocerans and copepods. Winberg concluded that oxygen consumption for all crustaceans complies with the equation, (Eq. 5.1):

$$R = 0.105W^{0.81} \quad (5.1)$$

in which R is the rate of oxygen consumption in mg/h at 15°C, and W is the weight in g.

As summarized later by Threlkeld (1976), the rate of oxygen consumption in cladocerans is: $R = 0.2935\,W^{-0.184}$ in *D. magna*; $R = 0.207\,W^{-0.124}$ (R in μg/day, W in μg) in *Daphnia pulex* var. *pulicaria*; and $R = 0.133\,W^{-0.107}$ in *Simocephalus vetulus*.

The rate of oxygen consumption is used as a measure of intensity of metabolism and, accordingly, of food requirements.

Oxygen consumption is labile and depends on various factors and on condition of cladocerans. It increases with increasing temperature (Skadovskiy, 1937), up to a maximum. For *D. middendorffiana*, the maximum was found to be 26°C, above which inactivation of respiratory enzymes started; for *Bythotrephes cederstroemi*, the upper limit is 23°C—thus, it is more stenothermic (Yurista, 1999). It should be kept in mind that, with increasing temperature, the oxygen content of water tends to decrease.

The oxygen consumption becomes less intensive in older specimens, as was shown with reference to *Simocephalus exspinosus* (Obreshkove, 1930). The rate of oxygen consumption in the third-instar specimens was by 3.6% less than that in first-instar specimens and 51–52% of that in the newly released young. Carbon dioxide production also decreased with age.

Oxygen consumption in *S. vetulus*, measured using Cartesian divers or 100–130 mL bottles, did not change within a pH range of 4.5–9.5 in immobile specimens, although their general oxygen consumption (including movement and filtration) did increase with increasing pH (Ivanova and Klekowski, 1972). O'Connor (1950) calculated that muscular activity in *Daphnia* requires 32% of their total metabolic energy. Similar value of energy consumption for the muscular activity was obtained by Ivanova and Klekowski (1972): *S. vetulus* immobilized in the small space in the Cartesian diver's bulb consumed 29.5% less oxygen than did moving animals. Respiration intensity varies depending on food quantity and quality as shown in *D. magna* (Lukas and Wacker, 2014a,b).

Unfortunately, intensity of gas exchange was never measured in the course of the intermolt cycle.

A model of oxygen transport from the environment into tissues has been suggested with reference to *D. magna* by Moenickes et al. (2010).

Oxygen consumption correlates with the respiratory electron transport system (ETS). Simčič and Brancelj (1997) reported that the ETS is localized in the inner mitochondrial membrane and comprises a multienzyme complex containing flavoproteins, metallic proteins, and cytochromes. This redox system transports electrons from NADH, nicotinamide adenine dinucleotide phosphate (NADPH), and succinate to O_2. Formazan produced in this process closely correlates with O_2 consumption; thus, a method of measuring formazan production spectrophotometrically in homogenates has been found to be extremely sensitive and applied to five species of *Daphnia*. Tissue oxygenation has been estimated by NADH fluorescence in the limb muscles of *Simocephalus* (Forasacco and Fontvieille, 2008), using the assumption that NADH content positively correlates with oxygen consumption.

5.3.3 Respiration in Littoral and Bottom-Dwelling Cladocera

Information on the respiration of bottom-dwelling Cladocera is very scarce. Benthic Cladocera, especially those that bury themselves in the bottom sediment, live under conditions of frequent oxygen deficiency and an unlimited supply of food material. *Chydorus ovalis* consumes 19–21 mg O_2/24 h/g wet weight (WW) at pH 9 and 23°C (Yatsenko, 1928), *Eurycercus lamellatus* consumes 14.4 mL O_2/24 h/1 g WW (Smirnov, 1962).

Littoral chydorids may survive at c. 1.9 mg/L O_2 or even as low as 0.4–1.7 mg/L O_2 (Pacaud, 1939; Bogatova, 1962). The minimum oxygen concentrations in their environment at 18–22°C is 1–1.7 mg/L O_2 (*Acroperus*, *Alona*, *Camptocercus*, *Eurycercus*, and *Pleuroxus*) (as summarized by Smirnov, 1975).

Reduced oxygen concentrations induces upward swimming in *C. sphaericus* and *Pseudochydorus globosus* (Meyers, 1980) at decreased light intensities (if not attached to a substratum). *C. sphaericus* reacts to higher oxygen levels (2.5 ppm) than does *Pseudochydorus* (1.15 ppm).

5.3.4 Respiration in Pelagic Cladocera

Pelagic species usually enjoy a relatively good oxygen environment (by living at normoxia). Water flow created by the beating of their thoracic appendages supplies both oxygen and food to pelagic species. Beating rates of the appendages depend on both the oxygen concentration and the concentration of available food particles (Pirow and Buchen, 2004).

Oxygen tension (pO_2) within the *Daphnia* body is about 4–5 times lower than that of the surrounding water (Fox, 1945; Wolvecamp and Waterman, 1960): in *D. magna*, it is 3.1 mmHg, but in *C. laticaudata* it is only 0.8 mmHg.

There have been numerous measurements of oxygen consumption rates in Cladocera, mainly for *Daphnia* species. At 20°C, *D. longispina* consume 19.1 mg O_2/24 h/g WW (Shcherbakov, 1935), i.e., 0.8 mg/h. Under an average "normal" temperature, *Daphnia* consume c. 1 μL O_2/1 mg dry weight (DW)/h (as summarized by Peters, 1987). Oxygen consumption is about 0.2–1.3 mL O_2/g WW/h at 20°C in *Daphnia*, 0.29 mL O_2/g WW/h in *Chydorus* (Wolvecamp and Waterman, 1960), and 0.14–1.18 μL O_2/individual (ind.)/day at 16°C in *Leptodora* (Hillbricht-Ilkowska and Karabin, 1970).

Oxygen consumption by *D. magna* is maintained at approximately the same level when the oxygen concentration in the water is 4–1.5 mg/L, but it abruptly declines with a further decrease in the external concentration (Skadovskiy, 1955). The respiration of *D. magna* increases at higher algal concentrations in the environment (Porter et al., 1982). Kersting (1978) also demonstrated that *D. magna* respiration reaches a maximum at a concentration of food algae that is close to the critical level (i.e., in which the food uptake does not increase further and becomes constant). With an excess

of food (*Chlorella*), *D. magna* respiration decreases somewhat (Kersting and van der Leew-Leegwater, 1976). It is higher in feeding animals, lower in animals provided with low-food levels, and still lower in fasting specimens (Jensen and Hessen, 2007).

From a quantitative aspect, the relationship between the body weight (W) and oxygen consumption (Q) in planktonic Cladocera can be described by the following equation (Eq. 5.2), and is somewhat below the average level for all crustaceans (Sushchenya, 1972):

$$Q = 0.143W^{0.803} \quad (5.2)$$

For *D. pulex*, Richman (1958) determined the following relationship between weight and oxygen consumption (Eq. 5.3):

$$\mathrm{Log}O_2 = \log 0.0014 + 0.881 \log W \quad (5.3)$$

The minimum oxygen concentrations in their environment at 18–22°C is 0.29–2.43 mg/L O_2 for pelagic cladocerans (*Daphnia* and *Simocephalus*) (as summarized by Smirnov, 1975). The lethal minimum oxygen concentration was found to be 0.29 mg/L in *Daphnia obtusa* and 0.99 mg/L O_2 in *Daphnia hyalina* (Herbert, 1954). Heisey and Porter (1977) investigated oxygen consumption and filtering rates in oxygen concentrations ranging from air saturation levels to almost zero. The oxygen consumption in *Daphnia galeata mendotae* turned out to be directly proportional to the oxygen concentration. In *D. magna*, it was slightly increased at O_2 concentrations above c. 3 mg/L; below this concentration, the rate of oxygen consumption declined rapidly.

Pirow et al. (2004) also investigated oxygen transport processes in *D. magna* using an oxygen-sensitive phosphorescence probe, Oxyphor R2, injected into the circulatory system. The resulting three-dimensional profiles were different for hemoglobin (Hb)-rich and Hb-poor specimens. A steep gradient was discovered in Hb-poor specimens, whereas Hb-rich individuals showed flat gradients (Fig. 5.1).

Oxygen consumption also depends on various other factors, such as age and clone (as shown for *Simocephalus* by Obreshkove and Banta, 1930), illumination (Buikema, 1972), and vessel size (Zeiss, 1963). Well-expressed clonal differences were found even in clones derived from a single female (Obreshkove and Banta, 1930).

Under conditions of insufficient food, the oxygen consumption in *D. magna* decreases about twice (Skadovskiy, 1937). The respiratory rate rapidly increased in previously starved *D. magna* provided with increasing concentration of algae until the "incipient limiting level" of food concentration was reached (Lampert, 1986). The oxygen consumption was significantly higher in negatively phototactic *D. pulex* than in positively phototactic ones (Skadovskiy, 1939a).

Water saturation with CO_2 does not influence the oxygen consumption by *D. magna* up to 0.0006 M (264 mg/L) but sharply decreases at 0.0076 M (334 mg/L) (Skadovskiy, 1937).

Drugs depress respiration of Cladocera either directly (nicotine, epinephrine, cyanide, strychnine) or by depressing the respiratory movements (i.e., corresponding muscles) (Sollman and Webb, 1941). According to Flückiger (1953), sympathomimetics (i.e., L-adrenaline, D-adrenaline, and L-noradrenaline) increase oxygen consumption by *Daphnia*. The presence of carbaminoylcholine chloride (a parasympathomimetic) also increases oxygen consumption.

5.4 HEMOGLOBIN AND IRON

Hemoglobin (Hb) has been reported in blood (hemolymph) of various littoral and pelagic Cladocera by many authors. With oxygen deficiency, Hb may appear in the blood of Cladocera, and its concentration increases over time (Chandler, 1954; Smaridge, 1956; Kobayashi, 1970). Fat cells are the sites of hemoglobin formation (Goldmann et al., 1999).

5.4.1 Hemoglobin in Littoral Cladocera

Although Hb is more important in littoral and bottom-living Cladocera than in pelagic Cladocera, Hb has mainly been studied in pelagic daphnids and in *Moina*. In littoral chydorids, such as *Acroperus harpae*, *Alona affinis*, *E. lamellatus*, *Picripleuroxus striatus*, *Pleuroxus trigonellus*, and *P. truncatus*, Smirnov (1970) demonstrated the presence of Hb using three different methods:

1. By producing crystals of pyridine hemochromogen in the bodies of the chydorids (according to the method of Hoshi, 1963a, p. 88);
2. By producing crystals of hemin (a crystalline heme) (according to the method of Hoshi (1963b, p. 94); and
3. By staining Hb blue with benzidine base (according to the method of Pearse, 1960).

The blue staining was clearly observed to be localized and the crystals produced by methods (1) and (2) were characteristic of each substance. Hb was found in both reddish specimens and specimens with no visible reddish coloration.

The red color of mud-living *Ilyocryptus sordidus* is caused by Hb, as shown by Fox et al. (1951). If its young are cultivated in well-aerated water, they grow into pale adults. It was also demonstrated that bottom-living and littoral Cladocera, such as *Ilyocryptus* and *C. sphaericus*, gain or lose Hb (Fox, 1955). The presence of Hb in bottom-living Cladocera may be related to an oxygen deficit in such conditions or even to anoxia. However, some species living on the mud surface are not colored red, as was observed e.g., in colorless *Drepanothrix dentata* (in Lake Glubokoe, Moscow region).

5.4.2 Hemoglobin in Pelagic Cladocera

Hb has been found in planktonic *Daphnia* and *Moina*, and also in *Simocephalus* (Fox, 1948; Hoshi, 1957; Hoshi and Nagumo, 1964). The maximum Hb absorption in an absorption spectrum is different in representatives of different species, and even in related species; for example, it is at 314 nm in *D. magna* and 418 nm in *Ceriodaphnia quadrangula* (Czeczuga, 1965).

Generally, Hb is formed in cladocerans when there is an oxygen deficit. The Hb concentration in *Daphnia* increases when there is a low oxygen concentration in the environment (Fox, 1955; Green, 1956a,b,c; Landon and Stasiak, 1983). The latter authors also demonstrated that the Hb concentration in *Daphnia* is directly proportional to depth in Arco Lake (Minnesota, United States). Studies on variations of Hb content in response to environmental factors, including oxygen concentration, have been reviewed by Kobayashi and Hoshi (1984). The Hb concentration in *D. magna*, as determined by Kobayashi (1981a), ranges from 0.1 to 1.7 g Hb/100 mL blood. According to later measurements, the Hb concentration of *D. magna* ranges from 0.24 to 241 mg Hb/g DW (Kobayashi and Nezu, 1986). It increased at low oxygen concentrations: rapidly in immature specimens and more slowly in larger animals, although animals longer than 3 mm did not become red.

The lethal oxygen concentration for pale *D. magna* is c. 0.22 mL O_2/L at 15°C and c. 0.5 mL O_2/L at 30°C; for red specimens, it is c. 0.1 mL O_2/L at 15°C and c. 0.12 mL O_2/L at 30°C. Under the same conditions, *D. magna* males accumulate more Hb than do females at low oxygen concentrations (Kobayashi, 1970). Hb is not just a passive chemical, however. In Hb-rich *D. magna* its contribution to the circulatory transport of oxygen to tissues is much greater than in Hb-poor specimens (Bäumer et al., 2002). Extra Hb ensures an adequate oxygen supply to limb muscle tissue in *D. magna* at moderate oxygen concentrations in the ambient water (Pirow et al., 2001).

The Hb content of *Daphnia* decreases before a molt, but increases in the ovaries at the same

time (Fox et al., 1949). During an instar (the intermolt period), Hb passes from the blood into parthenogenetic eggs, as has been shown for *Daphnia* (Green, 1956a,b,c), and this occurs a short time before molting. In aerated water, the Hb content in *Daphnia* decreases. The Hb content of pink *Moina macrocopa* was shown to be 1.7 g Hb/100 mL blood and 1/15 of this value in pale specimens (Kobayashi, 1981b).

Daphnia manage well without Hb; however, at low oxygen concentrations pale *Daphnia* make up for the absence of Hb by increasing the rate of movement of their thoracic limbs (Hoshi and Inada, 1973). Curiously, although Hb appears in animals at reduced oxygen concentrations, inactivation of Hb by carbon monoxide (CO) did not make daphnias much less vigorous or decrease their survival in short-term experiments. According to Fox (1948), *Daphnia* containing deoxygenated Hb survive for a long time: it was, therefore, thought that Hb takes no part in their respiration. It seems to have only a supplementary role. Long-term cultivation, however, revealed that red daphnias do live longer, swim more actively, consume more food, and produce more eggs (Fox, 1948; Fox et al., 1951).

According to Kobayashi (1981b), in poorly aerated water the respiration rates of pale *M. macrocopa* and of CO-treated red specimens are identical. The lethal oxygen concentration becomes higher in *Daphnia* at increasing CO concentrations and is more obvious in pink *Daphnia* (Kobayashi and Hoshi, 1980). Kobayashi and Hoshi (1982) believe that Hb functions only at low O_2 concentrations. In their experiments, all specimens of pale *D. magna* died at c. 0.5 mL O_2/L, whereas specimens containing 1−1.5 g Hb/100 mL blood died at c. 0.1 mL O_2/L. The critical values for *C. quadrangula* are: 0.9 mL O_2/L for pale specimens and 0.3 mL O_2/L for pink specimens (Kobayashi, 1983a). Above this level, the rate of oxygen consumption by *C. quadrangula* is constant: c. 12 μL O_2/ind./h for pale specimens and 11 μL O_2/ind./h for pink specimens.

Survival may also depend on the former environment of the tested specimens. For example, *S. vetulus* taken from a pond survive much longer in deoxygenated water than do specimens taken from a river (Shkorbatov, 1953).

Rudiger and Zeis (2011) found an inverse relationship between temperature and Hb concentration in *D. longispina* from oxygen-rich water, with the seasonal minima of Hb concentration coinciding with low food availability and the peak of reproduction. These authors suggested that in *Daphnia* Hb functions as both a respiratory protein and a protein store.

Generally, the role of Hb in Cladocera is facultative and these organisms may successfully live and reproduce without it (e.g., when it is inactivated). The Hb values indicated by different authors therefore depend on various reasons (e.g., environmental factors and species).

5.4.3 Iron

Hb formation in *Daphnia* is favored by the presence of iron in the environment (Fox and Phear, 1953; Goodwin, 1960). The iron component of Hb is present in water and more abundantly on the bottom of water bodies in both ferrous and ferric forms. Obviously, it can, therefore, be ingested by cladocerans directly or in algae. The iron content of Hb has been measured as 0.033%, in *D. magna* and 0.317−0.353% in *M. macrocopa* (Hoshi et al., 1967; Hoshi and Kobayashi, 1971). Dave (1984) found that the optimum concentration for Hb formation is 2 μg Fe/L ($FeCl_3.6H_2O$). Hb formation is also favored by higher temperatures and is not influenced by carbon dioxide levels (Fox and Phear, 1953).

In the body of *D. magna*, iron distribution was described by Smaridge (1956), who found three forms of iron in *Daphnia* tissues: "loosely bound" iron, "firmly bound" iron, and iron within heme compounds (Hb and cytochromes). Loosely bound iron can be stained by, for example,

Prussian blue, and firmly bound iron can be revealed by treatment with acid alcohol. The iron in Hb and cytochromes may be revealed by treatment with, for example, hydrogen peroxide or by microincineration. Radioactive iron−59 (^{59}Fe) is also incorporated by *Daphnia* (Smaridge, 1956).

Loosely bound iron is found in the gut wall, gut ceca walls, and fat cells (both as ferrous and ferric iron), as well as in ovaries, blood plasma, and appendage walls (ferrous iron). It is assumed that iron is incorporated into newly synthesized Hb in the fat cells and ovaries. Some iron may be accumulated in fat cells and then excreted. Iron is also incorporated into heme and is present in all tissues, even in the lenses of the eyes. Within the bodies of *D. magna* specimens with increasing Hb, iron is absorbed by the gut wall and is present in newly synthesized Hb in fat cells and the ovaries (Smaridge, 1956). In specimens that are losing Hb, it is especially present in walls of the gut ceca and fat cells, and in the excretory shell glands.

Tazima et al. (1975) also reported that in *Daphnia* ferruginous compounds accumulate in the gut ceca, and that during Hb breakdown they can be observed in fat cells and the shell glands.

Iron used for Hb synthesis is absorbed from the midgut and used by fat cells for Hb synthesis; Hb destruction is aided by gut and fat cells, and Hb is excreted via the maxillary glands (as loosely bound ferric iron).

5.4.4 Sites of Hemoglobin Synthesis

The presence of Hb in fat cells was noted by Green (1955). Goldmann et al. (1999) applied techniques of in situ hybridization with subsequent signal amplification and found that Hb is synthesized in the fat cells and epithelial cells of epipodites. Using radioactive iron, Hoshi and Sato (1974) found that there is a continuous process of Hb synthesis and breakdown in the *Daphnia* body.

Hb breakdown is now known to occur in fat cells (Smaridge, 1954, 1956; Green, 1955), whereas it was previously thought that Hb is normally excreted through the shell glands and the intestine (in the intestinal fluid chemochromogen in solution was noted) (Fox, 1948). Hb can also be decomposed in fat cells without yielding bile pigments (Green, 1971).

5.5 EVOLUTION OF CARBON DIOXIDE AND THE RESPIRATORY QUOTIENT (RQ)

Carbon dioxide (CO_2) production by *D. magna* is estimated to be 0.03 mg/g air-dried weight by females at 22°C and 22% higher in males (MacArthur and Baillie, 1929). In *D. pulex*, as determined by Richman (1958), its values are close to those of O_2 consumption. Carbon dioxide production decreases with age, as was shown with reference to *S. exspinosus* (Obreshkove, 1930).

Later, CO_2 production by *D. magna* was measured by Kolupaev (1989) to be 0.38 mg/g WW/h in January−April and 0.51 mg/g WW/h in May−August; oxygen consumption was 0.43 mg/g WW/h and 0.62 mg/g WW/h, respectively; and the frequency of thoracic limb beating 299/min and 346/min, respectively.

RQ is the ratio of the carbon dioxide released to the oxygen consumed. The RQ is 1.00 for carbohydrate metabolism, 0.711 for fat metabolism, and 0.781 for protein metabolism (Koshtoyants, 1951). RQ values <1 may indicate deposition of fat into eggs or anoxybiosis (CO_2 formation during fermentation in an oxygen-free medium). The RQ in well-fed *D. pulex* is 0.92−1.11 for those of 1.6 mm in length and 0.95−1.24 in larger specimens (Richman, 1958). The RQ in starved *D. pulex* decreases to 0.7, thus indicating a shift from carbohydrate metabolism to the utilization of fat and protein (Richman, 1958). According to data published by Kolupaev (1989), the RQ in *D. magna* is <1.

5.6 ENERGY BUDGET

Richman (1958) determined the energy budget of *D. pulex* (Table 5.1) to comprise expenditures for the growth and production of young, with the latter using the major part of the stored energy. An energy-channeling scheme for *Daphnia* was proposed by McCauley et al. (1990).

Basal metabolism (i.e., metabolism in *Daphnia* immobilized by D-tubocurarine) was determined by means of a Cartesian diver (O'Connor, 1950). It was found that the expenditure for muscular activities is 32% or more of the total metabolism. In *D. magna* immobilized with uretane, basal metabolism takes 1/4–1/6 of the metabolism of an active *Daphnia* (Postnov and Philippova, 1988). The expenditure for respiration was determined by Galkovskaya (1970) to be 21–44% in *D. longispina* and 32% in *Bosmina*. Male cladocerans have a higher rate of metabolism than females (Banta and Brown, 1924).

5.7 HYPOXIA

Hypoxia (low oxygen concentrations) is commonly experienced by Cladocera. Oxygen deficiency may occur at the bottom of a water body or in the open water in the early morning following the prevalence of algal respiration over photosynthesis, or when there is a complete covering of floating *Lemna*. Investigations of Cladocera respiration under conditions of low oxygen content, specifically for planktonic Cladocera, demonstrated that during an oxygen shortage *Daphnia* swim toward regions of higher oxygen concentration (Pardi and Papi, 1961).

At the concentration, 0.8 mg O_2/L and lower, the oxygen consumption by *D. magna* much decreases, movements become slow, and at aeration the normal activity is restored slowly (Skadovskiy, 1937). An oxygen concentration c. 0.2 mg/L O_2 suppresses the formation of large filtering screens in *D. magna* at a low food concentration, and thus reduces the filtering rate (Hanazato, 1996). Growth was also retarded.

The oxygen consumption in *D. magna* (in contrast to *D. galeata*) rapidly declines at O_2 concentrations below c. 3 mg/L (Heisey and Porter, 1977). It has been also shown with reference to *D. pulex* that at the concentration of oxygen 3 mg/L the filtering rate decreased sharply and at a longer exposure it increased and even surpassed that at the initial O_2 concentration, obviously due to compensatory processes (including appearance of Hb) (King and O'Brien, 1976).

Rivier (1986) reported the presence of *Daphnia* with numerous embryos during winter in the bottom of ice-covered Lake Siverskoe, at a low oxygen content (c. 2 mg/L O_2). She also found *D. longiremis*, *D. galeata*, *D. cristata*, and

TABLE 5.1 Energy Budget of *Daphnia pulex* at a Moderate Food Concentration

Age	Energy Consumed (Cal)	Energy for Growth (Cal)	Energy for Growth and Young (Cal)	Energy of Respiration (Cal)	Energy of Egestion (Cal)
Preadult	0.469	0.062	0.050	0.050	0.357
Adults after 34 days of growth (young not included)	5.671	0.071	–	0.791	3.872
Adults after 40 days of growth	6.140	–	1.070	0.841	4.229

The low relative value of energy for growth is to be noted in slow-growing adults.
From Richman, S., 1958. The transformation of energy by Daphnia pulex. *Ecological Monographs 28 (3), 273–291.*

Bosmina longirostris reproducing under the ice (Rivier, 2012).

In *D. magna* at 1.8 mg/L O_2, the following parameters decrease in comparison with controls at higher oxygen concentrations (Homer and Waller, 1983): dry weight, number of days to first brood, number of young in the first brood, total number of young produced over the 26 days of the experiment. The median lethal dose (or LD_{50}; after 4 h) is 0.2–0.7 mL O_2/L for *Daphnia* and *Simocephalus*, and 1–1.6 mL O_2/L for *Leptodora* and *Bythotrephes* (Herbert, 1954).

Pink *D. magna* have been shown to survive for at least 24 h at 5% air saturation whereas pale ones perish within 1 h at 7% air saturation (Usuki and Yamaguchi, 1979). At low oxygen concentrations, the lactic acid content in *Daphnia* increases (Fig. 5.2). In *D. magna* with embryos under hypoxic conditions, there is a higher rate of blood flow through the brood chamber (Seidl et al., 2002).

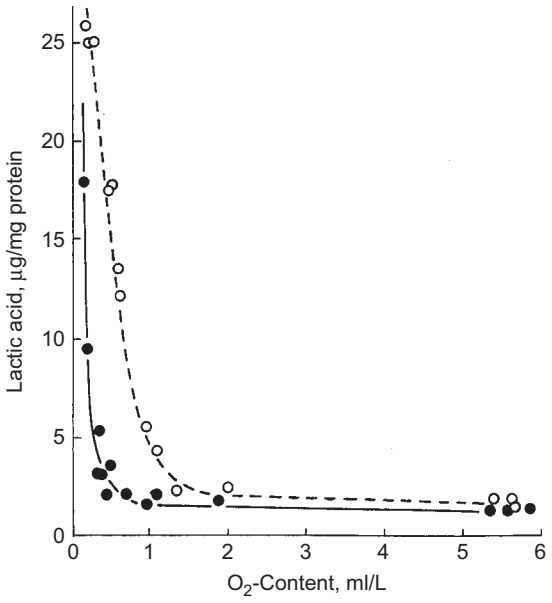

FIGURE 5.2 Lactic acid content in pale (*open circles*) and pink (*solid circles*) *Daphnia* at different oxygen concentrations during 1 h. *From Usuki, I., Yamaguchi, K., 1979. Physiological significance of the extracellular haemoglobin on the survival and lactic acid production of* Daphnia magna *Straus in low oxygen conditions. Science Reports of the Niigata University, Series D (Biology) 16, 5–12.*

Oxygen-dependent Hb induction takes place under hypoxic conditions, thus favoring enhanced oxygen transport, e.g., in *D. magna* (Gorr et al., 2004; Zeis et al., 2004a,b). In hypoxic *Daphnia*, the Hb content is increased in the blood, muscles, and nerve ganglia, and the content of cytochromes in the tissues also increases (Fox, 1945). At oxygen with a partial pressure of 3 kPa, newly synthesized Hb can be detected within 18 h and a steady state concentration is reached within 11 days (Zeis et al., 2003a,b); in the same species, hypoxia-inducible factors (HIFs) are accumulated and facilitate O_2 delivery to hypoxic tissues (Gorr, 2004).

Juvenoid hormones contribute to the elevation of Hb levels in *D. magna* (Gorr et al., 2006a,b): at high O_2 tension (pO_2), these hormones induce the robust reactivation of juvenoid response elements, but at low pO_2, this reaction is inhibited.

In addition, in *Moina micrura* not acclimated to hypoxia, the metabolism and the stroke frequency of their antennae is decreased (Hubareva, 2000a). At 0.7–0.8 mg O_2/L, pale *M. micrura* may stop filtration but continue swimming, in contrast to red specimens (Svetlichny and Hubareva, 2002b). In *D. magna*, there is no compensatory increase in the beating rate of thoracic limbs during hypoxia: it is maintained at c. 180/min (Colmorgen and Paul, 1995). It was later observed that the beating rate of their appendages does increase under hypoxic conditions and that compensatory tachycardia occurs, followed by an increase in the Hb concentration (Pirow and Buchen, 2004).

Under conditions of acute hypoxia in *M. micrura*, the ratio of consumed O to excreted N is 8.5 (this ratio increases to 34–40 under oxygen saturation); under brief periods of acute hypoxia, largely anaerobic protein catabolism occurs (Hubareva and Svetlichnyi, 1998). During longer periods of hypoxia, *M. micrura* become acclimated and use whatever oxygen is available

for lipid oxidation (Hubareva and Svetlichny, 1998; Hubareva, 2000b). Under O_2 concentration to c. 0.5 mg/L the typical protein−lipid catabolism of *M. micrura* changes completely to the anaerobic protein catabolism (Hubareva, 2000a,b; Svetlichny and Hubareva, 2002a,b, 2004). Further, the excretion of NH_3-N drastically decreases from 0.079 to 0.019 μg N/L/h, in proportion to the O_2 concentration.

Seidl et al. (2005) exposed both the parental generation and progeny of *D. magna* to 10−19% air saturation. Acclimation to hypoxia involved adjustments to the Hb level and the metabolic level: Hb concentration increased by 266% and oxygen affinity increased by 32%. Smaller offspring, combined with reduced Hb and metabolic levels, reduced the critical ambient oxygen tension by about 50%. Transgenerational effects were not observed.

Zeis et al. (2009) studied the composition of the *D. pulex* proteome under normoxic (pO_2 20 kPa) and hypoxic (pO_2 3 kPa) conditions. Hypoxia caused a strong inducement of Hb and carbohydrate-degrading enzymes, i.e., glycolytic enzymes (enolase).

In hypoxic water, Hb-rich *D. carinata* preferred a temperature of 19°C (vs. 16°C for controls), whereas in normoxic water all the animals gathered at regions of 23°C (Wiggins and Frappell, 2000). In *Daphnia*, hypoxic stress and heat (30°C) caused oscillation in reactive oxygen species, glutathione redox system activity, and Hypoxia-inducible Factor-1 (HIF-1) activity (Becker et al., 2011a,b).

It was found that there is some transgenerational effect of hypoxia. The neonates from hypoxia-exposed mothers of *D. magna* had a higher metabolic rate, this effect disappearing with their development (Andrewartha and Burggren, 2012).

5.8 ANOXIA

There are good reasons for discussing anaerobiosis in Cladocera. The inhabitants of mud in which the oxygen content is close to zero have long been known (Kurz, 1878): *Ilyocryptus* ssp., *Leydigia* ssp., *Alona quadrangularis*, *Pleuroxus uncinatus* (syn. *P. personatus*). Some species live in the oxygen-deficient water layer over mud: *D. pulex*, *Lathonura rectirostris*, and *S. exspinosus* (Brand, 1946). Many littoral species live on the bottom of water bodies, where there is, at least periodically, a deficiency of oxygen. For the lake bottom Martynova (2010) reported low presence of O_2 at 0.75−9 mg/L (the bottom water layer), N_2 at 42−175 mg/kg WW, CH_4 at 1−21 mg/kg, and CO_2 at 1.5−156 mg/kg.

In the absence of oxygen, *Daphnia*, *Simocephalus*, *Scapholeberis*, *Bosmina*, and *Alona* spp. have been shown to remain immobilized (Nikitinsky and Mudrezowa-Wyss, 1930). The lethal minimum oxygen concentration for chydorids is 0.4−1.7 mg/L O_2 (Pacaud, 1939; Bogatova, 1962). Under severely hypoxic conditions, movement of *C. sphaericus* and *Alona* in water saturated with hydrogen was discontinued (Mudrezowa-Wyss, 1933); and in nitrogen-saturated water, movement ceased in *C. quadrangula* in c. 35 min in red specimens and in 23 min in pale specimens (Kobayashi and Ichikawa, 1987).

It has been observed that *Chydorus sphaercus* does not feed in anoxic water layers (Lair, 1991). During anoxia the frequency and amplitude of the movements of thoracic limbs decreased in *D. magna*; the heart continued to beat at a rate similar to normoxia but the stroke volume decreased (Colmorgen and Paul, 1995; Paul et al., 1997). According to these authors, during hypoxia the frequency and amplitude of their thoracic limb movements remained at the usual rate of c. 180 min, but the heart rate increased (i.e., compensatory tachycardia).

Under anoxic conditions the metabolism of Cladocera changes radically. Anaerobiosis is generally characterized by the exploitation of carbohydrates in metabolism, suppression of protein metabolism (Brand, 1946), and high RQ

values due to the final stage of CO_2 production (Koshtoyants, 1951).

Lactic acid concentration in the body of *Daphnia* is generally very low, but at low oxygen concentrations *D. magna* accumulate more lactic acid prior to death (Usuki and Yamaguchi, 1979). In Hb-colored *Daphnia*, the lactic acid content is higher at lower oxygen concentrations (Fig. 5.2). The responses of *D. magna* to anoxia were studied further by Paul et al. (1998). During the first 2 h of anoxia, their anaerobic metabolism is characterized by L-lactate accumulation in the body (0.36 μmol lactate/g WW/min) and a decrease in pH in the body (metabolic acidosis). During the first 2 h of anoxia, the heart rate does not change much, but at longer exposures the heart rate decreases. Under subsequent conditions of normoxia, the heart rate and the body pH return to normal.

Exposure of *D. magna* to selective serotonin-reuptake inhibitors (fluoxetine, fluvoxamine, cyproheptadine) increased oxygen consumption rates and decreased carbohydrate levels in adults thus decreasing the capacity to survive under anoxic conditions (Campos et al., 2012).

In embryos of *S. vetulus* under anaerobic conditions, the glycogen content is 0.5% in the gastrula, 0.67% in the nauplius, 0.53% in the released young, and 0.64% in the third instar (Hoshi, 1953). Unlike in aerobic conditions, it does not increase during postembryonic development.

Loss of *D. pulex* under anoxic conditions was reduced by preconditioning by metformin (an antidiabetic drug) (Sheng et al., 2012). For preconditioning, the daphnias were kept for 3 h in solutions with the drug added at different concentrations and then in a regular medium for 2 h. Metformin somewhat decreased the glucose level in daphnias, increased the expression of HIFs, and caused various changes in regulation of metabolism.

Hydrogen sulfide (H_2S) is another factor that limits the availability of bottom habitats for Cladocera; for example, a low H_2S concentration (0.15 mM) causes irreversible immobilization of *C. ovalis* (Bryukhatova, 1928). In the presence of hydrogen sulfide, *Daphnia*, *Simocephalus*, and *Bosmina* became immobilized within c. 10–20 s (Nikitinsky and Mudrezowa-Wyss, 1930).

Metabolism of truly benthic Cladocera living under anoxic conditions is still inadequately known.

5.9 IMPACT OF XENOBIOTICS ON RESPIRATION

Information on this subject is very limited. Oxygen consumption in *Simocephalus* is inhibited by >5 mM 2,4-dichlorophenol-Na (Klekowski and Zvirgzds, 1971) and 2.5 mM 2,4-dichlorophenoxyacetic acid (2,4-D) (Andrushaitis, 1972). At 5–8 mg/L naphthalene, the Hb content and oxygen consumption of *D. magna* decrease by about 25% compared with controls (Crider et al., 1982). Phenol at a concentration of 0.1 mg/L reduces the beating rate of the thoracic limbs in *D. magna* from 305 beats/min (control) to 204 beats/min (Kolupaev, 1988). Oxygen consumption decreases in *D. magna* in the presence of copper and zinc during the first hour, then (as determined after 3 h) compensatory mechanisms are activated (Sladkova and Kholodkevich, 2012). Therefore, these authors suggest that measurements of this effect should be made after the first hour of exposure.

Respiration intensity of *D. magna* increased in the presence of tributyltin chloride (2×10^{-6} mg/L) (Kolosova and Stroganov, 1973). Copper stimulated oxygen consumption in *D. magna* at 0.01–0.03 mg/L and inhibited respiration at higher concentrations (Khangarot and Rathore, 2003). The respiratory demand increased in *D. magna* after a 23-day exposure to 0.99 mGy/h americium (Am-241), thus indicating an increase in metabolic expenditure (Alonzo et al., 2006).

CHAPTER

6

Circulation

6.1 ANATOMICAL BACKGROUND

Cladocera possess an open circulatory system and a myogenic heart. Heartbeats and the flow of blood (hemolymph) with blood cells (hemocytes) can be easily observed, as Cladocera are semitransparent. The heart has lateral ostia (Figs. 6.1 and 6.2) for the inflow of hemolymph and a short anterior vessel from which hemolymph is squirted at each heartbeat. In *Leptodora*, an additional propulsive organ has been described (Gerschler, 1910; Saalfeld, 1936; Maynard, 1960), which can easily be found and observed in living specimens in the first thoracic limb, at the distal end of the proximal segment; it takes the form of a disk supplied with a propulsive muscle.

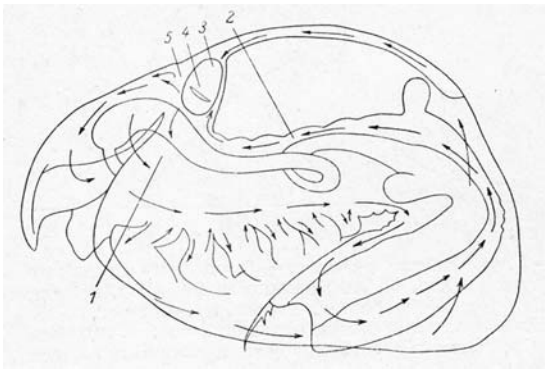

FIGURE 6.1 Blood flows in *Eurycercus*. 1, arterial flow; 2, venous flow; 3, heart; 4, ostium laterale; 5, eddy. *From Smirnov, N.N., 1971. Chydoridae. Fauna SSSR, New Series, No. 101, Nauka, Leningrad, 531 p. (in Russian).*

Membranes that divide the venous and arterial blood flows (venous and arterial sinuses) in the area of the heart (Fig. 6.2) were first reported in *Eurycercus* by Herrick (1884). It was later found that the whole body space is subdivided by ventral and dorsal membranes into three blood spaces: the ventral lacuna, the dorsal lacuna, and the intestinal lacuna. In addition, two vertical membranes divide the ventral lacuna and the limbs into the medial lateral compartments (Hérouard, 1905; Pirow et al., 1999b). It may be added that the inner membranes of the cladoceran body need better description, especially for representatives of various genera. The carapace covering the body is double walled, with the blood flowing into the internal carapace lacuna.

Cladoceran blood is light yellow, but may also be red due to the presence of hemoglobin. Artemocyanin (biliprotein) has been found in the blood of *Daphnia pulex* (Peeters et al., 1994).

6.1.1 Blood Cells

Blood cells were originally noted in the blood flow by early authors (e.g., Gruithuisen, 1828; Leydig, 1860, p. 56) and can be easily observed. Gruithuisen (1828) and many subsequent authors (e.g., Hardy, 1892; Fritsche, 1917) believed that Cladocera possess a single type of blood cell. Cuenot (1891) saw numerous amoebocytes of size 6–9 μm in the blood of *Daphnia*

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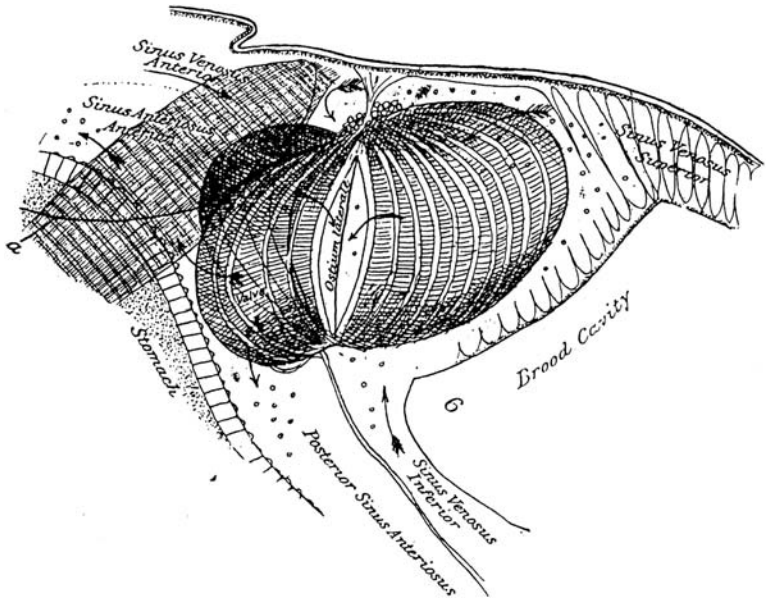

FIGURE 6.2 *Eurycercus* heart and membranes dividing arterial and venous blood flows. *From Herrick, C.L., 1884. A Final Report on the Crustacea of Minnesota Included in the Orders Cladocera and Copepoda. The Geological and Natural History Survey of Minnesota, Minneapolis, 192 p.*

schefferi, *Simocephalus vetulus*, *Chydorus sphaericus*, and *Sida crystallina*. According to Cuenot, amoebocytes produce pseudopods. He noted local aggregations of amoebocytes but did not locate any lymphatic glands. In *Eurycercus* (Smirnov, 1970, 1971) the average length of these cells is 15 μm, but in *Daphnia* they are only 7–8 μm in diameter. The blood cells of *Eurycercus* are of variable structure and have occasional pseudopods. There are many thousands of hemocytes in *Eurycercus*, and several hundred in the smaller *Acroperus* (Smirnov, 1975a,b). The process of hemopoiesis is poorly known.

Metchnikoff (1892) observed a single type of blood cell in *Daphnia*; he termed and understood them to be leukocytes. He also observed *Daphnia magna* hemocytes in the process of phagocytosis (Fig. 6.3) (Metchnikoff, 1884).

Closer examination of *D. magna* revealed two cell types circulating in the hemolymph (Auld et al., 2010): spherical cells (granulocytes) and irregular-shaped amoeboid cells (about 9 μm long), and identified them as the cells initially described by Metchnikoff (1884) (Fig. 17.3). The latter cells attack the bacterial parasite *Pasteuria ramosa*; in parasitized *Daphnia*, there is a large increase in the number of amoeboid cells.

6.2 BLOOD FLOW

The movement of hemolymph is powered by the heart and by body movements. Gruithuisen (1828) was probably the first to present a general scheme of circulation within *Simocephalus*. Following the movement of blood cells, he indicated arterial flows from the heart to the cephalic region, to antennae, over valves, and along the trunk, as well as reverse venous flow. According to this author, the blood flows along capillary canals. Then Lievin (1848) observed blood flows in *Sida* over the body and valves discerning the arterial and venous flows.

Blood flow can be clearly seen by the movement of blood cells (Fig. 6.1), and the patterns

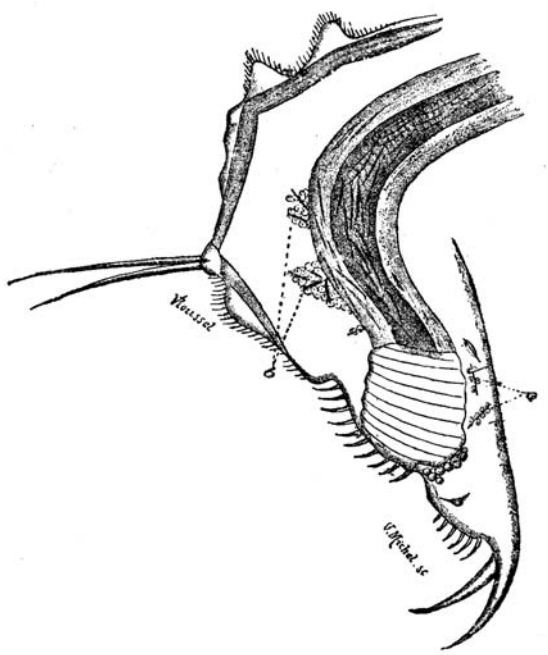

FIGURE 6.3 Phagocytosis in *Daphnia*. a,b, spores of *Monospora* surrounded by leukocytes. *From Metchnikoff, E., Mechnikov, I.I., 1884. Über eine Sprosspilzkrankheit der Daphnien. Beitrag zur Lehre über den Kampf der Phagocyten gegen Krankheitserreger. Archiv für pathologische Anatomie und Physiologie und für klinische Medicin 96 (2), 177–193.*

of flow are generally permanent, being separated and directed by membranes within the body. The time for the complete transit of a blood cell through the circulation of *Daphnia* is about 10–20 s (Dearborn, 1903; Maynard, 1960).

It has been determined, with reference to *D. magna*, that the hemolymph makes up 58% of its wet weight and 61% of its volume (Kobayashi, 1983b). The systolic heart volume was determined to be 2.53 nL in *D. pulex* and 1.28 nL in *Holopedium* (Maynard, 1960). In general, blood flow is directed forward from the heart (arterial flow) and along the dorsal side of the body. In the area of the head, it turns and then passes through the limbs, follows along the ventral side to the postabdomen, and then returns along the dorsal side of the body and of the shell, as observed in *Daphnia* by Hérouard (1905). In different groups of Cladocera, the system of blood flow varies, but it has not yet been accurately described.

In *D. pulex* and *Holopedium*, the systole is about 1.5 times longer than the diastole (Fig. 6.5) (Storch, 1931). At each contraction, about half of the blood is expelled from the heart. The systolic volume of the heart in *D. pulex* is 2.53 nL (Maynard, 1960). At its contraction in systole, the cranial region of the heart is first contracted, then the ostial region, and then the caudal region, resulting in complete contraction of the heart (Proksova, 1950). In diastole, the cranial region is dilated first, and so on, further resulting in a complete widening of the heart.

The blood flow in *D. magna* has been investigated in detail by Pirow and Buchen (2004) (Fig. 5.1). Pirow et al. (2004) also reported details of oxygen partial pressure throughout the body of hemoglobin (Hb)-poor and Hb-rich *D. magna*, and represented the data obtained as a three-dimensional diagram (Fig. 6.4).

6.3 HEART RATE

The heart rate can be determined by direct counting. Probably the first report of c. 200 bpm for *Daphnia* was made by Cuvier (1817). At rates over 400 bpm the visual counting becomes too difficult. Optoelectronic method of recording the heart rate in *Daphnia* was proposed by Kolupaev et al. (1977). A similar device was proposed by Présig and Véró (1983), based on an optoelectronic circuit that transforms changes in light intensity caused by heartbeats into changes in potential. Kimographic recording of the *Moinodaphnia macleayi* heart rate was made by Tonapi et al. (1984).

Numerous studies on heart rate in Cladocera have been mainly confined to *Daphnia.* In *D. magna* without embryos, the heart rate is 350 bpm; with embryos, it is 379 bpm (Kolupaev,

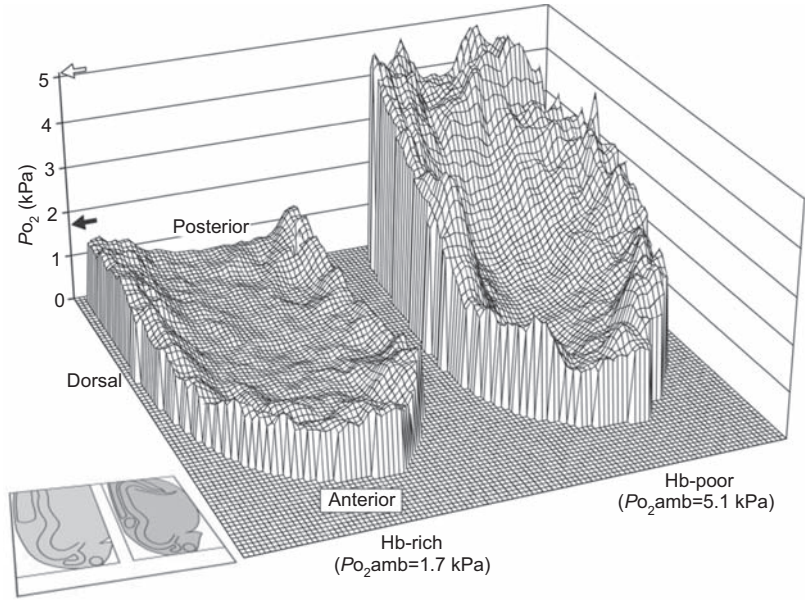

FIGURE 6.4 Oxygen partial pressure in the hemolymph over the body of Hb-rich and Hb-poor *Daphnia magna*. *From Pirow, R., Bäumer, C., Paul, R., 2004. Crater landscape: two-dimensional oxygen gradients in the circulatory system of the microcrustacean* Daphnia magna. *Journal of Experimental Biology 207, 4393–4405.*

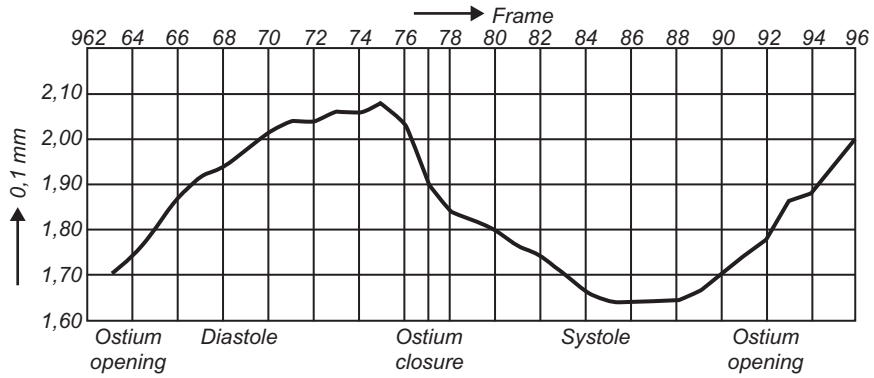

FIGURE 6.5 Diastole and systole in the *Daphnia* heart. Changes in heart size were measured. *From Storch, O., 1931. Ueber des Mechanik des Herzschlages bei Cladocere. Eine Analyse mit Hilfe der Mikrozeitlupe. Zeitschrift für vergleichende Physiologie 14 (4), 709–736.*

1989). For 22 species belonging to 16 genera from six families they have been reported by Smirnov (1965b). The heart rate in littoral and pelagic species, as determined by direct counting, ranges from 190 to 320 bpm at 17–18°C in females. The very slow-moving macrotricid, *Ilyocryptus agilis*, was a notable exception; it has a heart rate of only 120 bpm at 20°C. Another species with a low heart rate, *M. macleayi,* was found by Tonapi et al. (1984); it was 105 bpm in females without embryos, 120 in females with embryos, 90 in males, and 120 in males after molting (at 22°C).

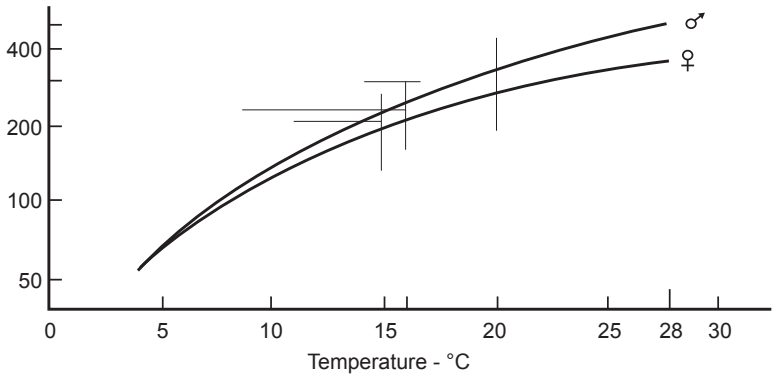

FIGURE 6.6 Heart rate in female and male *Daphnia* at different temperatures. *Modified from Maynard, D.M., 1960. Circulation and heart function. In: Waterman, T.H. (Ed.), The Physiology of Crustacea, vol. 1. Academic Press, New York, London, pp. 161–226.*

The Ponto–Caspian *Podonevadne*, *Evadne*, *Corniger*, and *Cercopagis* have a heart rate 132–170 bpm (as communicated by F.D. Mordukhai-Boltovskoi).

Remarkably, the heart rate increases in both females and in males by about 10–20% following every kind of disturbance, i.e., the increase is emotional (Smirnov, 1965b). When the disturbance is discontinued, the former heart rate is rapidly restored. Otherwise, the heart rate is stable and only significantly drops just before death.

There are numerous data characterizing the level of heart rate as being dependent on various factors. Jermakoff and Ermakov (1936) found that the heart rate of *D. magna* changes with starvation, molting, reproduction; potassium ion (K^+)-induced systolic arrest, and calcium ion (Ca^{2+})-induced diastolic arrest. The heart rate increases with increased body size, up to a certain limit, in *Daphnia longispina* (Ingle et al., 1937). It decreases in *D. pulex* exposed to CO_2 (Skadovskiy, 1939a).

Heart rate exhibits a diurnal cycle; in *Daphnia*, the maximal heart rate occurs in late afternoon (Tonolli, 1947), which may be linked to the fact that the heart rate generally increases at higher temperatures (Fig. 6.6) (Maynard, 1960). However, at temperatures above 37°C the heart rate of *D. magna* rapidly decreases (Bownik et al., 2014). The heart rate also decreases upon illumination with a beam of light directed at the heart (Schulz, 1928) and by ultrasound (up to full arrest) (Nikonov et al., 1970).

Salo (1960) presented data showing that the heart rate in *D. magna* and *D. pulex* may be equal to, higher than, or lower than the beating rate of the limbs. The sum of both parameters is far from constant, but a compensatory relationship was suggested between the rate of movement of the thoracic limbs and the heart rate. In view of this fact, their conclusion of a compensatory relationship between the movement rate of the thoracic limbs and the heart rate (as suggested by Salo, 1960) seems to be highly questionable and demands further investigation. However, the existence of compensatory tachycardia has been convincingly demonstrated in *D. magna* under conditions of hypoxia (Paul et al., 1997; Pirow and Buchen, 2004) and a lack of food (Pirow and Buchen, 2004). The heart rate is below 300 bpm at 20 kPa O_2 and increases to c. 400 bpm at 3–7 kPa O_2.

Skadovskiy (1955) thought that the heart rate in daphnias may serve as an index of metabolic intensity. It is, therefore, tempting to apply the heart rate as an index of metabolic intensity, assuming that they are quantitatively proportional (as was thought also, e.g., by Meijering, 1975). Indeed, the heart rate increases with

increasing temperature (Fig. 6.6). However, its relationship with the metabolic rate is certainly not direct (see also Maynard, 1960, p. 206) and, in view of the emotional reactions and the occasional retardation and arrest of the heart in active animals, it can hardly be used for estimating metabolic intensity. Our observations convince us that the heart rate is a parameter that is mainly changed by emotional factors and tends to maintain a constant average until death.

Normal work of the heart depends also on alimentation. The heart rate increases after feeding (Baylor, 1942; Maynard, 1960), depending on temperature and level of illumination, whereas starvation is followed by a decreased heart rate. If fed solely on starch, *D. longispina* consumes its fat resources during one to two ovarial cycles, the fat body is not seen any more, the work of different heart fibers becomes uncoordinated as well as the functioning of muscles of the antennae and thoracic limbs, and *Daphnia* die in about 6 days (Flückiger and Flück, 1952). Addition of vitamin B_1 (aneurin) to the culture medium of *Daphnia* fed solely on starch restored a normal heart rate. The heart rate decreases in starving *D. longispina* (Ingle et al., 1937).

Despite its variability, the heart rate of *Daphnia* has also been suggested as a measure for the estimation of pollution by means of a recording device (Kiknadze et al., 1983).

6.4 HEART REGULATION

Fischel (1908) described the heart nerve in *Daphnia* with its ganglia. According to Jermakoff and Ermakov (1937), the heart of *D. magna* is innervated which react to adrenaline (thus increasing the heart rate); however, the central nervous system does not take part in heart regulation, as has been shown by the elimination of its various parts (Jermakoff and Ermakov, 1936). Later, Jermakoff and Ermakov (1937) demonstrated the presence of nerve fibers that accelerate and retard the heart rate. Obreshkove (1942) also reported that the movements in the heart and intestine of *Daphnia* are accelerated or inhibited through nervous stimuli.

The heart is myogenic and acetylcholine is the inhibitory transmitter substance (Postmes et al., 1989); further, an extract of *Daphnia* has been reported to contain acetylcholine-like substances (Artemov and Mitropolitanskaya, 1938). Bekker and Krijsman (1951) noted the existence "of a myogenic pacemaker in the heart of *Daphnia*, inhibited by extracardiac cholinergic nerves," in the absence of a neurogenic pacemaker. This view is supported by the experimental evidence described later. Results obtained by Villegas-Navarro et al. (2003) suggest the presence of Na^+-ATPase receptors to verapamil and of adrenergic receptors in *D. magna* heart.

The influence of various substances on the heart rate has been studied either in specially prepared specimens (1) or in intact cladocerans (2).

1. For standardizing the testing of chemical effects on the heart of *D. pulex*, Lévy (1927a) placed the animal in Ringer solution and made two incisions in the dorsal integuments, one in front of the heart and another behind the heart. In most cases, the heart rate stabilized after about half an hour. Excess potassium ions in the solution resulted in a rapid intensification of the heart rate, whereas excess calcium ions inhibited the heart (Lévy, 1927b).
2. Other observations have been made on intact specimens. Various substances either accelerated or inhibited the heart depending on the chemical nature and concentration.

The heart rate of *Daphnia* is accelerated in the presence of atropine sulfate (3 mg/mL), caffeine (Pickering, 1894), thyroidine (Hykes, 1926), adrenaline (Hykes, 1926; Jermakoff and Ermakov, 1936, 1937; Suomalainen, 1939; Baylor, 1942), prolan (Skadovskiy, 1939b), L-adrenaline bitartrate, D-adrenaline bitartrate, L-noradrenaline bitartrate, L-ephedrine hydrochloride, dihydroergotamine

methansulfonate, and oxyphenyl ethanomethylamine tartrate (Flückiger, 1952).

The heart rate of *Daphnia* is retarded in the presence of acetylcholine (Baylor, 1942; Bekker and Krijsman, 1951), atropine (Baylor, 1942; Beim et al., 1970), pilocarpine (Jermakoff and Ermakov, 1927, 1936, 1937; Bekker and Krijsman, 1951), and ergotamine [1: 10,000 weight per volume (w/v)] (Jermakoff and Ermakov, 1927, 1936, 1937). In addition, the heart rate is also retarded in the presence of adrenaline, aminasin, aprophen, dihydroergotoxin, ephedrine, isadrin, noradrenaline, pilocarpine, pituitrin, and strophanthin (Beim et al., 1970; "with some exceptions"), choline (Suomalainen, 1939), $NaHSO_3$, and KCl (Baylor, 1942), tetraethyl pyrophosphatase, digitalin, rotenone (Bekker and Krijsman, 1951), caffeic acid, propranolol (Campbell et al., 2004), pituitrin (Hykes, 1926), and an extract of the thymus gland of mammals (Hykes, 1926). Phenobarbital strongly decreases the heart rate of *D. pulex* to about 25% at a concentration as low as 2.9 mg/L (Postmes et al., 1974). Lactose at 50−200 mM also inhibits the heart rate and causes severe arrhythmia in *D. pulex* (Campbell et al., 2004). It is thought that lactose directly affects ion channels in the heart, an effect that is reversible within 3−4 h.

Some substances, for example, adrenaline and atropine, as seen previously, may accelerate or inhibit the heart rate. Atropine retards the heart rate at a concentration 1:50 (w/v) according to Jermakoff and Ermakov (1936, 1937), but accelerates it at 2.5:10,000−6.25:100,000 (w/v), according to Bekker and Krijsman (1951). Adrenaline was found (Bekker and Krijsman, 1951) to retard the heart rate of *Daphnia* at lower concentrations (1:500,000) but to accelerate the heart at higher concentrations (2:10,000). Obviously, such differences and contradictions depend on the concentration of the tested substances.

MacCallum (1905) observed that 0.1−1% pilocarpine solutions do not change the heart rate of *Sida*. Dopamine in a culture solution was found to produce only a slight effect on the *Daphnia* heart rate; from c. 66 beats per 10 s in the control, it increased to c. 70 at a dopamine concentration of c. 1 mM (Peñalva-Arana et al., 2007).

According to Pickering (1894), diastolic stoppage in *Daphnia* occurs after about half an hour; muscarine nitrate, veratrine, theobromine, and xanthine do not greatly influence the heart rhythm. Caffeine in large doses culminates in systolic stoppage in *Daphnia* according to Pickering (1894) but increases the force of beats slowing the heart rate culminating in diastolic stoppage at toxic doses according to Viehoever (1936). The action of pilocarpine ends in arrhythmia and diastolic arrest, whereas that of atropine ends in arrhythmia and systolic arrest (Jermakoff and Ermakov, 1927). Exposure to strychnine (1:20,000) results in retardation of the heart rate and the movements of thoracic limbs (Viehoever and Cohen, 1937). The heart activity of *Daphnia* depressed by chloroform recovered upon addition of the leaf powder of *Digitalis* to the culture medium, recovery was more speedy at addition of gitalin and bigitalin (*Digitalis* glucosides) (Viehoever, 1936).

Eserine (physostigmine) produces a toxic effect on the heart of *D. magna* (Baylor, 1942). The heart rate of *D. pulex* increases in the presence of serum from mammals, frog (*Rana*), and carp, and then stops in systole (Vatovec and Timet, 1952). Such stimulation is irrespective of the sex of the donor animal (Vatovec and Timet, 1955).

In *D. magna*, parasympathomimetics (e.g., mecholyl) do not slow the heart, whereas cardiac tonic drugs (e.g., digitalis) cause slowing of the heart and its dilatation (Sollman and Webb, 1941). According to these authors, the *Daphnia* reactions differ qualitatively in many ways from those of vertebrates.

Postmes et al. (1989) studied the effect on *D. magna* of adrenoceptor agonists (1-epinephrine-bitartrate, 1-norepinephrine-bitartrate, 1-phenylephrine HCl, phenylterol HCl) and adrenoceptor antagonists (dl-metoprolol tartrate and dl-propranolol-HCl). It was found

that epinephrine could not be blocked by propranolol (an adrenoceptor antagonist), thus suggesting that the drug action is not meditated by adrenoceptors. Postmes et al. (1989) found epinephrine to inhibit the heart rate, contrary to previous reports by Sollman and Webb (1941), Baylor (1942), Bekker and Krijsman (1951). Dzialowski et al. (2006) investigated the effect of beta-adrenergic receptor antagonists on *D. magna*. The lowest-observed effective concentration for decreasing the heart rate was 55 μg/L for propranolol and 3.1 mg/L for metoprolol.

Baylor (1942) noted regulation by acetylcholine and potassium (inhibition) in the *Daphnia* heart similar to that of vertebrates. The consistent experimental results on the efficiency of cholinolytics, obtained using *D. magna* and rats by Tonkopiy et al. (1994b), led these authors to the conclusion that *Daphnia* possess M-cholinoreceptors of a structure similar to those in mammals, a case of parallelism between remote groups. The cholinolytics tested (amedin, amizil, artropine, cyclodol, glycine, and spasmolytin) provide a protective action following intoxication of *D. magna* by arecoline and armine (Tonkopiy et al., 1994a).

The heart rate of *D. magna* is decreased by diphenhydramine (DPHM) and increased in the presence of curcumin (Vaidya et al., 2009). These authors suggest the following explanations: DPHM may prevent the sympathetic action of histamine, and parasympathetic acetylcholine (Ach) may bind acetylcholine receptor (Ach-R) onto myocardial cells and reduce the heart rate. Curcumin may antagonize histamine *N*-methyl transferase and thus prevent histamine methylation. Vaidya et al. (2009) think that histamine may act as a primary cardiac sympathetic neurotransmitter.

An estrogenic substance (ethynylestradiol) causes a significant decrease (to 1/3 of the normal rate) in the heart rate of *Daphnia* (Walker et al., 1998).

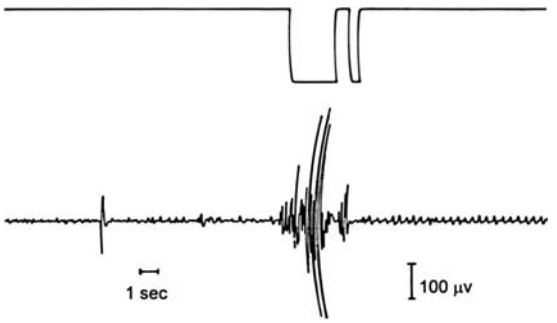

FIGURE 6.7 Electric activity of *Daphnia* recorded with remote electrodes (lower trace) is correlated with the observed body movements (upper trace). *From Tasse, J., Camougis, G., 1965. Electrographic studies on cardiac activity of the cladoceran,* Daphnia. *Crustaceana 8 (2), 197–205.*

6.4.1 Electrical Activity

Tasse and Camougis (1965) were the first to investigate the cardiac activity of *Daphnia* electrographically. They used three methods:

1. The application of remote electrodes revealed a correlation between muscle action potential and body movements (Fig. 6.7).
2. Metallic electrodes placed on the ventral abdominal region revealed slow, biphasic waves on which faster, smaller waves were superimposed. The magnitude of the biphasic waves varied from 200 to 500 μV, and their frequency from 1.5 to 4 Hz. There were periods of no electrical activity.
3. Glass-capillary electrodes punctured the carapace and were placed in the dorsal region of the heart. This revealed variations in the amount of electrical activity, ranging from a slow, biphasic wave to a fast monophasic and larger biphasic wave (Fig. 6.8). Fundamental frequencies ranged from 3 to 9 Hz, averaging at 5.4 Hz, with amplitudes from 100 to 150 μV.

In a $1/10^6$ dilution of γ-aminobutyric acid (or GABA), the frequency of electrograms decreased.

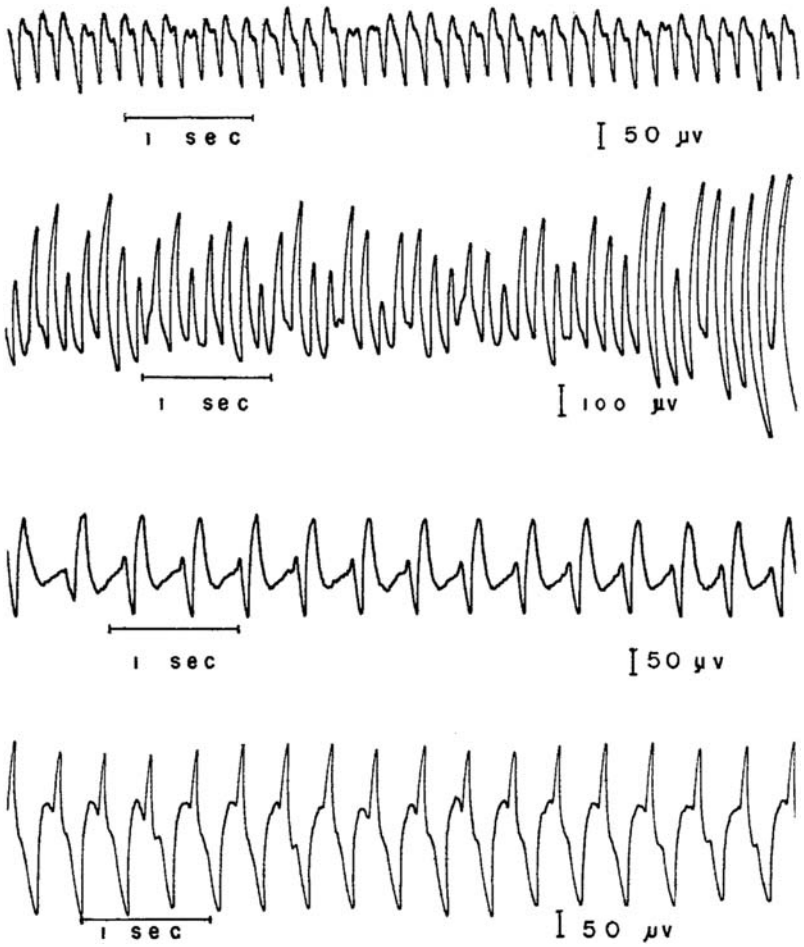

FIGURE 6.8 Electric activity of the heart of *Daphnia* recorded with capillary microelectrodes in different specimens. *From Tasse, J., Camougis, G., 1965. Electrographic studies on cardiac activity of the cladoceran,* Daphnia. *Crustaceana 8 (2), 197–205.*

6.5 HEART ARREST

The heart rate of Cladocera is stable and drops immediately prior to death. Despite this, the heart may stop for rather a long time (from minutes up to 1 h) in response to provocation without any detrimental consequences. For reasons that are unclear, regular activity of the heart is sometimes interrupted for long intervals in *Eurycercus*, *Camptocercus*, *Bosmina*, *Ilyocryptus*, and *Lathonura* (Smirnov, 1965b). In *Lathonura rectirostris*, after a prolonged interval the heart resumed its activity, without any obvious reason; the discontinuation of heartbeats had no negative effect on the animal whatsoever.

In copulating *C. sphaericus*, the heart rate either remains normal or decreases markedly in both males and females; in females, the heart sometimes completely stops.

It was observed that if the bend of the digestive tube of *Daphnia* is touched with a fine glass needle, then the heart stops immediately (in

systolic phase) and the posterior region of the intestine exhibits intensive peristalsis (Obreshkove, 1942). The period of heart arrest can reach 20 min. After a while, both the heart and intestine return to their normal activities. Heart arrest is also caused by touching the dorsal surface at the posterior heart area. It is thought that nerve endings convey the inhibitory impulses to the heart. After its arrest, the heart resumes beating, but, for several minutes, the beats are feeble and irregular.

Addition of 0.01—1 μg/L acetylcholine results in immediate normalization of the heartbeats. In *Daphnia* treated with 10 μg/L eserine (physostigmine) prior to heart arrest, the recovery from inhibition is rapid and complete (Obreshkove, 1942).

The general impression is that Cladocera manage well without their heart and do not need it much. Events related to the mechanism of heart arrest in Cladocera deserve further investigation.

6.6 ADHESION OF BLOOD CELLS

Blood cells sometimes stay on the surface of organs. This fact may be observed especially if a local irritation, injury, or induction shock, is inflicted—to such an extent that none may remain in circulation (Hardy, 1892). In *Leptodora* this happens when it sticks to the surface film and the cells become freely circulating again when the *Leptodora* is liberated and during ether narcotization (Saalfeld, 1936). Maynard (1960) indicated that in *Daphnia* blood cells adhere to tissues in the event of irritation. In *Acroperus*, all hemocytes sometimes become immovable (Smirnov, 1971). Hardy (1892) believed that blood cells that absorb fat may attach to the inner substrata and thus participate in the formation of the fat tissue.

There has been no explanation of such events in Cladocera and a mechanism for understanding this process has not yet been outlined.

6.7 PHAGOCYTOSIS

When consumed by *D. magna*, *Metschnikowiella bicuspidata* (syn. *Monospora bicuspidata*, Ascomycetes) spores are liberated from their coat and penetrate the body cavity through the wall of the intestine. Metchnikoff (1884) observed that *D. magna* leukocytes can surround and destroy these spores by phagocytosis (Fig. 6.3); this process allows some individuals of *Daphnia* to survive. However, leukocytes do not respond to *Saprolegnia* penetrating the body of *D. magna*, and to some other bacterial parasites. Metchnikoff initially described these facts in detail in 1884, and then reported them in 1885 and in 1892 in "Lectures on Comparative Pathology of Inflammation." He also observed (Metchnikoff, 1888) phagocytosis of spores of the bacterium *Pasteuria ramosa* infesting *Daphnia*. Metchnikoff (1885) had also earlier noted *Daphnia* leukocytes surrounding wounds. Phagocytosis was also observed by Metchnikoff in some other invertebrates and warm-blooded animals, and he developed these observations into a theory of general biological and medical importance.

Hardy (1892) observed that fat globules and other particles are carried from the gut lumen through the gut wall by blood cells, and that blood cells may attach to inner substrata, thus contributing to the formation of the fat tissue. According to Jaeger (1935) too, the blood cells loaded with fat may settle on tissues and turn into cells of the fat body.

Despite the convenience of transparent cladocerans, such observations on daphnids or their relatives were not continued for over a century. For a long time, there was no further direct information on phagocytosis in Cladocera. Moreover, Vonk (1960) and Quaglia et al. (1976) did not observe phagocytosis in the midgut of crustaceans and Quaglia et al. (1976) assumed that the food is liquefied within the gut and absorbed by the epithelium.

A closer examination of *D. magna* revealed the presence of two cell types circulating within the hemolymph (Auld et al., 2010), amoeboid cells (about 9 μm long, and identified as the cells initially described by Metchnikoff) can perform phagocytosis. They attack *Pasteuria*, and there is a large increase in the number of amoeboid cells in parasitized *Daphnia*.

6.8 IMPACT OF XENOBIOTICS ON HEART RATE

The growing pollution with pharmaceuticals (Flaherty and Dodson, 2005) would inevitably affect the heart rate of Cladocera. The available data indicate that xenobiotics mostly depress the heart rate of Cladocera spp. In *D. magna*, the addition of $CdCl_2$ is followed by a noticeable decrease in the heart rate and the beating rate of thoracic limbs (Smirnova, 1960); 500 mg/L of Dikonirt [a herbicide containing 2,4-dichlorophenoxyacetic acid (2,4-D)] caused reduction in the heart rate from 5 beats/sec to 1 beat/sec after 6 h of exposure (Présig and Véró, 1983).

As little as 0.1 mg/L phenol depresses the heart rate of *D. magna* from 368 bpm (control) to 254 bpm (Kolupaev, 1988). A 0.1 × lethal concentration, 50% (LC_{50}) of the pyrethroid pesticide, phosphatak, was shown by Saprykina (1996) to depress the heart rate in *Daphnia* (in the fourth, fifth, and sixth generations), stimulate the beating of thoracic limbs, and considerably stimulate oxygen consumption in the first, second, and fourth generations—by 39, 49, and 48%, respectively.

In contrast, hydroquinone stimulates heartbeats in *Daphnia* (Kiknadze et al., 1983). In solutions of acetates of Ni^{2+}, Cu^{2+}, Co^{2+}, Zn^{2+}, and Pb^{2+} (concentrations from 0.03 to 5.00 mg/L), the heart rate of *D. magna* increased up to 460 bpm against 140−160 in the control (Lobkova et al., 2009).

At a comparatively high concentration of $CuSO_4$, the thermoresistance of the cardiac muscle of *D. magna* is significantly decreased (Pashkova et al., 1998).

It may also be added that an alternating magnetic field with a frequency similar to that of *D. magna* heartbeats strongly intensified the heart rate (Usanov et al., 2001b, 2003). In an alternating low-intensity magnetic field, the heart rate of *D. magna* decreased; at low concentrations of phenol, inhibition of the *D. magna* heart rate by a magnetic field is higher than that by this chemical factor (Usanov et al., 2001b, 2003).

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CHAPTER

7

Excretion

7.1 ANATOMICAL BACKGROUND

The organ of excretion consists of the paired maxillary glands (shell glands) comprising the nephridium and its convoluted efferent ducts (Figs. 7.1 and 7.2). The maxillary gland is situated in the hemocoel, and is therefore exposed to the blood under the anterior area of the carapace, and opens to the outside in the anterior part of the body (Claus, 1875a). Judging by the detailed drawings of Claus, the opening to the outside is situated within the anterior part of the brood pouch. A detailed scheme of the structure of the nephridium is given by Gicklhorn (1931a) (Fig. 7.1).

Hérouard (1905) described the various stages of increasing complexity of the maxillary gland that occurs with age and stated that it is relatively simple in *Eurycercus*. He also noted that the carapace adductor muscles are attached at the inner surface of its convolutions. The lengths and arrangements of the convolutions of this gland are somewhat different in different representatives of particular families (Claus, 1875a, Plate XI; Hérouard, 1905). For example, the convolutions are numerous in *Daphnia* and *Sida*, whereas the gland is shorter in *Ceriodaphnia* and *Moina*. It is shortest in *Macrothrix* and *Acroperus*, but all of these have a convoluted efferent duct. The maxillary gland of *Penilia* (a marine species) is very different from those of other cladocerans (Leder, 1915): its duct is short,

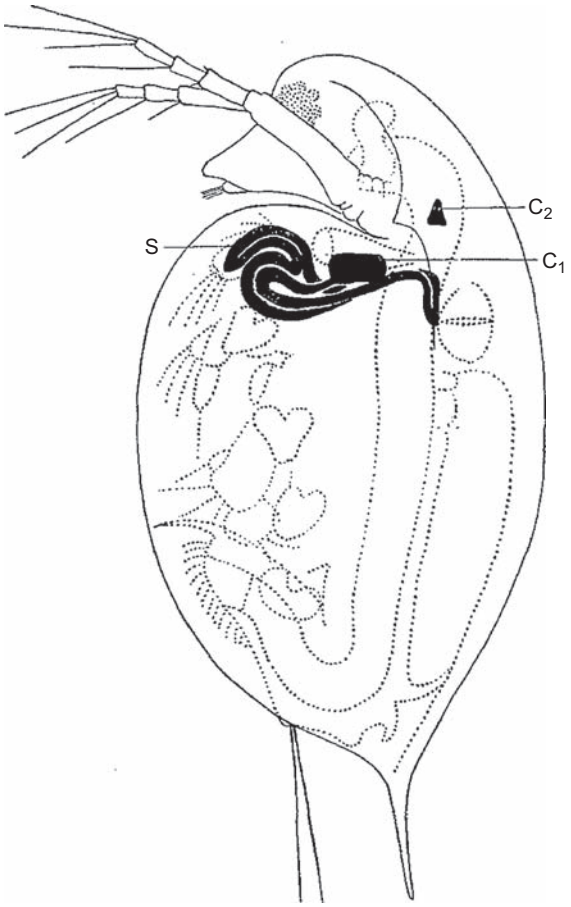

FIGURE 7.1 Position of nephridia in the body of *Daphnia*. *C1*, maxillar celomic sac; *C2*, antennal celomic sac; *S*, its ducts. *From Gicklhorn, J., 1931a. Elektive Vitalfärbungen im Dienste der Anatomie und Physiologie der Excretionsorgane vin Wirbellosen (Cladoceren als Beispiel). Protoplasma 13 (3/4), 701—724 (Sonderheft).*

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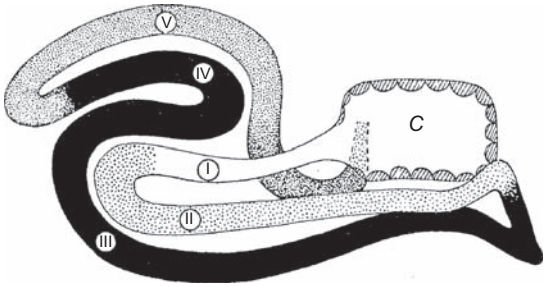

FIGURE 7.2 Nephridium of *Daphnia*. *C*, coelomic sac, *I-V*, its ducts, variously colored at intravital staining. *From Gicklhorn, J., 1931a. Elektive Vitalfärbungen im Dienste der Anatomie und Physiologie der Excretionsorgane vin Wirbellosen (Cladoceren als Beispiel). Protoplasma 13 ($^3/_4$), 701—724 (Sonderheft).*

has no convolutions, and is dilated in the distal part, producing a kind of urinary bladder (Fig. 7.3).

The nephridium of the maxillary gland and the antennal gland (the latter has no channel and is probably nonfunctional) are all that remain of the coelom in Cladocera. Although the transparent maxillary gland is discernible

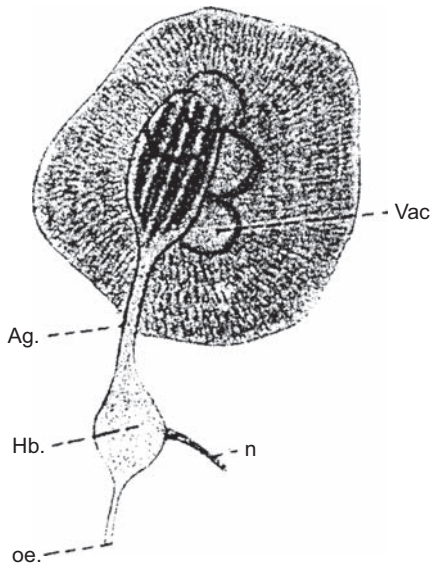

FIGURE 7.3 Nephiridium of *Penilia*. *Ag*, efferent duct, *Hb*, urinary bladder, *n*, nerve, *oe*, opening, *Vac*, vacuoles with liquid. *From Leder, H., 1915. Über* Penilia schmackeri *Richard in der Adria. Zoologischer Anzeiger 45 (6), 350—360.*

without any treatment, coelomic sacs may be made more clearly visible by means of intravital staining (Fischel, 1908; Dejdar, 1930; Gicklhorn, 1931a,c) with neutral red, methylene blue, Nile blue sulfate, or Bismarck brown.

Although Cladocera possess a functioning organ of secretion, Peters (1987, p. 219) believes that excretion occurs mostly through the body surface in Cladocera: "the soluble excreta of *Daphnia* are released through the general body surface."

7.2 THE PROCESS OF EXCRETION

The final products of metabolism are generally low molecular-weight compounds of nitrogen, phosphorus, dissolved organic carbon, carbon dioxide (CO_2), and water. Like other Crustacea, Cladocera are mostly ammoniotelic animals (Vonk, 1950), excreting mainly ammonia with some urea; at least, this is what occurs under aerobic conditions. What little information is available on Cladocera excretion relates mainly to daphnids, and occasionally to *Bosmina* spp.

As there is an obvious excess of intake of carbohydrates, it was found with reference to *Daphnia* that most of the carbon is liberated into the environment as dissolved organic carbon and undigested matter and algal cells (Lampert, 1978). The excess of carbon in the food of *Daphnia magna* and its fate during digestion were investigated by Darchambeau et al. (2003) and by He and Wang (2006a). See also Chapter 4.

Aladin and Plotnikov (1985) carried out an outstanding and unique investigation on the concentration, estimated by temperature depression of the freezing point, of the liquid excreted from the maxillary gland (i.e., urine) of *D. magna*, in comparison with the ambient water and the hemolymph. This is probably the only case in which a reduction in freezing temperature has been determined for these three media: the hemolymph, urine, and external water. Microcryoscopic techniques were applied. The

reduction in freezing point was −0.34°C for the liquid in the coelomic sac of the maxillary gland, −0.05°C for the convoluted duct, and −0.01°C for the external water. These data indicate that the urine excreted by *Daphnia* is hypoosmotic to hemolymph and slightly hyperosmotic to the external water. Thus, in freshwater, the urine of *D. magna* is isotonic with the hemolymph but hypertonic to the ambient water. The liquid in the excretory canal is hypotonic to both urine and hemolymph; this was interpreted as providing evidence of the reabsorption of salts from the urine and of water being excreted. In the case of *D. magna* acclimated to water with a salinity of 7‰ (i.e., 7 parts per 1000), the hemolymph, urine, and liquid in the excretory canal of the *D. magna* are isotonic to the water.

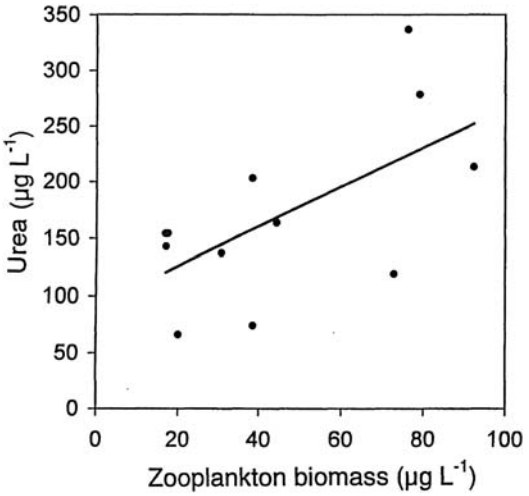

FIGURE 7.4 Urea concentration in lake water (Lake Schösee, Germany) in relation of crustacean zooplankton biomass. *From Wiltshire, K.N., Lampert, W., 1999. Urea excretion by* Daphnia*: a colony-inducing factor in* Scenedesmus*? Limnology and Oceanography 44 (8), 894–1903.*

7.2.1 Nitrogen Compounds

In Cladocera, the final products of nitrogen metabolism are principally ammonia derived from protein metabolism, along with a smaller percentage of urea (Fig. 7.4) (Parry, 1960; Wiltshire and Lampert, 1999). Although recent attempts failed to stain the coelomic sac of the maxillary gland with freshly prepared Nessler's reagent to demonstrate the presence of ammonia, further attempts would be worth making.

There are some quantitative estimates of the liberation of excretion products by Cladocera. According to Schmidt (1968), adult *D. magna* fed on green algae excrete 0.17–0.19 µg N/24 h/µg body N. The excretion of ammonia nitrogen by *Daphnia pulex* fed on the green alga *Chamydomonas* was found to be 0.20 µg N/individual (ind.)/24 h or 5.11 µg N/mg dry weight (DW)/24 h (Jacobsen and Comita, 1976). The mean, steady-state release of ammonia by *D. magna* was determined to be 11 nmol/mg DW/h by Gardner and Scavia (1981). The mean release of nitrogenous products by well-fed *Daphnia* was estimated to be for ammonia 0.76 µg/mg/h and urea 0.36 µg/mg/h (Wiltshire and Lampert, 1999). In other Cladocera (i.e., *Bosmina*, *Ceriodaphnia*, *Daphnia*, and *Scapholeberis*), the excretion rate was determined by Ejsmont-Karabin (1984) to be 0.8–2.6 µg/mg DW/h $N{-}NH_4$ and 0.26–0.58 µg/mg DW/h phosphorus. In *Daphnia*, the excretion rate (as summarized by Peters, 1987) is c. 1 µg DW/h for nitrogen and c. 0.5 µg DW/h for phosphorus. In addition, the rate of release of ammonia nitrogen by *Ceriodaphnia reticulata* has also been shown to depend on temperature: 1.0 mg/g wet weight (WW)/24 h at 15°C, 1.9 mg/g WW/24 h at 22°C, and 2.4 mg/g WW/24 h at 27°C (Gophen, 1976).

In contrast, starved *Daphnia* liberate 0.45 µg/mg/h ammonia and 0.06–0.1 µg/mg/h urea. A total of 6.5 min after feeding was discontinued, *D. magna* release ammonia nitrogen at a rate of 80 nmol/mg/h and phosphorus at 4.5 nmol/mg/h P (i.e., soluble reactive P) (Scavia and Garner, 1982).

The products of hemoglobin (Hb) decomposition are excreted by Cladocera through their maxillary glands (i.e., shell glands) termed by

Fox (1948) as hemoglobinuria. In the shell glands of *Daphnia* losing Hb, Smaridge (1954) noted Fe. The site of Hb breakdown is the fat body.

Amino acids are also excreted to the external environment, as shown with reference to *D. magna* by Gardner and Miller (1981). The estimated quantity is c. 21 nmol amino acids/mg DW per 1 h. They also release ammonia, urea, and phosphorus compounds. In the presence of *Daphnia galeata* fed on green algae, the concentration of dissolved free amino acids in the environment increases from 1 μM by up to 3 μM over 2 h (Riemann et al., 1986). Recalculated for carbon, the release rate was c. 0.2 μg C/ind./h, i.e., 12% of that ingested.

7.2.2 Carbohydrates

In addition to the release of carbon dioxide, it has been shown that excessively ingested carbon is excreted by *D. magna* as dissolved organic carbon (Darchambeau et al., 2003). In the presence of *D. magna*, the surrounding water is enriched with dissolved organic carbon, mostly by excretion following food digestion; much lower amounts are liberated from feces (He and Wang, 2006a,b).

7.2.3 Phosphorus Compounds

Inorganic phosphorus (PO_4—P) is released into the ambient water by *D. magna* at a rate of 8.4 ng/ind./h (Rigler, 1961a); phosphatase is also released. The release rate of phosphorus by *Daphnia* spp. is variously estimated to be 0.91 μg P/mg DW/h (Peters and Rigler, 1973), 1.1—1.5 μg P/mg DW/h (Olsen and Østgaard, 1985), or 0.05—1.5 μg P/mg DW/h [as summarized by Yuan Hua Wen (1994) from data from various authors obtained using radiotracer or chemical methods]. Adult *D. galeata* release 0.06—0.96 μg P/mg C/h into the surrounding water (Vadstein et al., 1995). The excretion rate of phosphorus for *D. galeata* was estimated by Pérez-Martínez et al. (1995) to be 6.3% of the total phosphorus/h for aduls and 16.2% for juveniles. Phosphorus excretion rates by natural populations in the Bay of Quinte (Lake Ontario) were estimated by Peters (1975) to be 2.2—4.8 mg P/m^2/day for *Daphnia*, 0.6—1.2 mg P/m^2/day for *Diaphanosoma*, and 0.6—1.3 mg P/m^2/day for *Leptodora*.

Pérez-Martínez et al. (1995) also obtained quantitative data on phosphorus metabolic rates in their three-compartment model: the gut, metabolic pool, and structural pool.

The specific P release rate in *D. pulex* decreases as the P:C ratio of its food decreases; there is no further release of phosphorus to the environment below a P:C ratio of 6—8 μg P/mg C (Olsen et al., 1986a,b).

The phosphorus release during the molting cycle of individual specimens of *D. magna* was measured by Scavia and McFarland (1982) in a specially designed incubation flow cell. The rate of phosphorus release was 6.7 times higher at and after ecdysis than at other phases of the life cycle.

Phosphorus release by Cladocera has seasonal fluctuations: in Swarzedzkie Lake (Poland), a peak of 10—15 μg P/L/day was reached in June (Kowalczewska-Madura et al., 2007).

7.2.4 Water

As noted earlier, Cladocera drink water and surplus water is rapidly excreted. According to Peters (1987), about 8% of the body's water is removed by *Daphnia* every hour.

7.3 BIOACCUMULATION OF TOXIC SUBSTANCES

It has been shown that *Daphnia* accumulate various substances that may be useful, alien, or harmful to their normal life, and some substances accumulated by Cladocera are unaffected by their metabolism. See also Chapter 3.

7.3.1 Accumulation of Inorganic Substances

If not killed by their high concentrations, *D. magna* may accumulate xenobiotics, for example, copper (Cu) or lead (Pb) (Holm-Jensen, 1948). When fed with green algae and *Euglena*, *Daphnia* selectively accumulated higher concentrations of sodium (Na), calcium (Ca), scandium (Sc), lanthanum (La), neodymium (Nd), zirconium (Zr), chlorine (Cl), bromine (Br), and nickel (Ni) than were present in *Euglena*, whereas the latter contained higher concentrations of various other elements [including mercury (Hg) and arsenic (As)] (Cowgill and Burns, 1975). The accumulation of such substances is represented by their bioconcentration factor (i.e., accumulation coefficient), which is the ratio of the concentration of a pollutant within the body to its concentration in the environment. The bioconcentration factor of *D. magna* for ^{54}Mn (manganese-54) was 65, reached after 8 h of exposure to the isotope solution, with excretion having started within 2 h of exposure (Kwassnik et al., 1978).

There are numerous studies on the accumulation of various metals and compounds, mostly restricted to daphnids (Table 3.11). According to Yu and Wang (2002), in *D. magna* the assimilation efficiency from algal food is 30–77% for cadmium (Cd), up to 44% for chromium (Cr), 24–58% for selenium (Se), and 7–66% for zinc (Zn). Routes for metal removal into the dissolved phase were different for different metals: excretion was the most important route, molting representing 50–70% of daily Cd efflux and 20–70% of Zn; and the major routes of Cr efflux were via excretion and feces egestion. Substantial amounts of Se were released via the production of offspring. The release of metals has an important impact on the biogeochemical cycling in lakes. Table 3.11 shows that bioaccumulation rates by Cladocera may vary widely.

Increased humic acid chelation of Cu, Cd, and Zn decreases their bioavailability (Winner and Gauss, 1986).

Radionuclides such as ^{110}Ag, ^{60}Co, ^{137}Cs, and ^{54}Mn ingested with food are accumulated in *D. magna* (Adam et al., 2002).

7.3.2 Accumulation of Organic Substances

Anthracene. Anthracene accumulated by *D. pulex* is removed at a rate of 1.6 μg/ind./h. However, within *Daphnia* there are three "compartments": a rapidly eliminated compartment (with c. 30% of accumulated anthracene); a more slowly eliminated compartment (60%); and a tightly bound one (8%) (Herbes and Risi, 1978). It is thought that metabolization of anthracene by *D. pulex* is much slower than its accumulation. Exposure of *D. magna* to anthracene plus ultraviolet radiation (UVR) reduced survival and fecundity more than exposure to anthracene alone (Holst and Giesy, 1989). Exposure to UVR in the absence of anthracene produced no significant effect.

Benzo(a)pyrene. *D. magna* mainly takes up benzo(a)pyrene (a polynuclear aromatic hydrocarbon) from solution; the presence of particles containing this absorbed chemical decreases its accumulation (McCarthy, 1983). Bioaccumulation of benzo(a)pyrene and fluoranthene by *D. magna* is reduced by humic substances (McCarthy, 1983; Gourlay et al., 2003); the effect is highest at pH 6.5 (Kukkonen, 1991). In addition, the accumulation of dehydroacetic acid from humic water by *D. magna* is lower than that from a nonhumic water control at a pH higher than 6; in contrast, at pH 5.5 and lower, the effect is reversed (Kukkonen, 1991). If fed with *Chlorella* contaminated with hexachlorobenzene (HCB), *D. magna* can accumulate 1.7 μg HCB/kg DW over 6 days (Muños et al., 1996).

Dichlorodiphenyltrichloroethane. Dichlorodiphenyltrichloroethane (DDT) is accumulated by *D. magna* from ambient water principally through their integuments (Table 3.11) (Crosby and Tucker, 1971). The total concentration of DDT in *Daphnia* may reach over 4.2 g/kg; a

large part of the DDT is found in the carapace and is removed during molting. The 100% lethal dose (or LD_{100}) was found to be 1100 ng/mL.

Fenvalerate. Fenvalerate causes a decreased rate of filtration of *Chlamydomonas* by *D. galeata* and *Ceriodaphnia lacustris* at sublethal concentrations (i.e., in *C. lacustris* that already contains 0.01 μg/L) (Day and Kaushik, 1987). Rates of algae assimilation also decrease, especially in fenvalerate concentrations >0.1 μg/L.

Lindane. According to Hansen (1980), *D. magna* accumulates lindane (a chlorinated hydrocarbon).

Pentachlorophenol is accumulated by *D. magna*, metabolized, and excreted mainly via the sulfate conjugation (Kukkonen and Oikari, 1988).

Polychlorinated Biphenyls. PCBs are accumulated by *D. magna*, from 2 to 130 ng/mg DW (Dillon et al., 1990) or c. 8 μg/g in 24 h from ingested algae and this concentration is maintained over the subsequent 72 h (Joaquim-Justo and Thomé, 1998), that is, no significant elimination occurs within 72 h. Concentrations reached up to 6 ng/g malathion in *Simocephalus vetulus* after 8 h of exposure (Olvera-Hernández et al., 2004) and up to c. 0.180 mg/g tetradifon in *D. magna* after 8 h of exposure (Ferrando et al., 1996).

Triphenyltin chloride is absorbed by *D. magna* via ineguments, according to Filenko and Isakova (1979).

There is also a dependence on temperature. It was shown by analyses of *D. pulicaria* homogenate fractions (Heisig-Gunkel and Gunkel, 1982) that at a temperature of about 8°C the accumulation of atrazine is correlated with protein content, whereas at higher temperatures (12−29°C), it correlates with fat, and protein becomes less important. Different sensitivity to chemicals of different species was shown. For example, *D. longispina* was sensitive to a solvent $[C_3mim][Tf_2N]$ whereas *D. magna* was tolerant (Ventura et al., 2010).

7.4 TRANSFORMATION OF XENOBIOTICS

Consumed toxic substances may be metabolized by Cladocera. Thus, arsenic (As) consumed by *Moina macrocopa* fed on algae containing Na_2HAsO_4 accumulates up to 111 μg As/g DW in the form of inorganic As (75%), mono-CH_3 (8%), and di-CH_3 (16.6%), and is then mostly excreted (Maeda et al., 1992).

An example of the transformation of an organic toxicant in the body of a cladoceran is the fate of heptachlor absorbed by *Daphnia magna* (Fig. 7.5). Heptachlor is metabolized in the daphnid body to 1-hydrochlordene, 1-ketochlordene, and 1-hydroxy-2,3-epoxychlordene, as well as derivatives such as glucosides and sulfates (Feroz et al., 1990). Pyrene consumed by *D. magna* is transformed into water-soluble metabolites (Ikenaka et al., 2006) Of the accumulated pyrene, after 24 h depuration time 7.8% remained in the body of *D. magna*, the initial substance was removed faster than its metabolites (Ruotsalainen et al., 2010).

7.5 THE ROUTES OF ELIMINATION OF XENOBIOTICS

Pollutants can penetrate the body of a cladoceran via its carapace or its gut along with ingested water and food. They then undergo biodegradation and, especially those that are only slightly biodegradable, are accumulated (to different extents in different tissues), and are gradually removed. The routes of elimination are excretion, defecation, molting, and transfer to eggs. The actual accumulation is the difference between the intake level and the capacity for elimination via these routes.

Cd (Carney et al., 1986) and ^{65}Zn (Winner and Gauss, 1986) obtained from solution are partly accumulated in the exoskeleton of *Daphnia* and molting frees the animal from a considerable part of these elements.

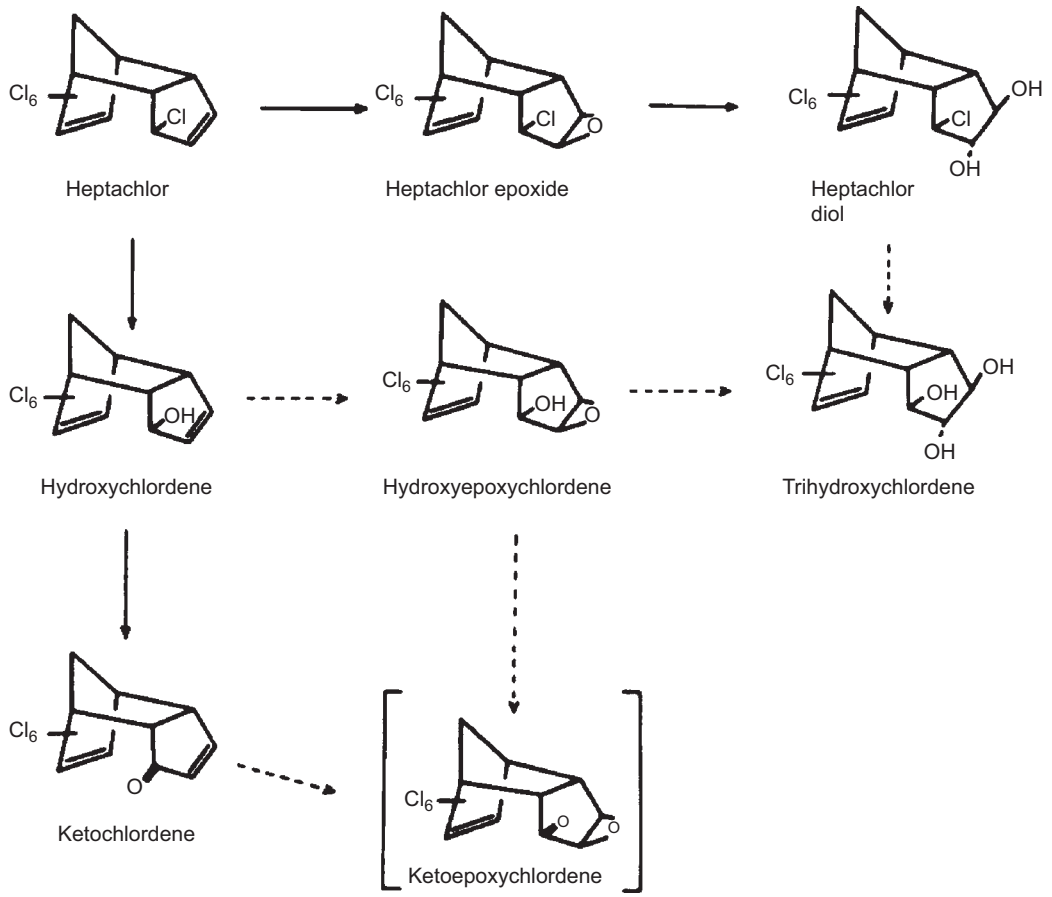

FIGURE 7.5 Biotransfromation of heptachlor within the body of *Daphnia*. *From Feroz, M., Podowski, A.A., Khan, M.A., 1990. Oxidative dehydrochlorination of heptachlor by* Daphnia magna. *Pesticide Biochemistry and Physiology 36, 101–105.*

The elimination rate of metals via these routes is little affected by the concentration of Cd, Se, or Zn in the ingested food (Guan and Wang, 2004a). However, rapid elimination of Se and Zn might depend on the transfer of these metals from mother to offspring. The principal pathway of Cd and Zn elimination from the body of *D. magna* is excretion to the water, a secondary pathway is transfer to neonates, and the pathways used least are by molting and through feces (Guan and Wang, 2004b).

Copper is principally accumulated by *D. magna* from its food; the Cu efflux rate is 0.20%/day at high food concentrations, and excretion accounts for 82–84% of the total Cu loss, whereas c. 6.6% is transferred to the offspring within 7 days (Zhao et al., 2009).

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CHAPTER

8

Osmotic Regulation

8.1 POTENTIAL ANATOMICAL BACKGROUND

Ion exchange in Cladocera is thought to be carried out via the neck organ and the epipodites of the thoracic limbs. Possible routes of ions through the neck organ of cladocerans were illustrated by Potts and Durning (1980) (Fig. 8.1). On the surface of the epipodite of *Daphnia*, which is reported to be slightly alkaline (pH 8) (Lavrentjeva and Beim, 1978; Beim and Lavrentieva, 1981; Beim et al., 1994), a special cell membrane, presumed to participate in active ion transport (Kikuchi, 1982), was identified on the side in contact with the external medium.

8.2 ENVIRONMENTAL BACKGROUND

Different species of Cladocera live in freshwater, brackish water, and saline inland waters, and a few live in seawater. A small number of polyphemid species and one ctenopod (*Penilia*) are oceanic. In freshwater, where most Cladocera live, they risk losing salts and absorbing too much water. Vice versa, marine Cladocera risk losing water and having to discard extra salt.

Different species within a genus may tolerate different salinities (such as e.g., *Moina* spp.). Some species can endure variable salinity

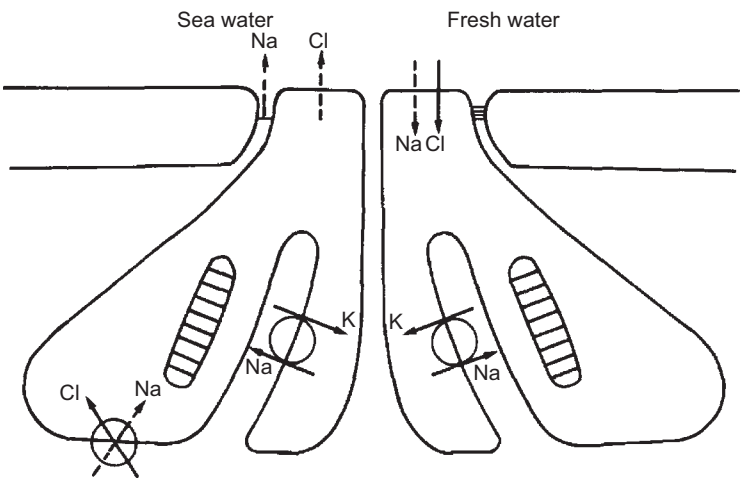

FIGURE 8.1 Possible routes of ion movements through neck organ in freshwater and in seawater. *Solid lines*, active transport; *broken lines*, passive diffusion. *From Potts, W.T.W., Durning, C.T., 1980. Physiological evolution in the Branchiopods. Comparative Biochemistry and Physiology 67B, 475–484.*

http://dx.doi.org/10.1016/B978-0-12-805194-8.00008-8

within rather limited ranges, but the presence of salts in the external water is obviously necessary. *Chydorus ovalis* perished in triple-distilled water (Winberg, 1933), and ordinary distilled water caused high mortality levels in *Daphnia magna* within 24 h (Stobbart et al., 1977). The obvious reason for this is disturbance of their salt metabolism.

Total salinity (i.e., total dissolved solids; TDS) of freshwaters, where most Cladocera live, is within 100–200 mg/L (see, e.g., Hutchinson, 1957), reaching c. 1000 mg/L in bodies of hard water (Kitaev, 2007). The salinity of some inland lakes may be c. 30‰ and may reach the level of saturated brine. As is well known, oceanic salinity is c. 32‰ and may be lower in enclosed seas (16‰ in the Black Sea—the conventional boundary between marine and brackish waters). It should be noted, however, that both the total salt content and the ionic composition of inland saline waters and marine waters differ; they may also be different in various saline lakes.

8.3 WATER BALANCE

The general water and salt balance of the cladoceran body is maintained by the process of osmotic regulation through their water intake, intake of minerals with food, excretion, and through special channels for the removal of ions. There are two methods of water intake: Cladocera take some water in through their integument and they also drink water. The latter seems to be the principal source of water intake.

8.3.1 Impermeability of Integuments

Because chitin is unwettable, cladocera are almost entirely isolated from their aquatic medium; they are connected to the external water by drinking, taking in water through the anal opening, and partly by osmotic regulation. Freshwater Cladocera must excrete any excess water.

Some direct permeability of the *Daphnia* body was measured using intravital staining (trypan blue) by Fonviller and Itkin (1938). They found that a 0.01% solution of trypan blue stained first of all the esthetascs of the first antenna, and then penetrated further to the basal grains, nerves, an antennal ganglion, and the brain. The contents of the intestine were then stained and from there the stain penetrated the nephridia, epipodites, and finally the intestinal epithelium. (See also Chapter 4).

8.3.2 Water Drinking

Cladocerans drink water and also take in some water with their food. Water intake was observed by Fox (1952) in *Daphnia hyalina* (24 swallowing movements/min), *Moina brachiata* (43 swallowing movements/min), *Diaphanosoma brachyurum* (29 swallowing movements/min), *Penilia avirostris* (37 swallowing movements/min), and *Bythotrephes longimanus* (95 swallowing movements/min). Water intake through the mouth serves to mix digestive enzymes with the food and pushes the food through the intestine. It is also involved in osmotic regulation.

8.3.3 Anal Water Intake

Anal water intake is a normal characteristic of Cladocera. Several antiperistaltic contractions of the hindgut follow defecation, and the evacuated volume is compensated for by anal water intake. Such water intake has been known for a long time in *Daphnia* (Hardy and McDougall, 1894; Chatton, 1920), *Sida*, *Leptodora*, *Bythotrephes* (Fox, 1952), *Ceriodaphnia*, *Moina*, and *Bosmina*, and observed later in chydorids and macrothricids (Fryer, 1970; Smirnov, 1971), but not in the marine *Evadne* and *Podon* (Fox, 1952).

Anal water intake may be conveniently observed in a specimen placed in a solution of neutral red: after defecation, red liquid fills the

posterior gut. Anal intake of stained water was also observed by Chatton (1920), Rankin (1929), and Fryer (1970). It is possible to see that after defecation, there generally follow several antiperistaltic contractions of the hindgut. If the fecal particles were not expelled sufficiently far away, some are occasionally drawn back inside with the water (as observed in *Daphnia* and *Sida*).

8.4 PROCESS OF OSMOTIC REGULATION

The majority of Cladocera species live in freshwater. However, some species can prosper in a wide range of salinities, up to highly saline water bodies (Aladin, 1981; Frey, 1993). However, it is notable that species that can live successfully in saline lakes do not penetrate marine environments. This may be caused by differences in the salt composition of these two environments. The osmotic pressure within Cladocera is thus supported by the intake and efflux of various substances and of water.

Cladocera can propagate in four different environments that differ radically in their salinity:

1. freshwater (hundreds of species of Cladocera);
2. slightly saline water (dozens of species), the critical boundary of salinity being 5–8‰ (Khlebovich, 1974);
3. highly saline continental waters (a few species); and
4. the marine environment (a few species).

There must, therefore, be special mechanisms among the Cladocera that enable them to exist in such different situations. Species within the same genus, for example, *Moina*, live either in freshwaters or in saline inland waters. Among the ctenopods, *P. avirostris* is a marine species, whereas its morphological counterpart, *Sida crystallina*, is strictly a freshwater species. Their capacity for osmotic regulation demonstrates that the physiological background of salinity tolerance varies within particular species.

Extensive investigations into osmotic regulation, mostly in Cladocera and ostracods, were made by Aladin (1978–96). Having applied the microcryoscopic method (a version of determination of osmotic pressure by the freezing point), Aladin (1981) described three principal types of osmotic regulation in Cladocera:

1. hyperosmotic regulation—in Sididae (except for *Penilia*), Holopedidae, Daphniidae, Moinidae (except for *Moina mongolica*), Bosminidae, Chydoridae, and Macrothricidae;
2. Hypoosmotic regulation—in *Penilia*;
3. Amphiosmotic regulation—in Aralo–Caspian Podonidae and *M. mongolica.*

8.4.1 Freshwater Cladocera

The freshwater Cladocera are hyperosmotic: they maintain a higher concentration of solutes than the external medium. They drink water (Fox, 1952), must excrete excess water, and must supplement ions lost through excretion by obtaining them from food and by reabsorption of salts via the nuchal organ (Aladin, 1991). It has been determined that there is sodium and chlorine ion uptake in *Simocephalus* kept in a solution of sodium chloride; in this way, the hemolymph is maintained at a level hyperosmotic to the medium (Nimmo, 1966).

According to Fritsche (1917), the freezing temperature (or the freezing point) in *D. magna* can be reduced from −0.20 to −0.61°C. It is higher in younger specimens, and there is some relationship with nutrition and egg production, because it is higher in fed specimens than in starving ones. It decreases during prolonged parthenogenesis and is, on average, high in specimens with ephippia. Belayev (1950) found there to be considerable interindividual variation in temperature depression (Δ°) of the blood in *Daphnia pulex*

(0.24−0.45°C), *Eurycercus glacialis* (0.36−0.39°C), and *B. longimanus* (0.35−0.46°C). He concluded that *D. pulex* can regulate its osmotic pressure if the external salinity is not above 5‰.

8.4.2 Marine Cladocera

Marine Cladocera (podonids and *Penilia*) have a hypoosmotic type of osmotic regulation, that is, they maintain a lower concentration of solutes than the external seawater (Khlebovich and Aladin, 1976; Aladin, 1979, 1994, 1996a,b). To achieve this, they must continuously excrete ions through their epipodites and the nuchal organ (Aladin, 1991). In these cladocerans, increased activity of succinate dehydrogenase was found in the cells of the nuchal organ, indicating intensive cellular metabolism and active salt excretion against the concentration gradient.

Using the microcryoscopic method, it was found (Khlebovich and Aladin, 1976; Aladin 1978, 1979, 1982a, 1996a,b) that temperature depression of hemolymph in marine podonids ranges from −0.5 to −0.76°C (depression of seawater at water salinities above 12‰ is −0.7 to −1.39°C), whereas in *Penilia* it is −0.72°C (that of seawater is −0.99°C). Aladin also determined that marine Cladocera support a lower osmotic concentration within the brood pouch, which favors normal development of the embryos. According to Aladin, marine Cladocera swallow water and secrete ions: chlorine ions in the area of the nuchal organ in podonids, and from the epipodites of the thoracic limbs in *Penilia*. At the sites of these organs a special succinate dehydrogenase activity was identified, also indicating excretion of salts against a concentration gradient.

8.4.3 Amphiosmotic Regulation

Aladin (1982b) also discovered that Caspian and Aral podonids can use hyperosmotic osmotic regulation at salinities below 8‰ and may exploit hypoosmotic osmotic regulation above 8‰. This is a special case of cladocerans living in continental hypersaline waters. *M. mongolica* was able to live in salinities up to 88‰ [with the (former) salt composition of the Aral Sea]. At salinities over 8‰, it exploits hypoosmotic osmotic regulation; under 8‰, hyperosmotic osmotic regulation occurs (Aladin, 1982c, 1983).

8.4.4 Sodium Exchange in Hyperosmotic Cladocerans

It has been established that sodium is retained better at pH 3 by an inhabitant of acid environments (pH 3.4−6.3), *Acantholeberis curvirostris*, than by *D. magna* (pH 6.9−10.2); its uptake is reduced in acid waters in both species, but more so in *D. magna* (Potts and Fryer, 1979). Sodium loss is lower at pH 3 than at pH 7 in *A. curvirostris*, but is four times greater in *D. magna* (Potts and Fryer, 1979). Havas and Likens (1985) studied the effect of pH on sodium regulation in *D. magna* and *Daphnia middendorffiana*. The rate of Na loss (efflux) at pH 4 and below (in hard water) was compared with that at pH 8.0: Na uptake was the same in both. In soft water, Na uptake was inhibited by 69% at pH 4.5 (compared with the pH 6.5 control), and loss (efflux) increased to 125% of the control at pH 4.5. Thus, there are problems with Na regulation below pH 5.5 in soft water and below pH 4.5 in hard water.

Glover and Wood (2005) found that sodium metabolism in *D. magna* may be disrupted by acidification and by ionoregulatory toxicants. Acidification inhibits Na intake. At low pH, calcium (in the form of $CaSO_4$ and as calcium gluconate) inhibits Na uptake by *D. magna*, whereas at higher pH and at high Na concentrations, calcium stimulates Na uptake.

Daphnias that are depleted of sodium can restore their normal content of 26.3 mM/kg WW within 15 h (Stobbart et al., 1977). They do not accumulate excessive Na. *Daphnia galeata mendotae* survive better when they have a higher Na content (at 6−10 mg/g DW) (Havens, 1992).

At pH 4.5, both the Na content and survival were reduced. A concentration of 200 g aluminum (Al)/L at pH 4.5 enhances Na content in the body of *Daphnia* and prolongs survival.

Experimental exposure of *D. magna* to natural organic matter promoted the Na loss from the daphnid to the water, thus resulting in reduced whole-body Na levels; this was a labile process, dependent on the period of preexposure (Glover et al., 2005a).

Na uptake and release in *D. magna* adults and neonates was investigated more recently by Bianchini and Wood (2008): these processes turned out to have different mechanisms in adults and in neonates (Fig. 8.2). According to these authors, in neonates, a proton-coupled Na^+ channel is important for whole-body Na^+ uptake at the apical membrane. In contrast, this membrane does not contribute to whole-body Na^+ uptake in adults: adults possess only the Na^+ channel associated with Na^+/H^+ exchange. In both cases, protons (H^+) for the transporters are supplied by carbonic anhydrase. In neonates, at the basolateral membrane Na^+ is pumped to the extracellular fluid by an Na^+, K^+-dependent adenosine triphosphatase (Na^+, K^+-ATPase) and a Na^+/Cl^+ exchanger, whereas K^+ and Cl^- move through specific channels. In adults, a Na^+/Cl^- exchanger is replaced by a $Na^+/K^+/2Cl^-$ cotransporter. Accordingly, the sensitivity of adults and neonates to osmoregulatory toxicants is different.

Special attention was paid to acid stress. At normal conditions (pH 7.8), the extracellular pH within the body of *D. pulex* was determined as 8.33, P_{CO_2} 0.56 kPa, bicarbonate concentration in hemolymph 20.9 mM (a major part of the total buffer value) (Weber and Pirow, 2009). Acid

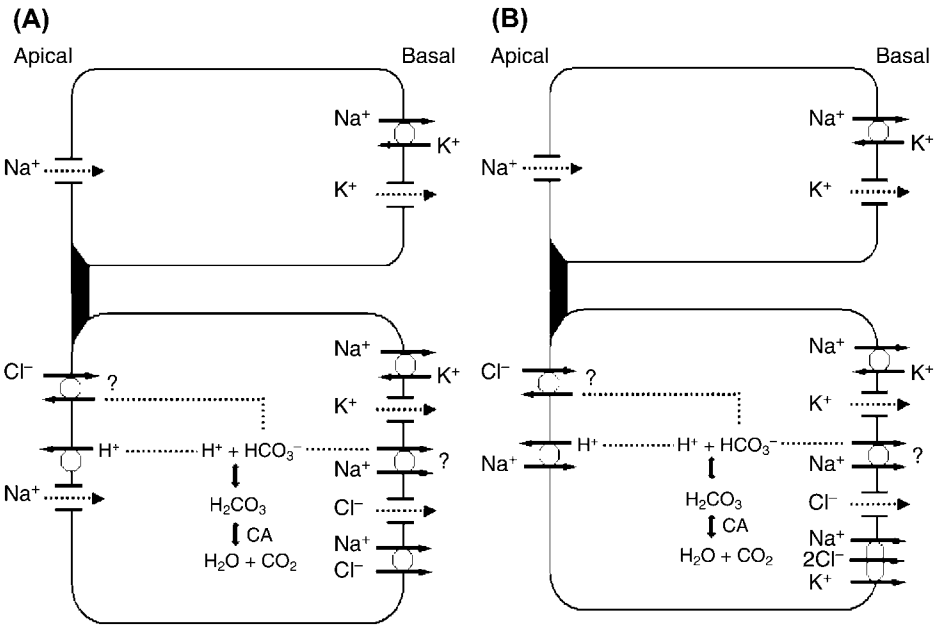

FIGURE 8.2 Ion transport in *Daphnia magna*. (A) Neonates and (B) adults. *From Bianchini, A., Wood, C.M., 2008. Sodium uptake in different life stages of crustaceans: the water flea* Daphnia magna *Strauss. Journal of Experimental Biology 211, 539–547.*

conditions (pH 5.5−6) caused 30−65% bicarbonate loss, decrease in the extracellular P_{CO2} to 0.33 kPa, tachycardia, hyperventilation, and hypermetabolism.

8.4.5 Turgor

Hydrostatic pressure, or turgor [termed also the hemocoel pressure by Downing (1974)], within the cladoceran body is supported in the same way as has been described for osmotic regulation. Its function is the upkeep of pressure necessary for mechanical actions. According to Krogh (1939), turgor requires only a small fraction of osmotic pressure.

Homeostasis is supported by both osmoregulation and excretion, in cooperation with other physiological processes.

8.5 EFFECT OF XENOBIOTICS ON OSMOTIC REGULATION

The following data have been reported. Ni is toxic to *D. magna* due to Mg^{2+} antagonism: exposure to Ni affects Mg^{2+} homeostasis (Pane et al., 2003). In the body of *D. magna*, Mg^{2+} is decreased by 18%, and its uptake also decreased. No noticeable effect of Ni on the Ca^{2+} or Cl^{-} balance was observed, and there was no acute (i.e., in short-term experiments) toxic effect on oxygen consumption. *N*-amidino-3,5-diamino-6-chloropyrazinecarboxamide hydrochloride (Amiloride) inhibits Na influx in *D. magna* (Glover and Wood, 2005). For perfluoroalkyl acids it was found that the main uptake route is the body surface of *D. magna* (Dai et al., 2013).

CHAPTER

9

Cell and Tissue Metabolism

9.1 ENZYMES IN THE BODY OF CLADOCERA

Metabolic processes are catalyzed by enzymes (specific proteins). For Cladocera, numerous individual enzymes have now been discovered. Those involved in food digestion are found in the digestive tract of *Daphnia*: these include proteases, lipases, amylases, and cellulase [Hasler (1937); summarized by Hebert (1978a); and in Chapter 4]. Enzymes involved in various metabolic processes have also been found in Cladocera homogenates (e.g., Hebert, 1973). As well, in homogenates of *Daphnia magna*, the protein content, antioxidant capacity, internal hydrogen peroxide, total ascorbic acid, and free proline levels (ascorbic acid and proline are both antioxidants) were assessed (Steinberg et al., 2010).

In addition, the following enzymes have been used to explore genetic variability in *Daphnia* spp.: alkaline phosphatase, esterase, fumarase, glucose-6-phosphate dehydrogenase, lactate dehydrogenase, glutamate oxaloacetate transaminase, leucine aminopeptidase, phosphoglucose isomerase, tetrazolium oxidase (Hebert and Moran, 1980; Hebert and McWalter, 1983; Hebert et al., 1989a,b; Hebert and Finston, 1996, 1997; Hebert and Wilson, 2000), malate dehydrogenase, xanthine dehydrogenase (Hebert and McWalter, 1983), aldehyde oxidase, amylase, phosphoglucomutase (Hebert et al., 1989a,b), aspartate aminotransferase, fumarate hydratase, and mannose-6-phosphate isomerase (Kořínek and Hebert, 1996).

Berges and Ballantyne (1991) found the following enzymes in whole-body homogenates of *D. magna*: citrate synthase (CS), lactate dehydrogenase (LDH; for anaerobic metabolic activity), pyruvate kinase (PK; for anaerobic metabolic activity), alanine aminotransferase (ala AT), aspartate aminotransferase (asp AT), glutamate dehydrogenase (GDH), nucleoside diphosphate kinase (NDPK; an anabolic enzyme), and glucose-6-phosphate dehydrogenase (G6Pdh; an anabolic enzyme).

The acetylcholinesterase (AChE) content in *D. magna* was determined to be 12.7 milliUnits per milligram (mU/mg) protein (Day and Scott, 1990). The AChE content in *Daphnia* is inversely proportional to the body size; this situation is probably caused by an increase in total protein that is not proportional to substrate hydrolysis (Printes and Callaham, 2003). AChE activity is 2.5 μM/L/min/g protein in 1-mm long *Daphnia* and decreases to c. 0.5 μM/L/min/g protein in 3-mm long *Daphnia*. In this system, parathion (*O*,*O*-diethyl-*O*-*p*-nitrophenyl phosphorothioneate) inhibits AChE activity and phenobarbital negatively affects the protein. *Moina macrocopa* AChE was extracted from homogenate supernatant by Martinez-Tabche et al. (1997, 1988), and differences in its activity relative to control has

http://dx.doi.org/10.1016/B978-0-12-805194-8.00009-X

been used to estimate water quality (Martinez-Tabche et al., 1988).

Choline esterase from *D. magna* hydrolyzes acetyl thiocholine iodide, propionylthiocholine iodide, and butyrylthiocholine iodide; it is highly sensitive to the organophosphate dichlorvos (2,2-dichlorovinyl dimethyl phosphate; DDVP) (Menzikova, 1988).

Phenoloxidase activity is increased in well-fed *D. magna*, and wounding stimulated an increase in enzyme activity. In addition, clones with higher phenoloxidase activity were more resistant to *Pasteuria* (Mucklow and Ebert, 2003).

Carbonic anhydrase, a metalloenzyme present in *Daphnia*, catalyzes the reaction $CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow HCO_3^- + H^+$, and participates in acid–base regulation, respiration, osmoregulation, and biomineralization (Culver and Morton, 2015).

9.2 ENDOCRINE AND EXOCRINE SECRETION

Endocrine secretion controls molting, reproduction cycle, and various metabolic processes.

Female and male hormones are discussed in Chapter 11.

Exocrine secretion comprises production of slimes and digestive enzymes. Slimes comprise saliva (Fig. 3.2), secretions of glands in thoracic limbs (Fig. 9.1), and slimes on the outer surface of some species (e.g., *Holopedium*) (see Section 3.2). Slime glands in the thoracic limbs are found in limb IV of *Eurycercus*, in which the slime gland secretions are stained bright blue with Mallory's stain (Fryer, 1962), and in *Alonopsis elongata* (Fryer, 1968) (Fig. 3.3). No other cladocerans have yet been investigated for the presence of slime glands in thoracic limbs.

The kinds of intestinal exocrine secretion have only been mentioned once for Cladocera (Avtsyn and Petrova, 1986): holocrine secretion in the epitheliocytes on the inner surface of the anterior part of the gut of *Daphnia*, and macrolemmocrine secretion and macroapocrine secretion in the middle part. (Note: the holocrine secretion is transformation of the whole cell for secretion, the apocrine secretion involves breaking off the ends of gland cells for secretion, whereas the functioning part of the cell with the nucleus is retained and continues the same kind of secretion).

9.2.1 Sites of Hormone Formation

Neurohormones, controlling reproduction and molting, are produced by groups of neurosecretory cells in the cephalic region of the nervous system (Fig. 13.2; see Section 13.2). It was supposed that ecdyson in *D. magna* is synthesized in the gut (Sumiya et al., 2014).

9.2.2 Hormonal Control

Endocrine secretion. The normal content of glucocorticoids in *D. magna* is 8.4–12.7 (Polunina, 1999) or 8.43 pmol/g hydrocortisone (cortisol) (Nikitina and Polunina, 2000) and 6.9–10.1 (Polunina, 1999) or c. 9–10 pmol/g (Nikitina and Polunina, 2000) corticosterone. Seasonal changes in the concentration of both glucocorticoids are found by Nikitina and Polunina (2000). Estradiol added to the culture medium increased the content of both glucocorticoids (Polunina, 1999), the content of hydrocortisone in spring only (Nikitina and Polunina, 2000). Oxytocin increased the concentration of hydrocortisone in all seasons (Nikitina and Polunina, 2000).

A steroid hormone hydrocortisone takes part in carbohydrate and protein metabolism. Exogenous oxytocin and estradiol increased fecundity (Nikitina and Polunina, 2000). Hydrocortisone at 0.25 mg/L increases the *D. magna* life span, as well as its heart rate and the number of eggs in the brood pouch, compared with controls (Kudikin, 2001).

Peptide Paracrines/Hormones

Applying transcriptonics and immunochemistry, Gard et al. (2009) identified in *Daphnia*

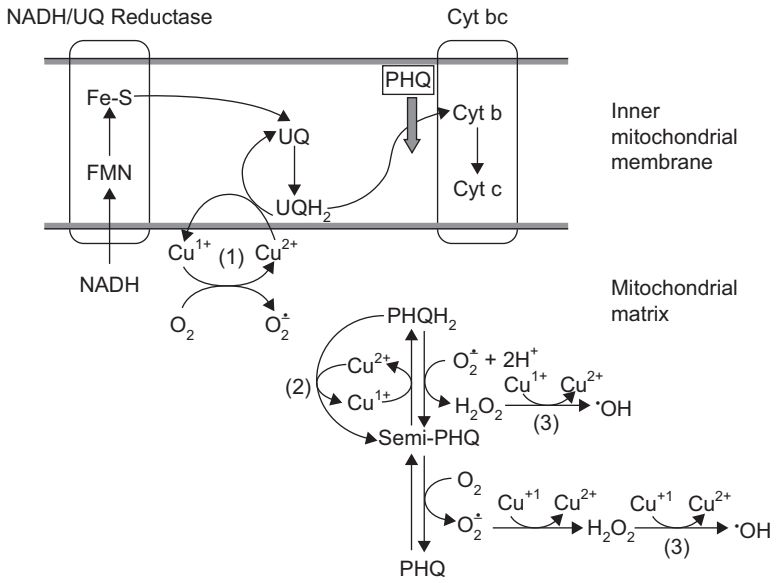

FIGURE 9.1 Proposed roles of copper in transformation of phenathrenequinone in *Daphnia*. *CYT*, cytochrome; *FMN*, flavin mononucleotide; *NADH*, nicotinamide adenine dinucleotide; *PHQ*, 9,10-phenanthrenequinone; *UQ*, ubiquinone. *From Xie, F., Koziar, S.A., Lampi, M.A., Dixon, D.G., Norwood, W.P., Borgmann, U., 2006. Assessment of the toxicity of mixtures of copper, 9,10-phenanthrenequinone, and phenanthrene to* Daphnia magna*: evidence for a reactive oxygen mechanism. Environmental Toxicology and Chemistry 25 (2), 613–622.*

pulex 14 peptide families/subfamilies that are supposed to be paracrines/hormones, including alltostatin, bursicon, cardioactive peptide, hyperglycemic hormone/ion transport peptide, diuretic hormone, and ecdysis-triggering hormone. All isomorphs found in *D. pulex* turned out to be novel. Localization in the central nervous system of *D. pulex* was shown for some of them: A-type allostatin, corazonin, cardioactive peptide, phenylalanine–methionine–arginine–phenylalanine–amine (FMRFamide), insect kinin, pigment-dispersing hormone (PDH), proctolin, red pigment-concentrating hormone (RPCH), alanine–tyrosine–arginine–lysine–proline–proline–phenylalanine–asparagine–glycine–serine–isoleucine–phenylalanine–amine (SIFamide), sulfakinin, tachykinin-related peptide (TRP). This suggests their role in local control and in neuroendocrine signaling. Gard et al. (2009, p. 271) note that their data "provide a strong foundation for future investigations on the effects of environmental/anthropogenic stressors on peptidergic control."

Eicosanoid Biosynthesis

Eicosanoids are considered in *Daphnia* by Heckmann et al. (2008a,b). They are signaling molecules taking part in reproduction (testosterone, estrogen), the immune system, and ion transport. These molecules are oxygenated molecules of C_{20} polyunsaturated fatty acids. In *Daphnia*, they (including prostaglandins) are synthesized from arachidonic acid (C_{20}:4ω6) through the cyclooxygenase pathway.

Prostaglandines make a group of hormones being the long-chain, unsaturated, hydroxy fatty acids possessing diverse effects.

9.3 ANTIOXIDANT SYSTEM

It is known that superoxide dismutase catalyzes formation of H_2O_2, which then is

transformed into water and oxygen by catalase. Antioxidant defense enzymes comprise also glutathione peroxidase and glutathione S-transferase (GST). These enzymes protect the cells from peroxidation. Such role in protection from peroxidation was shown for glutathione peroxidase (enzyme containing selenium) in *D. magna*; in specimens deprived of selenium, lysis of muscle fibers took place and in the fourth generation they rejected parts of second antennae (Elendt, 1990). An antioxidant enzyme Cu/Zn superoxide dismutase is found in *D. magna* (Lyu et al., 2013) (responsible for the conversion of superoxide to oxygen and hydrogen peroxide).

The system of protection of membranes from peroxidation is multilevel, with participation of selenium (Elendt, 1990): it comprises superoxide dismutase, glutathione peroxidase, and catalase; at the second level, it comprises vitamin E and lipid antioxidants.

There is a diurnal variation in the activity of *Daphnia* antioxidant enzymes (catalase, GST, and superoxide dismutase) (Borgeraas and Hessen, 2002b). Superoxide dismutase and catalase demonstrated diurnal variations in activity, with a maximum value at midday. In arctic melanic *Daphnia tenebrosa*, however, diurnal fluctuation was insignificant. Hyaline *Daphnia* showed higher GST and superoxide dismutase activities, but lower catalase activity in comparison with a melanic type. GST activity in *Daphnia carinata* decreased with age, as well as there was a sharp decline in GST activity toward *p*-nitrophenzyl chloride (Guo and Xie, 2011). Toxicity to *D. magna* of 1-octyl-3-methylimidazolium and methylimidazolium bromide affects the antioxidant system increasing activities of antioxidant defense enzymes (superoxide dismutase, catalase, glutathione peroxidase, and GST) (Yu et al., 2009).

The level of catalase in *D. magna* increased at higher temperatures; presence of ectoine in the medium moderated the catalase concentration (Bownik et al., 2014). The latter was explained as a manifestation of antioxidative properties of ectoine during hypertemia.

Furuhagen et al. (2014) measured the level of antioxidant capacity in *D. magna* as oxygen radical absorbance capacity and lipid peroxidation (assayed as thiobarbituric reactive substances). Assuming that caloric intake increases production of reactive oxygen species, the authors inhibited the feeding rate by haloperidol and lindane. There was a positive relationship between the level of oxygen radical absorbance capacity and feeding rate in *D. magna* exposed to haloperidol and lindane.

The antioxidant systems protect *Daphnia* against *Microcystis* toxins (Wojtal-Frankiewicz et al., 2013, 2014). These authors found lower activity of catalase and lower lipid peroxidation in *Daphnia longispina* in places with higher concentrations of *Microcystis* (and thus of microcystins). Presence of detoxification was also evidenced by the low glutathione concentration and the highest activity of GST in *Daphnia*.

Humic substances reduce antioxidant activity in *D. magna* (Steinberg et al., 2010).

Exposure of *D. magna* to TiO_2 nanoparticles led to accumulation of nanoparticles in the intestine followed by increased activities of antioxidant enzymes—catalase, glutathione peroxidase, and GST (Kim et al., 2010).

Acetylsalicylic acid caused the decrease in activity of superoxide dismutase and glutathione peroxidase, but increased activity of catalase in *D. magna*, thus inducing oxidative stress (Gómez-Oliván et al., 2014).

Cu_2O nanocrystals showed oxidative stress, octahedral crystals a higher one than cubic crystals; exposure of *D. magna* to them was followed by antioxidant response, antioxidant inactivation, and oxidation inhibition; toxicity caused by reactive oxygen species was due to accumulation of malondialdehyde (Fan et al., 2013).

9.4 EFFECTS OF TOXIC COMPOUNDS ON CYTOLOGY AND METABOLIC FACTORS

When investigating the lethal effect of solutions of NaCl and $HgCl_2$ on *Daphnia*, *Simocephalus*, and *Sida*, Beklemishev (1923a,b) found that the effect described a curve, with either one peak or two or more peaks. The latter was observed under conditions when some specimens survived and then suffered slower intoxication. This indicates that several physiological mechanisms are affected by the toxic agent.

The lethal concentrations are different for different substances, and can sometimes be very low. It has been shown that 100% of *Chydorus sphaericus* (Smirnov, 1971, 1974) perish (i.e., 100% lethal dose, or LD_{100}) within 24 h in a 0.00001% solution of $AgNO_3$ (precipitating mainly on the epipodites), but they can survive in a 0.0000003% solution. They also died in a 0.0005% solution of potassium dichromate, but survived in a 0.0001% solution; and died in 0.004% neutralized formalin, but survived in a 0.001% solution. In solutions of most other salts tested, death occurred at concentrations 0.5% or higher.

A series of similar measurements was made with *D. magna*: Ag, Cu, and Hg caused a loss of ions in this *Daphnia* species, for example, of Na^+ ions (Holm-Jensen, 1948). Prior to their death, Na^+ concentrations were reduced to one-third of the normal value. It is thought that this situation is caused by inhibition of the mechanism responsible for the active uptake of ions. Toxic effects could be eliminated by the addition of glutathione or (for Ag and Cu) of cysteine.

D. magna exposure to metals led either to an increase or decrease in the protein content of the body; the maximum reduction of total protein (9%) occurred upon exposure to Ni, and the maximum increase (40%) upon exposure to Mg (Biesinger and Cristensen, 1972).

In a lake with an elevated content of Cu, Ni, and Al, *Holopedium* had lower lipid content and lipid droplets in the body were smaller by 21%, compared with those in an unpolluted lake (Arts and Sprules, 1987). In the presence of $CuSO_4$, the excretion of amino acids to the external environment by *D. magna* was noticeably increased (Gardner and Miller, 1981).

Mercury is thought to produce soluble albuminates that may penetrate deeper into tissues. In addition, mercuric chloride ($HgCl_2$) has proven to be highly toxic to *Simocephalus*; the actual killing rates were determined at concentrations ranging from 0.5 M to 50 μM (Breukelman, 1932).

Gut diverticula (hepatic ceca) are the organs that are principally attacked by xenobiotics. In *D. magna* exposed to 12–52 μg Cd^{2+}/L, the gut diverticula became shrunken and paralyzed, and Cd granules were found in the mitochondria and microvilli of the distorted ceca (Griffiths, 1980). Sublethal solutions of $CoCl_2$, $NiCl_2$, $Al_2(SO_4)_3$, and $Cd(NO_3)_2$ cause degeneration of the hepatic ceca in *D. magna* (Luzgin, 1982a). Degradation starts from the apex of the cecum, thus indicating that this is the site of the most intensive assimilation of salts. Exposure of *D. magna* to sodium selenate (Johnson, 1989) also leads to Ca deposition in the mitochondria of hepatic ceca.

The impact of 3-amino-1,2,4-triazole (a herbicide) on newborn *Daphnia* was investigated by Schultz and Kennedy (1976a). Young *Daphnia* are immobilized at a concentration of 0.1 mg/L; immobilization of all individuals occurs after about 22 h. The length of exposure required to reach immobility is decreased in animals approaching ecdysis (molting). In particular, mitochondria, especially those of muscle cells, are affected. Other effects included tissue swelling, myofilament disarrangement, and dissociation of membranes. In *Daphnia*, Cu^{2+} and triphenyltin chloride cause chromosomal aberrations in cells of the intestines, and especially

in cells of the embryos (Filenko and Lazareva, 1989). At chronic levels of exposure, Zn accumulates mainly in cellular organelles and in heat-stable protein fractions in *D. magna* (Wang and Guan, 2010).

In *D. magna*, the toxic effect of phenanthrene and 9,10-phenanthrenequinone (PHQ) (polycyclic aromatic hydrocarbons) was tested both with and without Cu (Xie et al., 2006). Copper (Cu) and PHQ generated reactive oxygen species, and this process involved mitochondria. The proposed pathways for Cu cycling and the roles of Cu are shown in Fig. 9.1. In living *D. magna* cells, Cu^{+} or superoxide dismutase may reduce superoxide radicals to hydrogen peroxide and cause oxidative damage (Xie et al., 2006). At exposure of *D. magna* to diclofenac, formation of heat-shock protein (hsp)-70 was studied as a biomarker for proteotoxicity (i.e., toxicity caused by proteins) (Haap et al., 2008).

9.4.1 Disturbances to Enzyme Activity

Natural Toxicity

Microcystin-LR, a toxin from cyanobacteria, is a potent inhibitor of the protein phosphatase activity in *D. pulex* and *Daphnia pulicaria*, that of *D. pulex* was less sensitive (DeMott and Dhawale, 1995).

Effect of Xenobiotics

Exposure of *D. magna* to metals (Biesinger and Cristensen, 1972) leads to either an increase or a decrease in glutamic oxaloacetic transmutase activity within the body; the maximum decrease (26%) occurred upon nickel exposure, and the maximum increase (100%) upon exposure to Mn (a 65% increase occurred following exposure to Mg). Exposure of *D. magna* to xenobiotics inhibits enzyme activity; copper (II) chloride ($CuCl_2$) inhibition of succinate dehydrogenase activity occurs long before a lethal effect is observed (Luzgin, 1982b). At 0.05−0.1 mg/L, copper increases in *D. magna* the activity of glutamate oxaloacetate transferase but decreases the activity of glutamate pyruvate transferase and acid phosphatase (Khangarot and Rathore, 2003).

Parathion (at concentrations of 0.05−5 μg/L) (Dortland, 1978); and the surfactants dodecylbenzyl sulfonate and sodium dodecyl sulfate inhibit AChE (the enzyme that hydrolyzes acetylcholine, a neurotransmitter) (Guilhermino et al., 2000). AChE activity is inhibited by lead (Pb), sodium dodecylbenzene sulfonate, mixtures containing these components, and, especially, by crude oil (Martinez-Tabche et al., 1997). *D. magna* AChE is also inhibited in vivo by 48-h exposure to the median lethal concentration (LC_{50}) of sodium dichromate and sodium molybdate (Diamantino et al., 2000). Both substances inhibit growth and reproduction, with sodium dichromate being the more toxic.

Cholinesterases from *D. magna* are noticeably inhibited by zinc (Zn) (Diamantino et al., 2003). Cholinesterase (ChE) inhibition in *D. magna* by parathion, dichlorvos, and aldicarb [2-methyl-2(methylthio)propioaldehyde-*O*-(methylcarbamoyl)oxime] was studied by Sturm and Hansen (1999) and by paraoxon methyl (an organophosphate) by Duquesne and Küster (2010). The presence of pesticides inhibits ChE in a dose-dependent manner; parathion is efficient at 0.1 μg/L, dichlorvos at 1 μg/L, and aldicarb at 100 μg/L. There was a significant recovery after 24-h detoxification in a clean medium (Duquesne and Küster, 2010). The activity of ChE was suggested, with reference to *Pseudosida ramosa*, as a measure of toxicity (Freitas et al., 2014).

Anticholinesterase compounds are present in wastewater and, being physiologically active, represent dangers for animal and human health. They are released into the environment as final waste products from agricultural, medical, and military enterprises or because of accidents. Tonkopiy et al. (1993) investigated the cholinergic system of *D. magna* by applying various

anticholinesterase compounds, including reversible inhibitors, organophosphates, and carbamates. Anticholinesterase compounds increased the toxic effect of the myorelaxant ditilin. These authors demonstrated that central *m*-cholinolytics reduce the toxicity of armine and aminostigmine.

AChE is inhibited by physostigmine. AChE and carboxylesterase (CbE) inhibition by organophosphorus pesticides (malathion and chlorpyrifos) and carbamate pesticides (carbofuran) was assessed in *D. magna* by Barata et al. (2004). CbE is more sensitive than AChE to organophosphorus pesticides, but both are equally sensitive to carbofuran. Mortality increased at low levels of AChE inhibition by carbofuran, whereas upon exposure to organophosphorus pesticides mortality increased when the level of inhibition was >50%.

Glutathione is a thiol-containing tripeptide (γ-glutamyl-cysteinyl-glycine), which functions as a reducing agent in cells (Elliott and Elliott, 1997). It is found in *D. magna* cells and its concentration is reduced by treatment with acridine, 1,10-phenathroline, benzo(a)quinoline, phenantridine, and phenazine (*N*-heterocyclic polyaromatic hydrocarbons) (Feldmanová et al., 2006). Following an initial 10-h exposure, malathion decreased both protein content and AChE activity in *Simocephalus vetulus*; lipid content was reduced after 24-h exposure, but lipid peroxidation levels increased over the whole exposure period (4−50 h) (Olvera-Hernández et al., 2004). AChE activity decreased after exposure of *D. magna* to deltametrin, thus it may be used as a biomarker for deltametrin (Toumi et al., 2015). Strains of *D. magna* were differently sensitive to deltametrin.

Chitobiase, liberated in the process of molting, was affected by pharmaceuticals (atorvastatin, lovastatin, fluoxetine, sertraline) in *D. magna* (Richards et al., 2008).

Methoprene (a juvenile hormone agonist) is toxic to endocrine-related processes, that is, growth, molts, and fecundity, in *D. magna* (Olmstead and LeBlanc, 2001a,b).

Pyruvate kinase and malate dehydrogenase were inhibited after a 7-day exposure of *D. magna* to 0.05 mg/L 3,4-dichloroaniline (Morgado and Soares, 1995). However, after exposure for 14−21 days, the activities of these enzymes were stimulated relative to control. *D. magna* steroid hydroxylase is differentially modulated by exposure to the toxicants phenobarbital, β-naphthoflavone, piperonyl butoxide, and malathion (Baldwin and LeBlanc, 1994b). *D. magna* exposure to 811 μg/L di-2-ethylhexyl phthalate for 21 days resulted in decreased glycogen content (Knowles et al., 1987).

A polycyclic aromatic hydrocarbon, pyrene is transformed in *D. magna* with the participation of cytochrome P450 monooxygenase (Akkanen and Kukkonen, 2003), whereas the presence of piperonyl butoxide inhibits pyrene biotransformation.

Exposure of *D. magna* to 60 μg/L chlordecone for 7 days resulted in a reduction in RNA levels from 20 μg to c. 9 μg per individual (McKee and Knowles, 1986). The DNA concentration decreased from c. 0.6 μg to c. 0.2 μg per individual, respectively; and adenosine diphosphate (ADP) and adenosine triphosphate (ATP) concentrations decreased after longer exposure times.

Enzyme activation by xenobiotics may also occur under certain conditions. Carbohydrases (estimated by amylolytic activity and saccharase activity) in *D. magna* were activated by the herbicide Roundup (glyphosate) in vitro at concentrations up to 50 mg/L (Filippov et al., 2010). Upon exposure to Roundup, the overall proteolytic activity of *D. magna* increases and the overall amylolytic activity decreases (Papchenkova et al., 2009); this was interpreted to indicate an increased role for proteins in metabolism at sublethal concentrations of Roundup. No adaptation was observed over four generations.

The impact of cadmium (Cd) on delta-aminolevulinic acid (ALA-D) synthesis, an early step in the biosynthesis of heme (porphyrin), was tested by Berglind (1985). *D. magna* was exposed to cadmium (as $CdCl_2.2.5\ H_2O$) in

concentrations of 0, 0.1, 0.2, 0.4, and 1.6 μg Cd/L; ALA-D concentration fluctuated around the control values. However, after 16 days the hemoglobin (Hb) content decreased to 31–80% of the control. Further experiments (Berglind, 1986) were made to test the effect of Cd (at concentrations of 0, 0.2, and 2.0 μg/L) and other heavy metals, both separately and in combinations. Pb (in concentrations of 0, 0.26, and 260 μg/L) was added in the form of lead acetate $Pb(CH_3COO)_2$ and Zn (0, 020, and 200 μg/L) as zinc sulfate ($ZnSO_4.7\ H_2O$). ALA-D activity was enhanced by Cd and by high concentrations of phosphorus (P)+Zn, but inhibited by Pb, Pb + Cd, Pb + Zn, and Pb + Cd + Zn. Stimulation by Cd was abolished by Zn. Hb content did not decrease, even at 260 μg/L Pb. Estimated by immobilization, in seven clones of *D. magna* the sensitivity to Cd was the highest in clones displaying the lowest hsp-70 expression (Haap and Köler, 2009).

Fan et al. (2009) demonstrated that at increasing concentrations of Cd and Zn in the environment the body burden increased, as shown with reference to *D. magna*, followed by increased concentration of metallothioneins, as a part of detoxification mechanism. Activity of superoxide dismutase increased at low concentrations and increased at higher concentration of Cd and Zn. *D. magna* lactate dehydrogenase activity is noticeably inhibited by Zn (Diamantino et al., 2001).

The effect of 48-h menadione, paraquat, endosulfan, Cd, or Cu exposure on *D. magna* enzymes varied (Barata et al., 2005). A low response of antioxidant enzymes to menadione and endosulfan was observed to correlate with increasing levels of lipid peroxidation; antioxidant activities were enhanced by paraquat; there was a low antioxidant enzyme response to Cd; and high levels of both antioxidant enzyme activities and lipid peroxidation were induced by Cu. The most responsive biomarkers of oxidative stress were glutathione peroxidase, catalase, and GST (the key antioxidant enzymes). The toxicity of strophanthin for *D. magna*, determined by the median lethal dose (LD_{50}), was found to be different in different seasons, being highest in January–February and lowest in October–November (Rautenberg, 1953).

Exposure of *D. magna* to bisphenol A (an endocrine disruptor) increased GST activities measured with 1,2-chloro-4-dinitrobenzene as a substrate (Jemec et al., 2012).

The biochemical mechanism of resistance of clones of *D. magna* to fenitrothionin (an organophosphate insecticide) was studied by Damásio et al. (2007). The studied clones found sixfold difference in their resistance to fenitrothionin. These authors concluded that mixed-function oxidases play a principal role in bioactivation of fenitrothionin into its active oxon metabolite. Inhibition of carboxyl esterase and GST proved that their role is minor.

In *M. macrocopa,* following the increase of phenol concentration, the activity of lactate dehydrogenase gradually increased, whereas the activities of pyruvate kinase, succinate dehydrogenase, and glutamic pyruvic transaminase little changed; the activity of glutamic oxaloacetic transaminase after 24 h increased and then decreased (Wang et al., 2010a,b). Overall, these changes indicate to some shift from carbohydrate catabolism to protein catabolism.

D. magna β-galactosidase activity is decreased in the presence of fluoranthene (a phototoxic polycyclic aromatic hydrocarbon) following ultraviolet illumination (Hatch and Burton, 1999).

A sulfonamide antibiotic sulfathiazole in presence of UV-B caused in *D. magna* increased generation of reactive oxygen species (what caused phototoxicity), lipid peroxidation, concentration-dependent increase of catalase and of GST (Kim et al., 2009).

The activity of *D. magna* heme peroxidase is significantly increased in kerosene-contaminated groundwater (Connon et al., 2003).

In addition, the inhibition of *Daphnia* enzymes by pollutants, seen as reduced fluorescence, has been suggested as a sensitive toxicity test (Kubitz et al., 1995).

Daphnia exhibit a three-phase attraction or repulsion effect in response to coumarin or copper (II) sulfate ($CuSO_4$) (Heintz, 1964); the animals are repulsed at low and high concentrations, and attracted at intermediate concentrations. Maximum attraction occurred at 10 ng/L for coumarin and 600 mg/L for $CuSO_4$.

Difference in reaction of *Daphnia* of different age was found. Older *Daphnia* were less capable to deal with a prooxidant [Cr(VI) was tested as such] (Arzate-Cárdenas and Martinez-Jeronímo, 2011; Arzate-Cárdenas et al., 2011) due to decreasing antioxidant activity of their enzymes—superoxide dismutase, catalase, glutathion peroxidase, and glutathion reductase.

9.4.2 Disturbances to Endocrine Activity

Exposure to xenobiotics causes changes in metabolic processes. It was shown with reference to *D. magna* that at exposure to 4−128 μg triclosan/L the activity of superoxide dismutase appeared after 24 h but was inhibited after 48 h; the content of malonaldehyde and of 7-ethoxyresorufin 0-deethylase increased after 6 h and decreased after 48 h (Peng et al., 2013).

Methoprene (insecticide) affects the endocrine-related processes in *D. magna* at 5−50 nM concentration range (Olmstead and LeBlanc, 2001a). Molt frequency and the time of first brood deposition were reduced in a concentration-dependent manner.

With reference to *Diaphanosoma celebensis*, the deleterious effect of endocrine-disrupting substances was shown (of testosterone, 17β−estradiol, a herbicide mefenacet, and six other herbicides) (Marcial and Hagiwara, 2008).

9.5 DETOXIFICATION

Cladocera liberation from a toxic substance may result from depuration in a clean environment or of targeted metabolic processes. GSTs, the enzymes that participate in chemical detoxification, have been isolated from *D. magna* (LeBlanc et al., 1988; Baldwin and LeBlanc, 1996). *D. magna* GSTs are inhibited by 1,4-benzoquinone and 2,4-dichlorophenoxyacetic acid (Dierickx, 1987a). The presence of five major GSTs has been demonstrated in *D. magna*, as has detoxification of 1-chloro-2,4-dinitrobenzene (CDNB) (Dierickx, 1987b). Agents that inhibit or increase the GST content either increase or decrease CDNB toxicity, respectively. Pentachlorophenol is metabolized in *D. magna* by sulfate conjugation; it is then excreted at a rate of 2.65 nmol/g/h (Kukkonen and Oikari, 1988). In benzo(a)pyrene depurated from *D. magna*, 0.1 of its initial quantity remained after 40 h (McCarthy, 1983).

Detoxification of tannins, which are thought to be ingested with algal food, was studied in *Daphnia* and *Simocephalus* and found to be carried out by special enzymes (cytochromes P450, esterases, and GSTs) (Ray et al., 2000).

Cytochrome P450 enzymes may contribute to detoxification and acclimation of *D. magna* to chronic toxaphene exposure, as cytochrome P450 inhibition by piperonyl butoxide led to a decline in growth rate, fecundity, and survival (Kashian, 2004). Cytochrome P450 plays a major role in biotransformation of diazinon (insecticide) in *D. magna* (Kretschmann et al., 2011), the dominant elimination step being oxidative dearylation to pyrimidinol. When cytochrome P450 was inhibited the bioconcentration factor of diazinon much increased.

With reference to *Daphnia similis* exposed to a disperse azo dye (red I), it was shown (Yu et al., 2015) that detoxification proceeds in two phases: phase I with participation of cytochrome P450 and phase II with participation of GST. Upon exposure to disperse red I, GST activity increased. It was assumed that GST is involved in disperse red I metabolism and cytochrome P450 activity is necessary to induce GST activity.

On the other hand, there is no evidence of biotransformation by *Daphnia* of cyanobacterial

constituents (aerocyclamides B-D, cyanopeptolins A-C, microcylamide 7806A) (Sadler and von Elert, 2014a). The same authors (Sadler and von Elert, 2014b) point out that in *Daphnia* GST is involved in an oxidative stress response, not in the detoxification of microcystins.

Metallothioneins. Metallothioneins (special soluble proteins, the *proteins of detoxification*) with a molecular weight of c. 10 kDa are found in *Daphnia*. It was found that over 80% of the Cd burden in the body of *D. pulicaria* grown in Cd-containing water is bound by this 10-kDa protein (Gingrich et al., 1984). A similar percentage (up to 75%) of the Cd burden in the body of *D. magna* was also determined to be bound to metallothioneins; increased Cd in daphnids induces subcellular reorganization of essential metals (Cu and Zn), and a higher content of these metals in the soluble cellular fraction (Fraysse et al., 2006).

CHAPTER

10

Growth and Molting

10.1 SIZE AND WEIGHT CHARACTERISTICS

Most Cladocera species are small: from about 0.3 mm to c. 6 mm. One of the largest, *Leptodora*, is c. 10 mm in length. Due to their small size, their surface-to-volume ratio is high.

Several authors have investigated the length-to-weight ratios [e.g., Ivanova and Klekowski, 1972 (*Simocephalus*); Kawabata and Urabe, 1998]. The relationship between length (L) and wet weight (W) may be described by Eq. (10.1) (Kurashov, 2007):

$$W(\text{mg}) = qL^{b}(\text{mm}), \tag{10.1}$$

in which q is 0.133 for *Scapholeberis*; 0.075 for other Daphniidae, 0.127 for *Eurycercus*; 0.091 for *Alona* and *Alonella*; 0.203 for *Chydorus*; 0.083 for *Macrothrix*; and 0.140 for other Chydoridae and Macrothricidae. Variable b is 2.630 for *Scapholeberis*; 2.925 for other Daphniidae; 3.076 for *Eurycercus*; 2.646 for *Alona* and *Alonella*; 2.771 for *Chydorus*; 2.331 for *Macrothrix*; and 2.723 for other Chydoridae and Macrothricidae.

Similar equations were determined by Lynch et al. (1986):

for *Daphnia ambigua*, $W = 5.740\ L^{2.370}$;
for *Daphnia galeata mendotae*, $W = 5.480\ L^{2.200}$;
for *Daphnia parvula*, $W = 4.740\ L^{2.190}$; and
for *Daphnia pulex*, $W = 10.674\ L^{2.093}$.

Dry weight (DW) increases with length, as shown for *Daphnia hyalina* (Baudouin and Ravera, 1972). The relationship between DW (in mg) and length (L, in mm) was determined for *D. pulex* (Richman, 1958) to be Eq. (10.2) or for *Daphnia magna* (Porter et al., 1982) Eq. (10.3):

$$DW = 0.028\ L^{-0.022} \tag{10.2}$$

$$DW = 0.0119\ L^{2.3907} \tag{10.3}$$

and by Wen et al. (1994), for *Daphnia*, *Simocephalus*, *Ceriodaphnia*, and *Bosmina* combined, to be Eq. (10.4):

$$DW = 0.013\ L^{2.14} \tag{10.4}$$

Regression equations for DW and length for many planktonic and littoral species were supplied by Dumont et al. (1975), for five Amazonian species by Maia-Barbosa and Bozelli (2005), and for three species from Mexico by Nandini et al. (2005).

Weight at a certain length of Cladocera was also determined and presented in tabulated form by Mordukhai-Boltovskoi (1954), Kosova (1961), and Sokolova (1974). In addition, Vasama and Kankaala (1990) reported length–carbon regressions in Cladocera.

10.2 GROWTH

Increases in the body length of Cladocera principally occur between molts. For example,

http://dx.doi.org/10.1016/B978-0-12-805194-8.00010-6

within 10 s after molting, a *D. magna* was observed to increase in length from 1.3 to 1.6 mm (Green, 1956a). Newborn Cladocerans grow rapidly. Their growth during prereproductive instars is linear, and then it becomes slower, especially in species that bear two parthenogenetic eggs. Their growth slows down upon reaching maturity. In mature chydorids, for example, increments in length become very small; in contrast, in mature daphnids slow growth continues (Zaffagnini, 1964; Smirnov, 1971). Information on Cladocera growth was summarized by Frey and Hann (1985). In general, growth is accelerated at higher temperatures and food concentrations but is retarded at lower temperatures, low food concentrations, moderate illumination, and illumination with green light (Buikema, 1972). Within temperature range 5−20°C, maturation in *Daphnia* sp. sp. is much sooner attained at higher temperatures (Geller, 1987).

It was shown that environmental chemicals modify life−history parameters in *Daphnia*. Growth of *D. pulex* is retarded in the presence of chemicals produced by plants and it attains maturity later if exposed to vegetation (*Elodea*) (Burks et al., 2000).

A significant relationship was determined between somatic growth and aminoacyl- transfer RNA (tRNA) synthetase activity, both in terms of protein and dry weight (Yerba and Hernández-León, 2004).

10.2.1 Life Span

The life span of Cladocera species can reach several months. There are few experiments on lifelong cultivation of Cladoceran species and, accordingly, recording of events as Life Tables; obviously as this task is time-consuming. An early example is recording of life events in females of *Simocephalus* (20 generations, up to 2.5 months) and *Moina* (10 generations, up to 25 days) by Papanicolau (1910).

For littoral species the following individual life span (days) is indicated: *Pleuroxus denticulatus*—121 (Shan, 1969), *Alona costata*—140 (Smirnov, 1971), *Euryalona orientalis*—24, *Latonopis australis*—46, *Iyocryptus spinifer*—31, *Leydigia ciliata*—46, *Pseudosida bidentata*—34 (Venkataraman, 1990), nonswimming *Bryosplilus repens* — over 89 (Frey, 1980). In culture, *Macrothrix triserialis* attained 22 days (Venkataraman, 1990) or 50 days (Muro-Cruz et al., 2002), *Macrothrix flabelligera*—12.7 days (mean) (Güntzel et al., 2003) or 13 days (mean) (Huang et al., 2011), *Macrothrix rosea*—39 days (Huang et al., 2011). The latter authors indicate mean life span of 68 days for *D. magna*.

Bottrell (1975b) cultivated Cladocera at four levels of temperature and found length of life at 10°C to be (days) in *Alona affinis* 90, *Chydorus sphaericus* 59, *Eurycercus* 105, *Graptoleberis* 54, *Pleuroxus uncinatus* 79, *Sida* 74, and *Simocephalus* 74. Life duration somewhat decreased at higher temperatures and increased at lower temperatures.

For pelagic species, maximum life duration was reported as 182 days (Fritsch, 1953) or 57 days (25 instars) (Venkataraman, 1990). *Daphnia pulex* lived for 25 instars (Anderson et al., 1937), *D. magna*—for 17 instars on an average (40 days) (Anderson and Jenkins, 1942), *Daphnia similoides*—up to 45 days, *Ceriodaphnia affinis*—up to 75 days (Gershkovich and Isakova, 2013), *Simocephalus acutirostratus*—up to 44 days (22 instars) (Murugan and Sivaramakrishnan, 1973).

Much shorter life spans have been recorded by various authors for *Moina macrocopa*: a maximum of 14 days (Terao and Tanaka, 1928a), 11 days (Razlutskii, 1992), 32 days (Burak, 1997), 15 days (Garcia et al., 2004), 9 days (Sushchenya et al., 1990; Voronin and Makrushin, 2006; Makrushin, 2011), and an average longevity of 12.5 days (Chuah et al., 2007). The maximum life span in culture was indicated for *Moina weismanni* as 17 days (Venkataraman, 1990) and for *Moina rectirostris* (syn. *Moina brachiata*) as 28 days (Razlutskii,

1992). It may be reminded that most of *Moina* species are highly prolific.

In ctenopods, the maximum life duration is between 19 and 74 days, as summarized by Korovchinsky (2004).

The life span of *D. magna* is prolonged by heparin (Schechter, 1950). Interestingly, vitamin K reduces the life span of *D. magna* at a concentration of 400 μg/L, whereas it is somewhat stimulatory at a concentration of about 200 μg/L (Schechter, 1950). The longest life span of *Daphnia longispina* was recorded when calcium pantothenate was added to the culture medium (Ingle et al., 1937).

Meijering (1958a,b) suggested measuring the life span in heartbeats; *D. magna* reached 47 million heartbeats at 30 instars (about 65 days). In another communication, 50% of those that survived were reported to have had about 40 million heartbeats (Meijering and Redfern, 1962). About 8000 heartbeats occur between molting and the liberation of eggs into the brood pouch, according to Meijering (1960). Fritsch (1953) recorded that *D. magna* males live for about 30 days and have 19.1 million heartbeats.

Naturally, the longevity of any species depends on its living conditions, including food and temperature. The life span of *Daphnia* spp. decreases under conditions of high temperature (Green, 1957b; Armitage and Landau, 1982) and oxygen deficit (MacArthur and Baillie, 1929; Zhukova, 1955). At comparatively low temperatures (8−10°C), which slow down metabolic processes, the life span of *D. magna* is greatly increased (as determined by MacArthur and Baillie, 1929): it is 25 days at 28°C, 42 days at 18°C, 88 days at 10°C, and 108 days at 8°C. Female *D. longispina* can live for up to c. 66 days (1600 h) at 20°C, but for 234 days at 5°C, whereas male *D. longispina* lived up to 50 and 170 days, respectively, at the same temperatures (Munro and White, 1975).

It has been shown that other suboptimum conditions may also prolong the life span of *Daphnia* (Ingle, 1933; Green, 1957b). Limited food contributes to increased life span in *Daphnia* (Ingle et al., 1937; Hirosaki, 1953; Skadovskiy, 1955; Sterba, 1956b; Steinberg et al., 2010) and *Simocephalus* spp. (Hirosaki, 1953). Ingle et al. (1937) demonstrated that *D. longispina* live for 40% longer under conditions of minimum food compared to well-fed specimens. Starved *Daphnia* produced fewer young, but the total number of young produced is nearly identical in poorly fed (which live longer) and well-fed (with a shorter life span) *Daphnia*.

Extreme environmental conditions can reduce life span. For example, the mean life span of the low-salinity species *Diaphanosoma celebensis*, decreased from 24 to 5 days at a sea-water salinity of 30 psu (practical salinity units; undiluted seawater was 32 psu) (Achuthankutty et al., 2000). That of *D. magna* decreased from 56 days in fresh-water to 26 days at 6.6 g NaCl/L, and fecundity decreased (Martínez-Jerónimo and Martínez-Jerónimo, 2007).

Arctic *Daphnia* allocate more P to DNA (and also are more polyploid) than *Daphnia* of temperate latitudes (Van Geest et al., 2010), thus the former maximize their protein synthesis and growth and overcome brevity of the growing season.

10.2.2 Mortality

Mortality is caused by natural aging (see Section 10.6), diseases, or extreme level of natural or anthropogenic factors. Lethal limits of various substances were reported for *D. magna* by many authors (e.g., Dowden, 1961, 1962), for *Ch. sphaericus* by Smirnov (1971). Standard studies of sets of substances demonstrated different comparative toxicity of particular substances. Thus of 13 inorganic salts tested on *D. magna* by Dowden (1961) 24 h Median Tolerance limit of sodium sulfate was 8384 ppm, sodium nitrate was, 5980 ppm, sodium bisulfite was, 179 ppm, sodium dichromate was 22.43 ppm (as corrected by Dowden in his publication). These values were lower in case of 48 h exposure.

Gainutdinov et al. (1997b) found that the presence of sugars in the surrounding water, which is

controlled by algae, modifies *D. magna* mortality by a mechanism involving sensory cells, amplification of the signal, and modification of neuroendocrine units in the nervous system.

Mortality unrelated to predation and related to an unidentified infection that caused a 10% daily loss of *D. hyalina* was reported for Lake Constance (Germany) (Gries and Güde, 1999).

10.3 MODIFICATION OF FORM

There is sometimes a significant difference in the body forms of adults and juveniles of the same species, e.g., in some macrothricids. Adults of the same species of Cladocera are also variable in form; striking examples are species of *Daphnia* and *Bosmina*, which show numerous phenotypes. This morphological plasticity affects the general form, the body size, and the development of dorsal and posterior spines. It is shown with reference to *D. magna* that illumination influences the effect of kairomones: the kairomone-induced reduction in size at first reproduction is inversely related to the light intensity (Effertz and von Elert, 2014).

Previous reports have frequently investigated these morphological changes, but the physiological prerequisites for such modifications of form are currently unknown. Endocrine regulation is involved, as it is shown that the predator-induced morphological plasticity is regulated by juvenoid hormone (Dennis et al., 2014).

There are also malformations of various natures (Rammner, 1930) and mechanical damage inflicted, e.g., by predators. Cyanobacterial toxins were shown to cause malformation of neonates of *D. magna* (Dao et al., 2010).

10.3.1 Mechanical Damage and Regeneration

Mechanical Damage

Both littoral and planktonic Cladocera suffer mechanical damage (Rammner, 1930; Smirnov, 1971). Jermakoff and Ermakov (1927) collected a sample of *D. pulex* and *D. magna* in which about 5% of the specimens had damaged antennae or anomalous regenerated structures. In *Sida* (females), the percentage of damaged specimens can reach 25% (Jermakoff and Ermakov, 1929) or even 30% (Korovchinsky, 2004). Those living on substrata are well protected from mechanical damage by their comparatively thick carapaces, and their body form is modified to enable their penetration of various littoral obstacles. The thickest carapace is probably that of *Pseudochydorus globosus*, which can be up to 12 μm (Fryer, 1968).

Turbulence

With respect to modification of the general *Daphnia* form, according to Hutchinson (1953, p. 155), turbulence provides "a continual random stimulation of the sense organs." Hutchinson continues (1953, p. 156) that "there are an enormous number of interconnected internal things." However, the physiological mechanisms by which external turbulence modifies the body form are currently unknown. With moderate turbulence, the neonates of *Daphnia cucullata* developed large cephalic helmets at their older age (Laforsch and Tollrian, 2004a).

Wave beating kills a noticeable fraction of *Chydorus*, *Daphnia*, and *Bosmina* according to Manuilova (1955). She also recorded that the ratio of living to dead *Chydorus sphaericus* reaches 2.9:1 after storms (Manuilova, 1956). *Daphnia* can perish within 1 day in shaken or intensively aerated cultures (Harvey, 1972). In a shaker (at 200 shakes/min in 60 mL of water), 50% of *D. pulex* perished within 6 h, and 50% of *Eurycercus lamellatus* died within 7 h (Smirnov, 1971).

Predation

Broken structures are frequently present, as well as wounds inflicted by predators. Characteristic wounds inflicted to the carapace of various Cladocera by the copepod *Acanthocyclops* are indicated by Li and Li (1979). Specimens

(e.g., of *Bosmina*, *Daphnia*, or *Ceriodaphnia*) with obvious signs of injury and wound healing, caused, for example, by predatory cyclopoids are frequently found in Cladocera samples (Kerfoot, 1975; Murtaugh, 1981). The proportion of damaged prey may be used as a measure of predation pressure, but the abnormalities can also stem from disturbances in embryonic development.

Regeneration

In the case of a wound inflicted on a *Daphnia* as a hole in the valve and followed by regeneration, a decreased hole size was seen in successive instars (Anderson and Brown, 1930; Anderson, 1933). Over the course of successive instars, the wound area decreases until it is completely closed. It was shown that phenoloxidase is involved in wound healing of the cuticle in *D. magna* (Mucklow and Ebert, 2003). A lost appendage is usually regenerated, although not completely. It has been found experimentally that missing (amputated) segments of an antenna may not regenerate; nevertheless, the setae do (Fig. 10.1) (Kuttner, 1913; Agar, 1930; Anderson, 1935). Remarkably, regeneration either restores the normal structure or may produce hypermorphoses, hypomorphoses (submorphoses), teratomorphoses, or dichotomy (as defined by Jermakoff and Ermakov, 1927, 1929) (hypermorphoses—more structural elements than originally were, etc.). Impressive examples of hypermorphoses in *Daphnia* were obtained experimentally by Kuttner (1913).

It may be added that experimental extirpation of the eye has mainly been successful (see Section 13.4).

These facts are important for understanding the morphological (morphogenetic) potential of body structures. Strictly speaking, there is no hard boundary between variation and teratology (i.e., formation of abnormal structures). According to Shimkevich (1909), the trends shown by anomalies and teratologies may be exploited in the normal morphogenesis.

Teratologies may easily be reproduced experimentally and the events that occur during subsequent moltings may be observed.

Malignant tumors have never been reported for Cladocera and never been seen by the author.

Clot Formation

Formation of a blood clot was observed by Jermakoff and Ermakov (1927, 1929) at sites

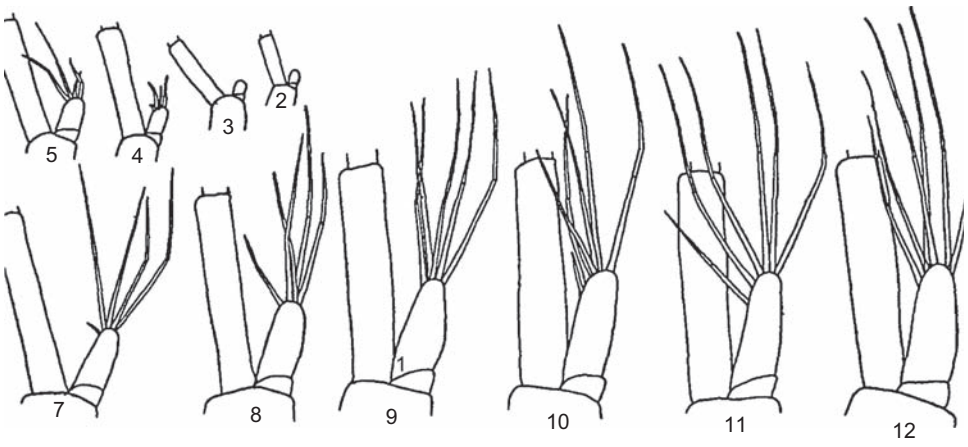

FIGURE 10.1 Antenna regeneration in *Daphnia* over successive instars. *From Agar, W.E., 1930. A statistical study of regeneration of two species of Crustacea. Journal of Experimental Biology VII (4), 349–369.*

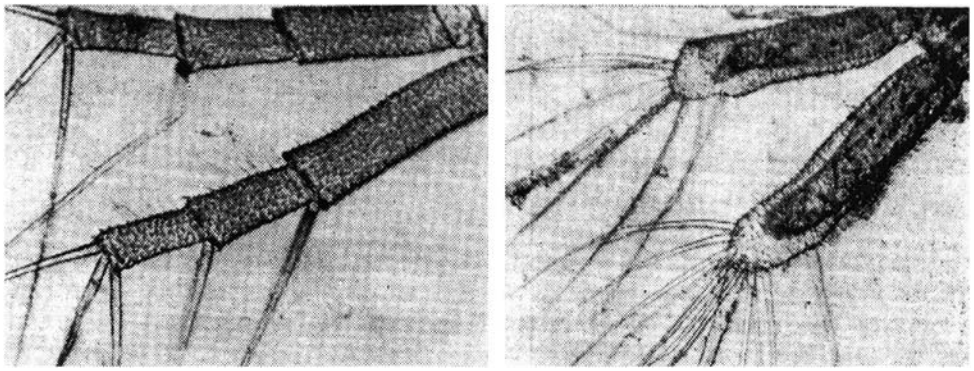

FIGURE 10.2 *Daphnia* antenna deformation in a toxic environment. Left, control; right, deformed by a toxic environment. *From Shcherban, E.P., 1980. Effect of the surfactant (R = C10–C13) on the major bioparameters and productivity of* Daphnia magna *Straus. Gidrobiologicheskiy Zhurnal 16 (2), 102–105.*

where an antenna or the shell had been dissected. Some blood initially flows from the wound, but then a clot is formed and hemorrhage stops. Most cladocerans can survive such operations. Within a few hours after an injury, the edge of the wound is surrounded by a brown material, which is assumed to be clotted blood with oxidized tyrosine (Anderson and Brown, 1930; Anderson, 1933).

10.3.2 Chemical Factors

The lowest calcium threshold for the growth of *D. pulex* juveniles was measured as c. 1.5 mg/L (Riessen et al., 2011). Early juveniles of *D. magna* (Hessen and Rukke, 2000a) and *Daphnia galeata* (Rukke, 2002) have especially high calcium requirements.

Somatic growth is constrained by food availability, including the availability of polyunsaturated fatty acids (Schlotz and Martin-Creuzburg, 2011). Cyanobacteria are characterized by a low content of eicosapentaenoic acid (20:5ω3) (Müller-Navarra et al., 2000) and a low sterol content (von Elert et al., 2003), which constrain the growth and reproduction of Cladocera.

D. magna growth is inhibited by the vertebrate antiandrogen, cyproterone acetate; this compound has no effect on molting and developmental parameters at concentrations up to 5 mM (which are nontoxic to *Daphnia*) (LeBlanc and McLachlan, 1999).

Chemomorphoses

The term *chemomorphosis* was suggested to describe morphological variation caused by exogenous chemical agents (Hebert and Grewe, 1985). It has been repeatedly reported that the formation of spined morphs of *Daphnia* (i.e., those with neck teeth) is caused by the presence of a predator (*Chaoborus*) and its excreted kairomones (Havel, 1985; Hanazato, 1991; Hanazato and Ooi, 1992; Hunter and Pyle, 2004). Both females and males of *Daphnia* develop larger protective helmets or dorsal denticles on their shells in medium enriched with an extract derived from predators (*Chaoborus* larvae or *Anisops*) (Grant and Bayly, 1981; Krueger and Dodson, 1981; Hebert and Grewe, 1985; Spitze, 1992; Hanazato, 1990, 1995; Hanazato and Ooi, 1992; Repka and Pihlajamaa, 1996). They also develop a cephalic spine and an elongated tail spine (Brancelj et al., 1996).

The morphology of various *Daphnia* sp. sp. has been shown to change under the influence of chemicals (kairomones) liberated into the environment by predators (e.g., invertebrates and fish) (Black, 1993; Larsson and Dodson, 1993; Tollrian,

1990, 1993, 1994, 1995). In *D. pulex* grown in the presence of predators, characteristics of their life cycle are also changed (Black, 1993), for example, including the comparatively rapid growth of juveniles. Generally, the effects include elongation of both cephalic helmets and the tail spine. This reaction also causes an increased rigidity of *Daphnia* carapace (Laforsch et al., 2004). Morphological changes induced in *Daphnia* by kairomones released by predators were stronger if the predators were fed with the same *Daphnia* species (Laforsch and Beccara, 2006).

While working with *Daphnia*, Jacobs (1980) concluded that there are specific growth determinants that preferentially act on mitotic rates within the Cladocera helmet. Helmet cells are not supplied with nerve fibers, and their growth determinants are carried in the hemolymph. Later, Beaton and Hebert (1994a,b, 1997) identified polyploid cells in the cephalic epidermis of *Daphnia*; the DNA content in these cells is higher than in the thoracic regions, and mitotic activity is concentrated around them. Thus, these cells are assumed to be developmental control centers that govern the shape of the head.

Morphological reactions to chemicals start in *Daphnia* embryos that have shed the third membrane; these have liberated chemosensilla that can detect chemicals (Laforsch and Tollrian, 2004b). However, morphological changes have been observed in the third instar in *D. cucullata* and *D. pulex*, but not in three other species. As well, Naraki et al. (2013) found that the kairomone-sensitive period in *D. pulex* is confined from late embryos to the postembryonic first instar; formation of neck teeth are formed due to proliferation of epidermal cells and modification of cuticle caused by kairomones.

A reverse morphogenetic process was discovered by Riessen (1984): loss of helmets was observed in generations of helmeted *Daphnia retrocurva* produced in the absence of predators and with abundant food. The opposite process, i.e., a reduction in tail-spine length, was also observed by Burns (2000), who cultivated nine species of *Daphnia* in water from crowded cultures of the same or another species. A reduction in body size and changes to the carapace morphology have been observed, including reduction in the tail-spine length (in *Daphnia lumholtzi* and *D. ambigua*). Kairomones or physical factors seem to either induce or inhibit the proliferation of tissues. Thus, identification of the chemical controlling factor(s) is the next priority.

However, identification of the chemical nature of this factor is easier than elucidating the actual pathway through which it effects changes in morphology. It is much more difficult to find out how proliferation is channeled to produce the modified structure. Barry (2002) applied substances to *D. pulex* that differentially affect neurotransmission, including those that either enhance (nicotine and physostigmine) or inhibit (atropine) cholinergic transmission; or stimulate (*cis*-4-aminocrotonic acid, diazepam, and muscimol) or antagonize [bicuculline, picrotoxin, and gabazine (SR95531)] the action of γ-aminobutyric acid (or GABA). Neck-teeth development was enhanced by physostigmine and picrotoxin but suppressed by atropine. It is thought that these compounds influence the cells that release hormones responsible for the development of neck teeth. In the first instar *D. pulex*, juvenoids (methyl farnesoate, fenoxycarb, and juvenile hormone III) stimulated formation of neck teeth; however, this happened only in the presence of kairomones released by *Chaoborus* larvae (Miyakawa et al., 2013).

Khlebovich and Degtyarev (2005) assumed that two alternative hereditary programs, corresponding to the typical and defensive (long-spined) forms, are present in *D.pulex*, as actinomycin D inhibits the transformation between these forms.

10.4 MOLTING

In Cladocera, molting (termed also ecdysis) and growth are inherently interconnected. The integuments of freshly molted specimens are

soft and thin and permit enlargement of the body. At molting, all integuments of the body, including those of its minutest parts, are removed. Thus, exuvia may provide a convenient material for the investigation of morphology.

The number of molts is likely to be different in different cladocerans. Molts may be numerous, reaching, e.g., 48 in *Acroperus* (Smirnov, 1965a). In Anomopods, an embryo undergoes a molt just after leaving the brood pouch (Kotov, 1997a,b). After this follow two molts (and accordingly two instars) prior to maturation in female chydorids and *Bosmina*, and up to six or more in daphnids and *Eurycercus* (Frey and Hann, 1985); and 2 to 6 molts in ctenopods (and even up to 10 in *Sida*) (Korovchinsky, 2004). In *D. magna*, five or more prereproductive molts have been observed (Anderson, 1932).

Juveniles are liberated from the brood chamber a few hours before molting, and eggs are released to the brood chamber a few minutes after molting (Rammner, 1929; Shan, 1969). However, molting also occurs in the absence of eggs or when eggs within the brood pouch decompose (Rammner, 1929). In some benthic forms, such as *Ilyocryptus*, *Monospilus*, and *Oxyurella*, valves are retained during molting in parthenogenetic specimens. However, prior to bisexual reproduction, the accumulated valves are discarded. *Daphnia obtusa* grown on severely phosphorus-limited green algae (*Scenedesmus*) are often observed with a postmolting exuvium attached to their posterior end (Sterner et al., 1993).

Cladocerans are attacked by various epibionts and endoparasites. Some of the epibionts are removed by movements of the postabdomen, but some stay in inaccessible places until the next molting. As molts occur every few days, they can be considered an instrument of sanitation for the animal's surface. Green (1974) counted an increasing number of attached epibionts during the intermolt period. He also discovered that some Peritricha leave *Daphnia* prior to molting, probably due to changes in the cuticula.

Exoskeleton degradation and recycling occurs with participation of chitobiase (*N*-acetyl-β-D-glucosaminidase), as shown in *D. magna* (Espie and Roff, 1995a, b). There is some flux of chitobiase from the old to the new cuticle. Chitobiase activity varies significantly over the molt cycle, with a fivefold increase 6 h or less before the next molt (Fig. 10.3). During molting, chitobiase is released with the molting fluid into the aqueous environment. Duchet et al. (2011) found a positive correlation between chitobiase activity in the water and the number of *Daphnia* neonates produced during the observation period. It was therefore assumed that chitobiase activity may be used to assess the condition of the population without the destruction of specimens.

Molting is controlled by neurosecretion. Bosch de Aguilar (1969, 1972) concluded that the esophageal group (see Section 13.2) of the anterior region of the nervous system produces a factor that inhibits molting. Martin-Creuzburg et al. (2007) measured ecdysteroid levels during a complete molt cycle in *D. magna*. Free ecdysteroids predominated in whole-body extracts: their

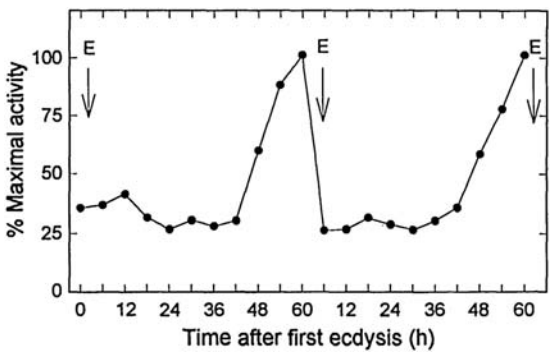

FIGURE 10.3 Activity of chitobiase of *D. magna* over the molt cycle. E, ecdysis. *From Espie, P.J., Roff, J.C., 1995a. A biochemical index of duration of the molt cycle for planktonic Crustacea based on the chitin-degrading enzyme, chitobiase. Limnology and Oceanography 40 (6), 1028–1034; From Espie et al. (1995), Limnology and Oceanography 40 (6), Fig. 1 on page 1030.*

concentration increased sharply in the early premolt stage, followed by a sharp decline prior to ecdysis. It was concluded that molting is probably induced by 20-hydroxyecdysone. Only small amounts of ecdysteroids are found in newly deposited eggs.

It is notable that treatment of *D. magna* with ecdysteroids (ecdysone and 20-hydroxyecdysone) at concentrations of up to 5 μM in the culture medium led to unsuccessful exuviation (Bodar et al., 1990c). When tested on *D. magna*, estrogens (i.e., 17β-estradiol, diethylstilbestrol, and 4-nonylphenol) also inhibited molts, whereas bisphenol A did not (Baldwin et al., 1995).

In general, molting does not depend on illumination or darkness. It was reported that illumination of *Daphnia* with green light accelerates molting (Buikema, 1972).

Under natural conditions, *Daphnia* feeding on blue-green algae (*Microcystis*) were unable to shed their old integument although a new one had already been produced (Rohrlack et al., 2004). These authors found that blue-greens contain the protease inhibitor microviridin J. From the gut of *Daphnia*, it penetrates into the blood and disrupts normal metabolism, which leads to a disturbance in molting, and thus of swimming and filtration, and is followed by death. Cladocera undergo periodic molting.

Phylogenetically, Livanov (1955, p. 211) observed that "molting delimits the size of arthropods—abundant small forms are created—and in this way they adapt to exploit the smallest life spaces and, accordingly, undergo extreme adaptation to the highly restricted life possibilities of the 'empty cavities' in nature that they fill" [translated from Russian].

10.4.1 Metabolism During the Molting Cycle

Periodical molting is interconnected with periodic physiological processes. An intermolt carotenoid pigmentation cycle of fat cells in *Simocephalus* was reported by Green (1966b); a sequence of development of the fat body and the ovaries (Sterba, 1956a) also follows the intermolt cycle (Fig. 3.9). Martin-Creuzburg et al. (2007) measured ecdysteroid levels during a complete molt cycle in *D. magna*; free ecdysteroids predominated in whole-body extracts: their concentration increased sharply in the early premolt stage, followed by a sharp decline prior to ecdysis. In juvenile *D. pulex*, an elevated DNA content was measured in the postmolt period, followed by an increase in RNA during the intermolt and premolt periods (Gorokhova and Kyle, 2002).

The phosphorus release during the molting cycle of individual specimens of *D. magna* was measured by Scavia and McFarland (1982): the rate of phosphorus release was 6.7 times higher at and after ecdysis than at other phases of the life cycle. Cd obtained from solution is concentrated in the exoskeleton of *D. magna*, and ecdysis frees the animal from a considerable part of the accumulated Cd (Carney et al., 1986).

There is a regular variation of the activity of chitobiase over the molt cycle, with a fivefold increase at c. 6 h before the next molt (Fig. 10.3) in *D. magna* (Espie and Roff, 1995a, b).

The principal site where Ca and Sr are accumulated is the carapace as shown with reference to *D. magna* (Porcella et al., 1967). Molting (e.g., in *Daphnia*) is accompanied by a drain of calcium (Ca), P, and carbon (C) (Hessen and Rukke, 2000a). The uptake of ^{85}Sr (strontium-85) takes place after molting, ^{85}Sr is then lost at every molting (Fig. 10.4) in *D. magna*; this was recorded over several moltings (Marshall et al., 1964). Sensitivity to Cr varies over the molt cycle (Fig. 10.5, Lee and Buikema et al., 1979).

Zinc-65 (^{65}Zn) is partly accumulated in the exoskeleton and is removed at molting (Winner and Gauss, 1986). During one molt cycle (from one episode of neonate liberation and molting to the next one) the content of K and Zn decreased but that of Ca and iron (Fe) did not in *D. magna* (Yukleevskikh, 2000). At 600 μg

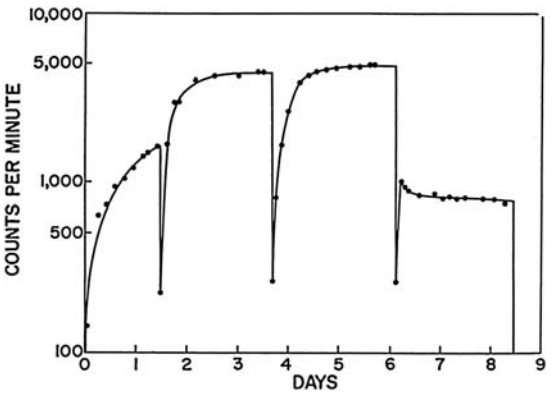

FIGURE 10.4 Accumulation of Sr by *Daphnia magna* and its removal in the course of three complete molt cycles (followed by unlabeled medium). *From Marshall, S.M., Beeton, A.M., Chandler, D.C., 1964. Role of zooplankton in the freshwater strontium cycle and influence of dissolved salts. Verhandl. Internation. Vereinig. Limnol. XV (2), 665–672; From Lee, D.R., Buikema, A.L. jr., 1979. Molt-related sensitivity of* Daphnia pulex *in toxicity testing. Journal of the Fisheries Research Board of Canada 36 (9), 1129–1133.*

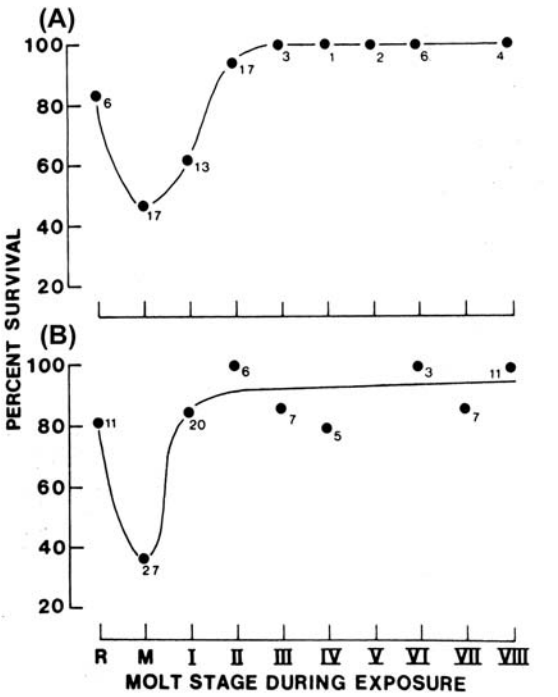

FIGURE 10.5 Sensitivity to Cr (0.56 Cr/L) of *Daphnia pulex* over the molt cycle. (A) In dechlorinated tap water, (B) in reconstituted water. R on the release of young. M on the event of molting. Numbers—sample size. *From Lee, D.R., Buikema, A.L. jr., 1979. Molt-related sensitivity of* Daphnia pulex *in toxicity testing. Journal of the Fisheries Research Board of Canada 36 (9), 1129–1133.*

Zn/L, Zn concentrations in the body fluctuate over 2–3-day intervals, confirming a role of molting in the elimination of Zn (Muyssen and Janssen, 2002).

The heartrate increases before molting, decreases after molting, and then increases again within several minutes as shown in *D. magna* (Meijering and von Reden, 1962).

More experimental work on metabolic events, such as respiration, during the molting cycle is necessary.

10.5 SENESCENCE

There is only circumstantial evidence concerning senescence in Cladocera. Some issues of senescence are discussed by (Pietrzak, 2011). There are morphological signs of senescence as well as physiological signs, e.g., decreased heart rate (Dudycha, 2003). It is demonstrated that such events may be different in different species (Dudycha, 2003) or clones (Pietrzak, 2011) of *Daphnia*.

In senile females, growth is inhibited and may become *negative*, as noted by Frey and Hann (1985). The time between moltings increases, as shown in old *Simocephalus vetulus* and *Moina* (Papanicolau, 1910). Decreased fecundity was indicated in old *S. vetulus*, *Moina* (Papanacolau, 1910), *Daphnia* (Dudycha, 2003). Fecundity, as was shown in *Macrothrix rosea*, increases up to 7th instar, reaching over 20 eggs per individual, then it decreases to c. 3 at the 17th instar (Huang et al., 2011).

In moribund *D. magna*, the heart rate decreases (MacArthur and Baillie, 1929), and in old age fat within the fat body is depleted, the midgut epithelium is progressively degraded,

the muscles degrade, and general activity decreases (Schulze-Robbecke, 1951).

Meijering (1958a,b, 1962) suggested measuring biotic time in heart beats, and found that mortality increases at the *age* of 18 million heart beats for *D. magna*. As observed in *D. magna*, old males may become sterile (Meijering, 1962). According to Skadovskiy (1955), if well-fed daphnias are given limited food, then their fecundity decreases by 2.5 times and they manifest signs of senescence (e.g., decreased heart rate).

Meijering and Redfern (1962) have named the period before death in *D. magna* the *debile phase*. It was noted that moribund *D. magna* lose sodium (Na) (Stobbart et al., 1977). Makrushin (2011) observed and confirmed histologically that natural death occurs in *Moina macrocopa* before they lose the ability to reproduce, whereas senescent *Moina* sink to the bottom as their antennae stop beating.

With reference to *D. magna*, the hypothesis was proposed by Gainutdinov et al. (1997a) that death results from a program of elimination of the organism that is regulated by changes in the environment, perceived via thermo- and chemoreceptors and resulting from the integrative processing of sensory information.

In *Ceriodaphnia affinis* cultivated in a 5.5 nM solution of the antioxidant SkQ1 [10-(6′-plastoquinonyl)decyltriphenylphosphonium], longevity increased due to interruption of the senescence program involving the mitochondria (Anisimov et al., 2008). In a 55 nM solution, the opposite effect was observed, obviously due to toxicity.

10.6 IMPACT OF XENOBIOTICS

Toxic effects

Cladocera live in a highly dynamic solution containing a great many inorganic and organic substances, including xenobiotics. There are numerous factual data on increasing mortality rates at increasing concentrations of xenobiotics, and only some are discussed here. *D. magna* mortality was used as a parameter for the assessment of water quality and bottom sediments by Romanenko et al. (2011). With reference to *Sida*, Beklemishev (1923a, 1924) found that the lethal effect of bichloride of mercury ($HgCl_2$) (recorded by heart arrest) is multiple, the components not determined closer at that time.

There are differences between species in the effect of xenobiotics. The larvicides spinosad and diflubenzuron significantly affect *D. magna* and *D. pulex* life spans; *D. magna* is more severely affected than *D. pulex* by diflubenzuron (Duchet et al., 2011). Number of young per brood in *D. similoides* (Xiang et al., 2011). For *D. magna*, a 100% lethal dose (or LD_{100}) of Cd comprised a 2-day exposure to 2 mg/L Cd^{2+}; and a median lethal dose (or LD_{50}) was provided by a 2-day exposure to a lead (Pb) ion concentration of 1 mg/L (Mardarevich et al., 2001). Copper (Cu^{2+}, as copper acetate) was especially toxic for *D. magna*, contrary to Ni^{2+}, Zn^{2+}, and Co^{2+} (Shilova et al., 2010).

Increasing NO_2-N concentrations (as $NaNO_2$) were followed by decreased longevity, number of moltings, number of offspring per female, number of broods per female.

The acute toxicity of 17 differently substituted benzaldehydes to *D. magna* was studied by Jin et al. (1998); the bioreactive toxicity of these compounds was quantitatively dependent on the nature of the substituted groups. Eleven triazoles and benzotriazoles were differently toxic for *D. magna* as determined by immobilization in 48 h (Durjava et al., 2013). Triazoles turned out to be not readily biodegradable. Lifelong exposure of *Ce. affinis* to low concentration of KCl (10 mg/L) or potassium dichromate (0.01−0.1 mg Cr/L) resulted in decreased life duration (Gershkovich and Isakova, 2013). Crude oil caused death of all *D. magna* at concentrations above 400 mg/L, the medium-sized specimens being the most sensitive (Lennuk et al., 2015).

Positive effects

Life duration and fecundity of *Ce. affinis* were stimulated by ethanol at concentrations

0.002−0.02 mg/L, by KCl at 0.1 mg/L, potassium bichromate at 0.0001 mg Cr/L (Filenko et al., 2012).

10.6.1 Impact of Xenobiotics on Morphology

Inorganic and organic xenobiotics cause deformities of the carapace, the formation of abnormal setae on antenna, the disappearance of antennal segments, and the abortion of eggs and embryos (Fig. 10.2) (Shcherban, 1986).

Chromium: Short-term exposure of the initial culture of *D. magna* to potassium dichromate (1 mg/L) induces morphological abnormalities in the following generations (0.4% of young specimens in the second generation) (Isakova and Kolomenskaya, 2002). In the generation F2, small-sized juveniles, juveniles with deformed rostrum, and a unique specimen with two eyes. The progeny of the latter (198 specimens) had no morphological deviations. Generation F3 manifested anomalies of antennae, and one dwarf female possessed enlarged antennule.

Copper (Cu) and nickel (Ni) reduced the induction of neckteeth in *D. pulex* in the presence of a *Chaoborus* kairomone (Hunter and Pyle, 2004); in contrast to Cu, 200 μg/L Ni decreased the length of neck teeth but increased their number. Later, Mirza and Pyle (2009) found that *D. pulex* neonates from mothers exposed to a kairomone + Cu had fewer and shorter neck teeth than those from mothers exposed to kairomone alone.

In presence of lead (Pb) (as lead acetate) in concentration 1.25 mg/L *D. magna* developed shell spine longer than in the control (Lobkova et al., 2009).

D. magna and *D. pulex* require c. 1 ppb of selenium (Se); in media deficient of Se *Daphnia* lost distal segments of antennal branches and manifested cuticle deterioration (resembling senescent specimens) (Keating and Dagbusan, 1984). In further experiments (Elendt, 1990), *D. magna* suffering shortage of Se-rejected parts of second antennae in the fourth generation, lysis of muscle fibrils was observed due to peroxidation. These changes are attributed by Elendt to glutathione peroxidase (a selenium containing enzyme) which normally protects the tissues from peroxidation.

Zinc (Zn) insufficiency leads to "an increased demand on the animal's pool of available selenium" in *Daphnia*, resulting in cuticle deterioration and depressed reproduction. The situation was improved by the addition of 5 ppb (parts per billion) Se (Keating and Caffrey, 1989).

In *D. magna*, banlen solution (a herbicide mixture of 2-methoxi-4-chlorphenoxiacetic acid and 2-methoxi-3,6-dichlorbenzoic acid) at a concentration 12.5 mg/L produced a high percentage of dwarfed juveniles with a deformed carapace, obviously due to disturbance in embryonic development; at 25 mg/L numerous deformities are produced, including a deformed eye; both concentrations result in increased fecundity, however the eggs, especially at higher banlen concentrations, either develop into deformed embryos or are aborted (Trofimova, 1976, 1979). Isophos (a derivative of thiophosphoric acid) at the concentration 0.0005 mg/L caused in *D. magna* disturbances in embryonic development producing specimens with underdeveloped setae of antennae, deformed spine on shell, or dwarfs (Bruskova, 1976).

Laurox-9 causes teratogenic changes in *Daphnia* progeny such as deformation of the carapace, reduction in the number of segments in the branches of their antennae, and an increased number of setae on their segments (Shcherban, 1980).

The effects of the insecticide carbamyl and the *Chaoborus* kairomone on the formation of neck teeth in *D. pulex* are thought to be synergistic (Hanazato and Dodson, 1992). Carbaryl inhibits the formation of antipredator morphological features in *Bosmina longirostris* (Sakamoto et al., 2009).

A terpenoid hormone methyl farnesoate (MF), known to cause formation of males, also was found (Oda et al., 2011) to affect the body form. In *D. galeata*, MF or fenoxycarb caused elongation of the helmet, but the tail spine was

reduced, but were concentration-dependent. In *D. magna*, embryonic abnormalities are also caused by fenarimol (an agricultural fungicide) via its antiecdysteroidal activity (Mu and LeBlanc, 2002a,b).

Malformations of the carapace and swimming setae are caused by a mixture of fluoxetine and clofibric acid (Flaherty and Dodson, 2005). At 30 mg/L and higher of polystyrene nanoparticles, neonates of *D. magna* were less numerous and had malformations (shorter terminal antennal setae on both branches) (Besseling et al., 2014).

Following exposure to tributyltin chloride, *D. magna* large fat cells (storage cells), which are especially numerous at the posterior curve of the digestive tract, become smaller, glycogen is lost, rough endoplasmic reticulum became less abundant and more vesicular, and their mitochondria are modified (Bodar et al., 1990b).

Pathrological modifications of the midgut and of "placenta" observed in *Bythotrephes* and *Cercopagis* (Makrushin, 1995), delamination of the brood pouch and shell in *Leptodora* (Makrushin and Zaprudnova, 2000; Golubkov and Makrushin, 2012) were attributed to anthropogenic pollution.

10.6.2 Impact of Xenobiotics on Molting

Sensitivity to toxicants is different in the course of the molting cycle, being especially high at the event of molting, as shown with reference to *D. pulex* (Fig. 10.5) (Lee and Buikema, 1979).

When exposed to polychlorinated biphenyl (PCB) 29 (2,4,5-trichloribiphenyl), arochlor 1242, and diethyl phthalate, *D. magna* takes longer than the controls to complete molting (Zou and Fingerman, 1997). Exposure of *D. magna* to a solution containing 20-hydroxyecdysone or ponasterone caused incomplete ecdysis, followed by premature death (Baldwin et al., 2001). Exposure of *D. magna* to sublethal concentrations of polybrominated diphenyl ethers disrupted molting in neonates, probably disrupting endocrine functions (Davies and Zou, 2012).

Some xenobiotics can interfere with the hormone-regulated molting process in arthropods by acting as antagonists of endogenous ecdysteroids. The exposure of *D. magna* to 20 μg Cd/L for 8 days led to a decrease in the whole-body ecdysterol (ecd) concentration from c. 200 to 70 pg ecd equivalent/mg DW (Bodar et al., 1990b); this was probably the reason for their unsuccessful exuviation.

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CHAPTER

11

Reproduction

11.1 ANATOMICAL BACKGROUND

Cladocera is one of the rare groups of animals which, together with aphids and some hymenopterans, reproduce principally through parthenogenesis. After an indefinite number of parthenogenetic generations, a cladoceran species may pass into gamogenetic (bisexual) reproduction, during which dormant eggs are produced, either surrounded by an ephippium [a reinforced part of the shell (in anomopods) or without a special case (in ctenopods)]. Accordingly, males are usually rare. With the exception of *Leptodora*, Cladocera have no larval stages in the free-living period of their life cycle.

Female Cladocera have paired ovaries and males have paired testes, along sides of the trunk. In the ovary, there are several four-cell groups: one cell develops into an egg, whereas the others are *nurse cells* that are resorbed during egg formation (Weismann, 1877a,b). The process of oogenesis in Cladocera was investigated more recently by Makrushin (1966–80). Spermatogenesis was described in detail by Wingstrand (1978). The spermatozoids of different species were found to be significantly different in form, as was previously noted by Weismann (1918), sometimes even in representatives of the same genus.

Parthenogenetic females either lay many eggs into the brood pouch (i.e., they are polyembryonic) or normally lay only two eggs (most chydorids). In polyembryonic species, the number of eggs is not fixed and may decrease if the food supply is insufficient. The number of latent eggs laid by one female ranges from one to several, depending on species.

11.2 CYCLICITY

Although they may be quite long, periods of parthenogenetic reproduction are interrupted by gamogenetic (bisexual) reproduction, which results in the formation of dormant eggs that serve for the latent period of the life cycle, termed *diapause*. The period of parthenogenetic reproduction along with the following period of gamogenetic reproduction was termed a *cycle* by Weismann (1880). If there is only one cycle per year, the population is called *monocyclic*; if there is more than one cycle, then it is called *dicyclic*, *tricyclic*, or *polycyclic*. When there is no period of bisexual reproduction, it is called *acyclic*.

According to Vereshchagin (1912), this cyclicity depends on the latitude (i.e., on the length of the ice-free period), but varies in different species. Frey (1982) considered the generation change further and noted that in the tropics gamogenesis is rather irregular and is unrelated to any particular season. He also gathered further evidence that in the northern United States, as in Europe, gamogenesis occurs prior to winter for most

http://dx.doi.org/10.1016/B978-0-12-805194-8.00011-8

species. Further south, gamogenesis in "northern species" becomes less intensive and tends to occur in spring, whereas in "tropical species" gamogenesis occurs irregularly.

If the vegetational period is long enough a female may produce many parthenogenetic broods. Under laboratory conditions, the period of parthenogenetic reproduction, i.e., when only diploid females are produced, may be almost indefinitely long. Continuous parthenogenetic reproduction of some Cladocera spp. has been observed in the laboratory for up to 13 years (Banta, 1925). In culture, over 700 uninterrupted parthenogenetic generations have been recorded for *Daphnia pulex* and *Moina macrocopa* (Banta and Brown, 1929). Cyclic reproduction in Cladocera, with special reference to *Daphnia*, was also analyzed by Berg (1934).

On the other hand, at a very short vegetational period (in high latitudes) only a brief life cycle is possible. In Alaska, at 7−10.5°C, exephippial females of *Daphnia pulex* var. *tenebrosa*, according to Edmondson, in spring (early in July) produced either the first brood with ephippial eggs or (a small fraction of the population) one brood with nonephippial eggs which developed into females producing ephippial eggs in September; no males were found. However, males of *D. pulex* were recorded from Novaya Zemlya (Jaschnov, 1925).

Progression of the reproductive cycle in Cladocera is regulated by ecdysteroids. Before molting and ovulation in *Daphnia magna* the ecdysteroid titer is low (Sumiya et al., 2014). These authors suppose that ecdyson may be synthesized in the gut.

11.3 PARTHENOGENETIC REPRODUCTION

Parthenogenetic reproduction yields progeny that immediately start an active life of their own upon liberation from the parental brood pouch. Liberation of the young is combined with molting.

The oocytes growing in the ovaries derive their fat from the fat body, and thus the content of fat in the fat body is inversely proportional to the development of the oocytes (Sterba, 1956a). Fat reserves (especially triglycerol) are transferred to the ovaries late in each intermolt period (Tessier et al., 1983).

Cladocera seem to be able to reproduce through an almost indefinitely long number of parthenogenetic generations. However, it has been shown that inbreeding *Daphnia longispina* after the 278th, 279th, 442nd, and 570th generations results in an increasingly lower hatching rate of sexual eggs and the production of dwarfs, sterile individuals, specimens that produce nonviable parthenogenetic eggs, fewer young, and mainly or all-male young (Banta and Wood, 1937).

Following the addition to the culture medium of gonadotropin, oxytocin, or prednisolone, *D. magna* reach maturity earlier; embryogenesis is accelerated by gonadotropin, whereas prefison retards maturation and embryogenesis (Vinokurova, 1977). Kairomones released by predators induce *Daphnia* to reproduce at a smaller size than without this cue; triglyceride content of the egg material was also lower in presence of the kairomone (Stibor and Lűning, 1994; Stibor and Műller-Navarra, 2000).

11.3.1 Fecundity

Cladocera may produce dozens of litters during their lifetime; thus, one specimen may potentially produce large numbers of progeny. This can be up to 15 litters in *D. magna*, totaling up to 1055 juveniles (Wojnarovich, 1958). For one specimen of *D. magna*, the potential progeny was estimated as to 10^6 individuals (Shpet, 1968). Some *Moina* species are even more prolific.

Cladocera species produce either numerous small eggs (polyembryony) or normally (Chydoridae except *Archepleuroxus*) produce two large eggs. In the latter case, exceptions are very rare. Two *Chydorus sphaericus* females contained three parthenogenetic eggs each

(instead of the usual two) were found in Kosino Lakes (Moscow), a phenomenon never previously seen during decades of work with thousands of samples from every continent. A still rarer case was reported by Michael and Frey (1984, p. 94) for the usually two-egged *Disparalona rostrata*: "quite a number of *D. rostrata* females were carrying from 4 to 10 small parthenogenetic eggs. This is the first time in our examination of many thousands of parthenogenetic females from many different species."

Polyembryonic families include the Daphniidae, Moinidae, Sididae, Holopedidae, Eurycercidae, and some of the Macrothricidae. In polyembryonic daphnids, the number of eggs per individual is highly variable, e.g., in *Simocephalus*, the number of eggs in a single female may reach 40, but is 20 on average (Green, 1966a). In polyembryonic cladocerans, the number of parthenogenetic eggs produced is directly proportional to body size, as was shown, e.g., for *Daphnia* (Green, 1954; Zhdanova and Frinovskaya, 1977) and *Sida* (Fairchild, 1983). *Daphnia cephalata* produce broods of up to 295 eggs, *D. magna*—c. 110, *Daphnia galeata*—6–7.5 (Hebert, 1978a,b). The number of eggs also varies with season, and depends on environmental conditions. Under unfavorable conditions, the number of eggs may drop to one or two, or a specimen may miss the next egg-formation cycle.

Dormant eggs are less numerous: 1 or 2 are present in the ephippium of chydorids, daphnids, *Moina*, and up to 11 in *Eurycercus lamellatus* (Smirnov, 1971). In an obligate parthenogenetic population of *D. pulicaria*, the number of eggs per ephippium increases at greater food availability (Conde-Porcuna et al., 2014).

The size of juveniles within the same brood may vary, as noted with reference to *Simocephalus* (Shkorbatov, 1953) and *D. magna* (Green, 1956a). Nonsynchronous development of embryos was also noted for *Daphnia*, *Evadne*, and *Podonevadne* by Rivier (1974). Moreover, Ramult (1926) found variations in the vitality of eggs within the same brood following exposure to solutions of mineral salts.

Dependence of Fecundity on Food Abundance: Lag Effect

The diversity of life histories within Cladocera, with respect to predation and food resources, was analyzed by Lynch (1989). Planktonic Cladocera exist under conditions of fluctuating food stocks due to factors including the periodic development of algae. When food is in short supply, they produce smaller (as shown e.g., for *D. pulex* by Lynch, 1989) and fewer eggs. Tessier and Consolatti (1991) also observed that the quantity and weight of *Daphnia* neonates depend on the level of available food and the C:N ratio of the food: they called this "adaptive plasticity in reproduction."

Makrushin (1966) demonstrated that in *D. pulex* and *D. longispina* ovicells decompose if starvation occurs at stage I or early stage II of their development, but not if starvation occurs later. Stage I was characterized by Makrushin as the state of the ovary when either no fat is present in the cytoplasm of ovicells or it is present as tiny droplets; the brood pouch may contain eggs that have not started cleavage. Such a time lag, dependent on the energy reserves of the body, was also reported by Goulden and Hornig (1980), who noted that smaller *Bosmina*, with insufficient energy reserves, die comparatively sooner.

Reaction to food limitation is somewhat delayed (lag effect, see also Section on Starvation).

As indicated for *Daphnia*, the critical time point at which starvation determines the number of eggs produced is about halfway through (0.5) the intermolt period; variation in the abundance of food at later stages does not change the number of eggs (Bradley et al., 1991a,b). Ebert and Yampolsky (1992) investigated how food shortage leads to the production of fewer eggs in *Daphnia*. They found that the number of eggs decreased when the "females were starved at least for 0.6 of the adult instar duration before

egg laying." In addition, the number of eggs increased when abundant food was given to the 0.6 instar, before the eggs were deposited into the brood chamber.

Taking into consideration that the yolk protein is the main material used in reproduction, Stibor (2002) found that *D. magna* demonstrate "parental optimism" producing more yolk protein for the offspring than they really release. Using a radial immunodiffusion technique for quantitative estimation of yolk protein in homogenized eggs or bodies of *Daphnia* he demonstrated that the production of the yolk protein starts at the beginning of the past juvenile instar and the yolk protein levels increase "during the first half of the last juvenile instar" (Stibor, 2002, p. 366). Further use of the yolk protein is flexible and *Daphnia* may reduce the brood depending on changes in the environment, including kairomones from predators.

Reproductive Constraint at Low Food Concentrations. Under unfavorable low-food conditions, *D. pulex* produces fewer eggs, e.g., two, one (Pyatakov, 1956), or none. When there is an extremely large reduction in the number of eggs and the formation of only one egg during the intermolt period, the ovaries function alternately. The fewer eggs produced are also larger. The clutch size is smaller when food supply is limited, as shown for *Daphnia* (e.g., by Gliwicz and Noavida, 1996). As shown under experimental conditions by Gliwicz and Guisande (1992), when food is scarce, *Daphnia* produce small clutches of comparatively large eggs; in contrast, eggs produced with abundant food are larger, and offspring hatched from larger eggs can survive for longer during periods of starvation.

Effect of Food Resource Quality

Fed on N-sufficient *Scenedesmus, D. pulex* produced larger broods than those fed on N-deficient green algae containing more lipids (Groeger et al., 1991). Vitamins have been shown to affect reproduction. In *D. pulex*, the number of progeny was highest at a vitamin B_{12} concentration of 0.75 g/L; when deprived of vitamin B_{12}, they did not produce viable progeny (Keating, 1985). Following the addition of pantothenic acid to food consisting solely of *Chlamydomonas*, the life span of *Daphnia* increased by a factor of three (Fritsch, 1953).

It was experimentally shown with reference to *D. pulex* that certain amino acids (namely, arginine and histidine) avert production of resting eggs (Koch et al., 2011).

11.3.2 Impact of Environmental Factors

Natural Factors

The most intensive *M. macrocopa* reproduction was recorded at 28°C by Terao and Tanaka (1928b). Within the temperature range to which they were exposed, the highest fecundity was measured at >30°C in *Latonopsis* cf. *australis* and at 20–30°C in *Diaphanosoma brachyurum* (Chaparro-Herrera et al., 2011).

Reproduction and molting in Cladocera are profoundly influenced by the photoperiod (Parker, 1966). It is thought that the photoperiod influences production of a corresponding neurohumor. It was reported that marine representatives of Polyphemoidea (*Pseudevadne* and *Evadne*) release neonates during total darkness, i.e., between 10 p.m. and 4 a.m. (Onbé, 2002).

Egg production in *D. magna* is reduced at low concentrations of calcium (Hessen and Rukke, 2000a,b). Volcanic ash so negatively affected fecundity and survival of Cladocera that they disappeared in 6 months after the eruption; direct negative effect was experimentally confirmed in *Daphnia commutata* exposed to 2–8 mg/L ash (Wolinski et al., 2013). At 8 mg/L *Daphnia* died before reaching reproductive age. Reproduction is stimulated by enrichment of water with potassium, as shown with reference to *Daphnia dentifera* (Civitello et al., 2014).

Effect of Xenobiotics on Fecundity

Since the first publications on toxicological experiments, numerous data have been reported on fecundity decreasing upon exposure to xenobiotics. For example, following exposure to

25–50 nM Cd^{2+}, *D. magna* fecundity decreased from c. 6.8 to 2 neonates/female, and at 100 nM Cd^{2+}, it declined to zero (Baillieul and Blust, 1999). In chronic toxicity 8-day tests the number of young in *Ceriodaphnia dubia* decreased proportionally to concentration of α-cypermethrin from 18 to 5 (at 250 ng/L), of deltamethrin—from 16 to 3.8 (at 100 ng/L) (Shen et al., 2012).

Exposed to ions Cu^{2+}, Zn^{2+}, Co^{2+}, or Ni^{2+} in concentrations 0.0001–0.01 mg/L *D. magna* decreased natality c. by 80%, at combined action and addition of NaCl natality decreased by 1.5–4 times (Shilova, 2014). At exposure to nanoscaled TiO_2 (1.19–6 mg/L) the culture of *D. magna* collapsed after five generations due to decreasing reproduction (Jacobash et al., 2014). Cytostatic drugs (especially, cisplatin and doxorubicin) at few milligrams per liter were toxic for *D. magna* and *C. dubia* and caused 50% reproduction inhibition (Parrella et al., 2014).

Tributyltin chloride at concentration 2×10^{-6} mg/L retarded maturation of *D. magna* and caused abortion of eggs and embryos (Kolosova and Stroganov, 1973). Toxicity decreased if tin was lead, methyl, or biphenyl.

In the stirred suspension of nanoparticles of TiO_2, at 5 mL/L, not all females of *D. magna* were gravid (Kim et al., 2014a,b).

Stroganov et al. (1977) demonstrated molting modification by stimulation of fecundity of *D. magna* by polyethylenimine at concentration 1 mg/L in subsequent generations, as well as formation of dwarfs.

11.4 GAMOGENETIC REPRODUCTION; DIAPAUSE

After an indefinite number of parthenogenetic generations at the onset of unfavorable conditions, Cladocera form males and gamogenetic females, and switch to bisexual reproduction producing resting (latent) eggs. Males emerge from eggs that show no external differences from the usual parthenogenetic eggs (Banta and Brown, 1924). The following latent period is termed diapause. This general pattern is, however, more complex.

Some cultures did not form males even after many hundreds of parthenogenetic generations. It was shown for *Daphnia*, *Simocephalus*, and *Moina*, that the same female may, in the same brood, produce (1) parthenogenetic females, (2) parthenogenetic females and males, or (3) parthenogenetic females, gamogenetic females, and males (Papanicolau, 1910; Agar, 1920; Orlov and Cherepanov, 1986; Zadereev et al., 1998). Hebert (1978a,b, p. 396) notes for *Daphnia*, "Normally, a particular brood of eggs is single sexed, although mixed broods do exist."

A single female *D. pulex* may produce only females, only males, or a mixture of both sexes (Innes, 1997).

In some cultures it was not possible to obtain males at all. In some samples, males were present whereas a part of females continued parthenogenetic reproduction. i.e., male-producing and nonmale-producing females may coexist, e.g., as noted for *D. longispina* by Manuilova (1956) and for *D. pulex* by Innes (1997). Within the same species, the sequence of parthenogenetic and bisexual reproduction may differ in different water bodies. In ponds of temperate Russia (of the Yazhelbitsy Fish Farm) a gradual increase in bisexual reproduction of *D. longispina* from its initiation was found until the middle of July, when parthenogenetic reproduction was completely discontinued (Manuilova, 1951). In other ponds, the same species reproduced both parthenogenetically and bisexually throughout the summer. The latter was also the case for *Ceriodaphnia reticulata* in ponds of the Yazhelbitsy Fish Farm, in contrast to those in Lake Kolomenskoe, also in temperate latitudes (Tverskaya Oblast, Russia).

Latent (resting) eggs are contained in the ephippium in anomopods or are liberated in ctenopods. The ephippium takes various forms in different species. In *Lathonura* and *Alonopsis*, it consists of an upper-posterior part of a shell, which is reinforced by special glands (Fig. 11.1)

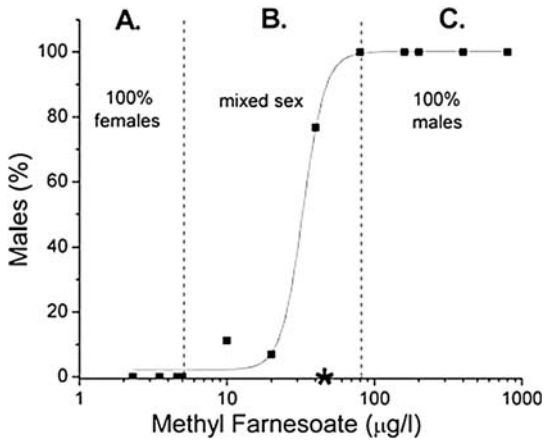

FIGURE 11.1 Percentage of males in broods of *D. magna* at various concentrations of methyl farnesoate. The asterisk denotes the concentration at which gynandromorphs were produced. *From Olmstead, A.W., LeBlanc, G.A., 2007. The environmental-endocrine basis of gynandromorphism (intersex) in a crustacean. International Journal of Biological Sciences 3 (2), Fig. 4 on page 81.*

(discovered by Makrushin, 1970). In *Daphnia*, the same species and a clone may produce both buoyant and nonbuoyant ephippia, as shown in *D. pulicaria* (Cáceres et al., 2007). Latent eggs are well protected from mechanical damage. In *D. pulex*, the envelope of latent eggs is 2.2 μm thick, whereas the outer wall of nonresistant eggs is only 0.35 μm (Seidman and Larsen, 1979).

The shell composition of resting eggs of *Daphnia* includes crystalline calcium phosphate and magnetic material; their distinctive chemical composition and honeycomb structure are thought to ensure survival under harsh conditions (Kawasaki et al., 2004b). The distribution of elements over the integument of *D. magna* ephippia was found inhomogeneous (Kawasaki et al., 2004b): P, Ca, and K were present mostly over the embryos, whereas sulfur (S) is distributed over the eggshell. Resting eggs of *Ceriodaphnia quadrangula* contain chitin (15–167% DW) and chitosan (11% DW) (Kaya et al., 2014).

Protective melanistic coloration of ephippia varies from 0.5% to 96.5% of their surface, as shown for *D. pulicaria* (Gerrish and Cáceres, 2003), and is caused by genetic and environmental components. In most daphnids, ephippia with resting eggs mainly float on the water surface (Makrushin et al., 1990), whereas in other anomopods they sink or are glued to underwater surfaces (Fryer, 1972; Makrushin et al., 1990). In macrothricids, at least, the ephippia are attached to substrata, thus ensuring "continuity of an already established colony" (Fryer, 1972, p. 79).

Diapause plays a big role in life and phylogeny of Cladocera (Fryer, 1996). Berg (1934) advocated the idea that gamogenesis is correlated with phenomena of depression in Cladocera.

The previous facts suggest the presence of different clones within a single population of a species, as shown by Carvalho and Hughes (1983) by differential photoperiodic induction of ephippia formation in *D. magna*.

11.4.1 Natural Factors That Influence the Formation of Males

Various factors that cause the onset of gamogenesis also lead to the formation of males (Spaak, 1995): low temperature (8°C) and limitation of nutrition (*Simocephalus*) (Issakovitsch, 1905), crowding (Banta and Brown, 1929; Lopatina and Zadereev, 2015), food shortage (Tauson, 1931; Stuart and Cooper, 1932), changes in the food quality (fat accumulation by declining algal populations) (von Dehn, 1948, 1950, 1955), photoperiod (Stross, 1965, 1969c; Buikema, 1968). Maximum formation of males of *D. magna* happened (Korpelainen, 1986) at 14°C and photoperiod light/dark ratio (L:D) 16:8 h. On the other hand, the formation of males is induced by increased temperature (Fries, 1964), a decrease in day length to 12 h (Stross and Hill, 1965). In *Scapholeberis armata*, ephippial females appeared under conditions of a short photoperiod (11.4 h light vs. 15.5 h light) (Berner et al., 1991), usually after one or more parthenogenetic broods.

Males and gamogenetic females are produced by *M. macrocopa* in a medium supplemented

with metabolites that accumulate in their culture (Orlov and Cherepanov, 1986). Gamogenetic reproduction was observed to be caused by the accumulation of metabolic products in the medium (in *Moina brachiata* and *M. macrocopa*, but only by those from the same species) (Lopatina and Zadereev, 2007).

Ephippia formation has been experimentally induced in *D. magna* at low food levels, high culture densities, and short-day photoperiods (12 h: 12 h L:D periods) (Carvalho and Hughes, 1983).

Transition to gamogenesis is likely to be a result of the combined action of several factors. It has been shown experimentally for *D. magna* that formation of gamogenetic eggs is induced by a combination of three factors: food limitation and quality, short-day photoperiod, and chemically induced crowding (Kleiven et al., 1992). In addition to fat derived from diatoms, fat is known to be accumulated by algae under conditions of depleted nutrients in senescent algal cultures. Thus, two processes seem to co-occur in nature: (1) the accumulation of fat by cladocerans due to feeding on fat-rich algae and (2) stimulation of male production by substances derived from the algal fat. The maximum percentage of males is formed at the low P concentration in food (0.05 mg/L) in *Moina irrasa* and higher P concentrations in *Daphnia similoides*(10 mg/L) (Meng et al., 2014).

Daphnia schoedleri switches to gamogenesis when there is an increased content of neurosecretory hormones in its body (Alekseev, 2010).

In energetic terms, the formation of an ephippial clutch is equivalent to laying eight to nine parthenogenetic eggs (Zadereev et al., 1998).

In addition, parthenogenetic reproduction may be resumed after the formation of gamogenetic eggs and ephippia, as has been shown in *Daphnia* (Sklayrova, 1938; Green, 1956a; Makrushin, 1969) and *Moina* (Makrushin, 1969).

As males are generally rare, some species were suspected to have lost bisexual reproduction. As well, stimulatory effects of environmental conditions on formation of males are diverse. Reasons of such complexity are being investigated.

The term *pseudosexual reproduction* has been applied to some clones (races) which produce ephippial eggs and ephippia that develop without fertilization in the absence of males (with reference to *Daphnia*) (Banta, 1925; Schrader, 1925). Such races have been called *thelytokous*, with the ephippial eggs being known as *pseudosexual eggs*. Later, obligatory asexual clones were noted for *Daphina*, producing dormant eggs in ephippia in the absence of males which then hatch in due time (Dudycha and Hassel, 2013). Thus, transition to gamogenesis is related to the clonal composition.

Gynandromorphism. Sometimes, individuals with mixed female and male features (gynandromorphic) occur. Such specimens have been reported for daphnids, chydorids, and *Leptodora* (Frey, 1965). Experimentally, the formation of gynandromorphs was caused in *D. magna* by MF treatment (Olmstead and LeBlanc, 2007): especially high numbers of gynandromorphs were produced at concentrations of c. 12 μg/L, which is intermediate between the concentrations that produce 100% females and those that produce 100% males.

Hybrids. The presence of hybrids between species is a rather common occurrence (Agar, 1920; Hebert, 1985; Spaak, 1995; Flössner, 2000; Korovchinsky and Boikova, 2009). Naturally occurring hybridization was recorded in *Simocephalus* (Hann, 1987) and in *Daphnia* (Flössner, 2000; Hobæk et al., 2004; Korovchinsky and Boikova, 2009). The possibility of crossing *Pleuroxus denticulatus* and *Pleuroxus procurvus* was experimentally demonstrated (Shan, 1971).

11.5 THE PHYSIOLOGY OF EGGS AND EMBRYOS

The available data on physiological traits of eggs and of the early developmental stages will be described next.

11.5.1 Parthenogenetic Eggs and Embryos

The density of *D. magna* eggs is about 0.37 mg DW/mm^3, irrespective of food availability to the females (Trubetskova and Lampert, 1995). The eggs are very strong and the egg envelopes of *Simocephalus* have been shown to withstand centrifugation at 1058 g (Hoshi, 1950a).

The size (volume) of parthenogenetic eggs varies within the same species and depends on the season and the length of the females (Green, 1956a). In *Bosmina longirostris*, Kerfoot (1974) found that the females carry small parthenogenetic eggs in spring–summer; in late fall, larger eggs are produced that contain double the yolk volume and produce larger young. This cycle is, however, not strongly associated with nutritional conditions.

The eggs obtain their chemical constituents from mothers via the ovary. The volume, dry weight, fat content, and carbon content of *D. magna* eggs were the highest at low food levels and lowest at high food levels (Boersma, 1997). Egg size in *D. magna* was largest under conditions of low food availability and small at starvation level in the study of Trubetskova and Lampert (1995).

The chemical composition of *Daphnia hyalina* eggs was reported as 9.3% DW of nitrogen (N), 53.6% DW of carbon (C), and 1.2% DW of phosphorus (P) (Baudouin and Ravera, 1972). The P content of *D. magna* eggs did not depend on its level in the mother's food and varied only slightly, within c. 14–14.6 mg P/g DW, with large variations in the C:P ratio (Becker and Boersma, 2005).

The color of parthenogenetic eggs is mostly brownish, but may vary within the same species. Eggs may be green [as mentioned by Müller, 1785, p. 81 ("ova plerumque viridis") and Sars, 1862], greenish-blue, orange, or brown in *Daphnia* (Konovalov and Konovalova, 1955) and blue or violet in *Moina* (Papanicolau, 1910). Clearly depending on the type of food available, parthenogenetic eggs of *Acroperus* may be gray, green, orange, or violet, all of which develop into viable progeny. Teissier (1932) especially noted that the pigmentation of eggs depends on the mother's food and if fed with food completely void of carotenoids *D. pulex* produced eggs with oil drops uncolored (losing their usual green coloration) and the cytoplasm red [with hemoglobin (Hb)].

The carotenoid content of Cladocera eggs depends on the quality of the food consumed by the adult (Green, 1957a,b). Developing eggs are colored green or blue by carotenoid protein; in contrast, in fully developed embryos that are about to be released, the fat globules become orange colored due to the release of carotenoid from the bound protein, as was observed by Green (1957a,b) in many littoral and planktonic species. About half of the mother's carotenoids are transferred to the eggs of each brood (Herring, 1968). This pigment is not utilized by the developing eggs or embryos and its presence does not enhance either the viability or fertility of the eggs. Carotenoids have never been observed in *Leptodora* eggs (Green, 1957a,b).

Carotenoproteins may function by protecting eggs and embryos of *Daphnia* from solar radiation (Green, 1957a,b, 1965).

Hemoglobin (Hb) is passed into developing oocytes in the *Daphnia* ovary, and the transfer is especially intensive in the last few hours prior to egg laying (Dresel, 1948). Two possible explanations for this were suggested by Dresel: either the Hb molecules can pass through the cell membranes or they must be broken and rapidly resynthesized in the oocytes. Before parthenogenetic eggs are laid, they receive Hb from the blood at the end of each instar, as shown for *Daphnia* by Green (1965); the parthenogenetic eggs of *Simocephalus* also contain Hb (Hoshi, 1957). The Hb content in eggs is 0.17 mg Hb/g DW from pale *D. magna* and 0.53 mg Hb/g DW from red *D. magna* (Kobayashi and Nezu, 1986). At a high concentration of Hb, it is included in fat cells as soon as these are formed (Green, 1965).

Probably, Hb in the eggs is a supply of protein. It is broken in the course of development (Fox, 1948; Green, 1965). Green (1957a,b) suggested that the breakdown of Hb in *Daphnia* involves oxidation with participation of linoleic acid. However, the embryos with their Hb blocked by carbon monoxide (CO) remain normally active (Fox, 1948). The Hb content is higher in early embryos of *D. magna* than in the hemolymph of adults, and in an anoxic environment a longer time is required for Hb deoxygenation in early embryos than in adults (Kobayashi and Takahashi, 1993). The Hb content in a single *D. magna* embryo was measured as 0.12 μg (Kobayashi et al., 1987). Moreover, the oxygen affinity of Hb in *D. magna* embryos is higher than in adults (Hoshi et al., 1974).

The hatchability of *D. magna* eggs is best (100%) at an oxygen tension c. 15 torr (approximately 2000 Pa), and it dropped at c. 12 torr (approximately 1600 Pa). Hatchability was also highest at temperatures of 10–27°C and abruptly decreased at 30°C; 50% hatchability was recorded at 29°C for embryos from pale animals and at 30°C for embryos from red animals (Kobayashi et al., 1987).

After being laid into the brood pouch, the parthenogenetic eggs of most Cladocera develop using the resources contained within the egg, independent of the maternal metabolism. The eggs may, therefore, be cultivated in a watch glass (Krogh, 1939; Murugan, 1977; Murugan and Venkataraman, 1977; Kobayashi et al., 1987; Sobral et al., 2001). This has been demonstrated for *Simocephalus*, *Scapholeberis*, *Eurycercus* (Rammner, 1933; Hoshi, 1951b), *Daphnia* (Obreshkove and Fraser, 1940a,b), *C. reticulata* (Shuba and Costa, 1972), and *Chydorus* (Green, 1961b). The eggs develop well if removed no earlier than the first hour after transfer to the brood chamber (Krogh, 1939).

However, moinids and polyphemids are an exception (Weisman, 1877a,b) in that *Polyphemus* females produce small eggs and possess placenta (the original German term is *Nährboden*) to supply nutrition for the eggs developing in the brood chamber. Patt (1947) presented cytological evidence of this process. A kind of placenta is also present in *Moina*.

As shown for *Simocephalus vetulus*, lipid oxidation predominates in early embryos, whereas free-living individuals rely on carbohydrate oxidation (Hoshi, 1951a). The principal sources of material for the development of cladoceran eggs are the yolk and oil. In a developing egg, the initial large fat globule diminishes in size and splits (Green, 1957a,b). Later in the developed embryo, the fat is distributed as small globules in the fat cells. Relatively larger eggs convey to the embryos a greater relative amount of triacylglycerol that is left over from embryonic development (Goulden et al., 1987). A hemochromogen (daphniarubin) is found in the gut of later-stage embryos of *Daphnia* (Fox, 1948).

The fate of glycogen in parthenogenetic eggs is discussed by Green (1965) mainly with consideration of the data obtained by Hoshi (1951a, 1953) for *Simocephalus*. Glycogen, present in the cytoplasmic fraction but not in the yolk, first appears in the muscles, in the wall of the gut and of the axillary gland. Its content is 0.7% of WW of the gastrula, rises to 0.91% WW, and then decreases again to 0.71% WW in the released young. Green (1965, p. 586) notes that "It is formed rather than utilized in the early stages of development."

The egg membrane is slightly permeable to water (Krogh, 1939). The osmotic pressure in parthenogenetic and fertilized winter eggs is 57–72 mM and reaches 216 mM within 50–80 h (Krogh, 1939). In Cladocera with an open brood chamber, the total osmotic concentration of fluid in the embryo is supported at a level of about 5‰; during embryogenesis, this does not decrease below 4‰ (Aladin and Valdivia Vilar, 1987). When they start feeding, hypertonia abruptly increases because the total osmotic concentration of the hemolymph initially depends on the amount of ingested salts.

The respiration rates of *D. magna* eggs and early embryos are about a third of those of neonates and adults (Glazier, 1991). Thus, in brooding females they take a small proportion of the total oxygen consumption. It was reported in *Daphnia* embryos that oxygen consumption per unit weight increases at later developmental stages, in contrast to the postembryonic period (Eremova, 1991). Under anaerobic conditions the embryos of *Simocephalus* survive the longest at the gastrula stage (10−12 h), the survival time decreasing with growth, being 1−2 h in the released young (Hoshi, 1952).

In eggs free ecdysteroids dominate as shown with reference to *D. magna* (Martin-Creuzburg et al., 2007).

The peak of RNA concentration occurs at the last stages of *D. pulex* embryo development and is followed by a gradual decrease during juvenile development (Gorokhova and Kyle, 2002), whereas the DNA concentration is highest in the early stages of postnatal development. Vitellogenin-like proteins are found in whole-body homogenates of *D. magna* (Jubeaux et al., 2012). The role of precursors of the major egg-yolk protein is ascribed to these proteins.

In their early stages, *Simocephalus* embryo development is mainly due to fat metabolism; at later stages, close to the emergence of juveniles, the respiratory quotient (RQ) shifts toward predominantly carbohydrate (i.e., glycogen) metabolism (Hoshi, 1950b, 1951a): at the gastrula stage, the RQ is 0.74−0.8; in an embryo liberated from the egg's envelope, the RQ is 0.88; and in newborn juveniles, it is 0.99. In the embryo, glycogen is firmly bound to protein and fat; the glycogen content increases in the organs of the developing embryo and then drops in the released young (Hoshi, 1951a). Hoshi believed that free-living *Si. vetulus* young predominantly use carbohydrate as a metabolic substrate.

In *Simocephalus*, the longest survival under anaerobic conditions occurs during gastrula, suggesting that anaerobic metabolism predominates in this period (Hoshi, 1957).

It has been suggested that *Ceriodaphnia* eggs can delay hatching in the presence of chemical cues from predators (Blaustein, 1997).

Bottrell (1975a) cultivated eight species at four levels of temperature and found the duration of development of eggs to be inversely proportional to temperature, longer in larger species, and longer in ephiphytic species than in planktonic species.

Finally, it may be added that *D. pulex* parthenogenetic eggs are reported to have remained viable (at least some of them) and to have hatched after passing through the digestive tract of a fish (*Carassius auratus*) (Samchyshyna, 2002).

11.5.2 Effects of Extreme Limits of Environmental Factors and Xenobiotics on Eggs and Embryos

At the extreme limits of temperature, Cladocera do not reproduce. During several weeks at 5°C, no reproduction occurred in *C. sphaericus* and *P. denticulatus* (Keen, 1971). A pH above 10, which often occurs in lakes, is detrimental for *D. galeata* egg development (Vijverberg et al., 1996). Food limitation results in decreased fecundity and interrupted reproduction.

The effect of xenobiotics has mainly been tested in adult cladocerans. However, it has been shown, with reference to *D. magna*, that eggs and embryos are sensitive to cadmium (Cd), copper (Cu), dodecyl benzyl sulfonate, and 3,4-dichloroaniline (Sobral et al., 2001).

Mercury (Hg) at concentrations of 0.32 μg/L and higher is highly toxic to embryos, as shown with reference to *D. magna* (Khangarot and Das, 2009). The number of *D. magna* embryos was also affected by 15 aniline derivatives in a dose-dependent manner, although no morphological abnormalities were induced (Abe et al., 2001).

Postdiapause embryos of *Wlassicsia pannonica* and *M. macrocopa* were destroyed by peroxides (H_2O_2) at concentration 0.3% (Alekseev et al.,

2010). All embryos of *M. macrocopa* were also killed by ammonium.

Prometryne (at 3—4 mg/L) is embryotoxic, as shown in *D. magna* (Pushkar and Usacheva, 1977): only one gonad was functioning in several specimens, some ovicells had no integuments, some embryos lagged in development and finally perished, abnormal specimens appeared (especially in the third generation), there were disturbances in development and functioning of the endo- and exoskeleton, the eye was absent, etc., etc.

Propanid at the concentration 0.2 mg/L affected embryogenesis of *D. magna* (Pushkar, 1973): organs appeared in eggs (not in embryos), eggs had a black spot, some eggs turned into a swollen transparent mass, embryos were abnormal (e.g., had no head); ovogenesis and embryogenesis were discoordinated: final ovicells were formed in the ovaries, but the egg chamber was filled with abnormal eggs and embryos.

Maternal exposure of *D. magna* to the fungicide propiconazole at 0.25 mg/L led to developmental arrest at later stages of embryonic maturation, and thus to embryonic abnormalities and death (Kast-Hutcheson et al., 2001). Exposure of D. magna to fungicide tebuconazole at a concentration higher than 0.41 mg/L decreased the total number of broods, number of neonates, brood size per female, and time of the first brood (Sancho et al., 2016).

The insecticide tanrek blocks the oocyte development in *D. magna* and is directly toxic to newborns and adults (Papchenkova, 2012). Sensitivity to carbendazim is higher during egg development in *Moina micrura* (Miracle et al., 2011). These authors suppose that carbendazim adsorptive transfer stripping is an endocrine disruptor.

Disinfectants hypochlorite, formaldehyde, and m-cresol, according to Ton et al. (2012), cause teratogenic effects in embryos of *D. magna* in the late stages of organogenesis.

The actual stage of sex determination at toxaphene exposure of *D. magna* is the oocyte before its final maturation cleavage, before its extraction to the brood chamber (Ignace et al., 2011). These authors draw attention to the fact that thus the sex determination is transgenerational.

The effect of mixture of polychlorinated biphenyls and tributyltin chloride on reproduction of *D. magna* was synergistic, whereas the effect on swimming was mainly additive (Schmidt et al., 2005).

The total number of offspring was slightly decreased in *D. magna* exposed to 17α-ethynylestradiol and norethindrone (Goto and Hiromi, 2003). In *D. magna*, 4-nonylphenol can be directly toxic and embryotoxic, depending on its concentration (LeBlanc et al., 2000): this substance interferes with the metabolic elimination of testosterone. Exposure of maternal *Daphnia* to testosterone caused abnormalities in the development of their embryos. However, 4-nonylphenol-induced abnormalities are different from those caused by testosterone. The concentration threshold for the induction of developmental abnormalities is rather low (c. 44 μg/L). Methyl farnesoate (MF), pyriproxyfen, and fenoxycarb [ethyl 2-(4-phenoxyphenoxy) ethylcarbamate; a juvenile hormone analog] all disrupt ecdysteroid-regulated aspects of embryo development in *D. magna*; exposure of ecdysteroid-responsive cells from *D. magna* embryos to 20-hydroxyecdysterone reduces cell proliferation (Mu and LeBlanc, 2004). Yu et al. (2006) investigated the effect of four estrogens on reproduction of *D. magna*. 4-Nonylphenol decreased the number of progeny in the first and second generations, diethylstilbestrol—in the second generation. 17β—estradiol and bisphenol A caused no statistically significant effects.

Hanazato and Dodson (1992) considered the effect of the *Chaoborus* kairomone and the insecticide carbamyl on *D. pulex* neck teeth formation to be synergic.

Abortion of eggs is a rather common event in Cladocera. A considerable proportion of the parthenogenetic eggs within a brood may be nonviable. In *Daphnia cucullata* and *D. galeata*,

20–35% of females carried a number of nondeveloping eggs and the maximum proportion in such females was 70% (Boersma and Vijverberg, 1995). These authors noted that "it might be a more common phenomenon than is supposed" and mention nutritional deficiency as a possible cause. Threlkeld (1979, p. 611) noted that "it is unclear how widespread such egg abortion is." According to more recent evidence, this is a widespread and quantitatively significant event under normal, natural conditions. In addition, some of the embryos may be resorbed during development.

In *Daphnia*, egg mortality and the abortion of embryos seem to be caused by nutritional deficiency (Gajewskaja, 1949; Boersma and Vijverberg, 1995). Burgis (1967) recorded the presence of "sterile" (nondeveloping) eggs in *Ceriodaphnia*. Resorption of embryos under extreme conditions has been observed in *D. pulex*, *Daphnia pamirensis*, *Evadne*, and *Podonevadne* (Rivier, 1974). For *Bythotrephes*, this has been demonstrated to be a natural process, not necessarily related to water pollution (Rivier, 2005). In *Daphnia rosea*, under natural conditions eggs degeneration can reach 30% of their total number when there is an inadequate concentration of algal food (Redfield, 1981).

In daphnids, parthenogenetic eggs have been shown to develop successfully outside the brood chamber (as mentioned earlier: Rammner, 1933; Obreshkove and Fraser, 1940a,b; etc.); therefore, when liberated into the brood chamber, they do not receive any nutrition from the female and egg mortality must therefore be caused by inadequate food during the period of egg formation. Abortion of resting eggs (accordingly, laying of empty ephippia) in *D. pulicaria* was also recorded (Conde-Porcuna et al., 2011).

Influence of toxic substances on egg abortion. Egg abortion is aggravated by extreme or toxic conditions. In a suspension of blue-greens, the abortion of eggs in *D. pulicaria* increased up to 2.6–12.9% versus much lower values in suspension of green algae (Bednarska and Ślusarczyk, 2013). A noticeable percentage of eggs were also aborted by *D. magna* in the presence of a decomposing mass of the blue-green alga *Microcystis* (Barros et al., 2000). It has also been shown that *Daphnia* can lose eggs because of postabdominal movements that clean blue-green algae from their limbs (Bednarska and Ślusarczyk, 2011).

Daphnia pressed eggs out from the brood pouch following a prolonged exposure to phenols or oil (Lesnikov, 1967). A noticeable percentage of eggs were also aborted by *Daphnia* in the presence of the surfactant, laurox-9 (Shcherban, 1980), of 3,4-dichloroaniline (Ivanova et al., 1989). The active ingredient (isopropylamine salt of glypohosate) of Roundup ay 1.35 mg/L caused 100% abortion of eggs and embryos of *D. magna* (Cuhra et al., 2013).

Long-term exposure of *D. magna* to testosterone at 0.31–2.48 mg/L increased abortion of eggs, to 4-hydroxyandrostenedione at 0.84 mg/L increased mortality of neonates (Barbosa et al., 2007).

Irradiation of *D. magna* with light-emitting diode (650 nm, 0.9 $mWcm^2$) caused abnormalities in the progeny and increased mortality (Vorobyova, 2013a,b).

11.5.3 Resting Eggs

The following notes are clearly incomplete; however, they characterize the current state of investigations in this field. Resting (gamogenetic, dormant, or latent) eggs are produced by all Cladocera. In ctenopods, resting eggs are not enveloped in the ephippium (Korovchinsky, 2004), in anomopods they are. Ephippia are produced of valves and contain one, two, or many resting eggs. In the absence of males, gamogenetic eggs are usually resorbed, either in the ovary or in the ephippium (Zhukova, 1955), or empty ephippia may be produced. Dormant eggs invariably hatch out females, as was shown, e.g., for *Moina* (Grosvenor and Smith, 1913), *P. denticulatus* by Shan (1969).

The same female may form several ephippia, one after another, as has been observed in *Moina*

(Murakami, 1961) and *Eurycercus*. After gamogenesis, parthenogenetic reproduction may be resumed (Sklayrova, 1938; Green, 1956a). Cladocerans may pass not only from the production of parthenogenetic eggs to the production of latent eggs in ephippia but may also backtrack from ephippia formation to formation of parthenogenetic eggs, as reported, e.g., for *Moina* and *Daphnia* (Makrushin, 1969).

During their formation in *Acroperus*, *Alona affinis*, and *Pleuroxus truncatus*, resting eggs are enveloped in a special membrane formed by the paired protoephippial gland (described by Makrushin, 1972). In *Lathonura* (Macrothricidae) and chydorids, Makrushin (1970) discovered glands that contribute to the formation of ephippia (Fig. 11.2). These glands accumulate a secretion during the formation of the resting eggs,

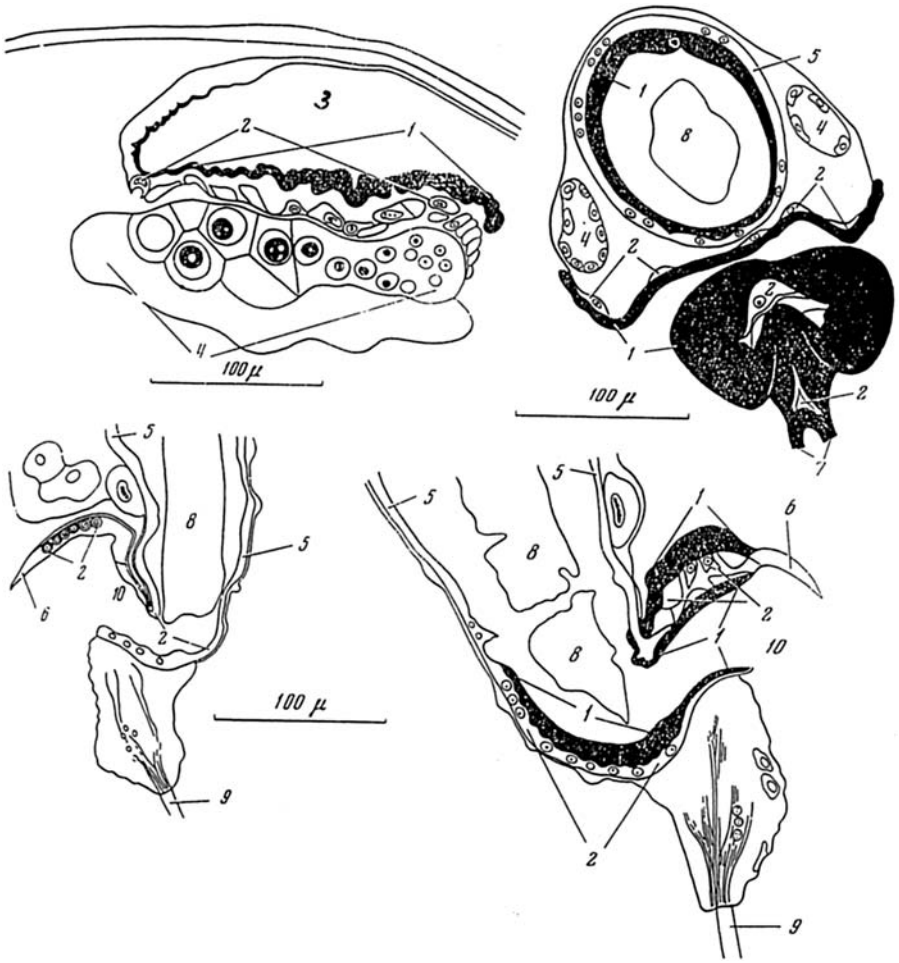

FIGURE 11.2 Glands that participate in ephippia formation in Lathonura and Alonopsis (Upper left, *Alonopsis elongatus* female prior to molting. Latent egg is outside of the optical section. Upper right and bottom right, *Lathonura* gamogenetic female. Bottom left, *Lathonura* parthenogenetic female. 1, gland secret; 2, gland cells; 3, brood chamber; 4, ovary; 5, gut; 6, claws; 7, bases of setae natatoriae; 8, gut contents; 9, seta natatoria; 10, anus. *Makrushin, A.V., 1970. Changes in the organism of females of some species of Cladocera under their transition to gamogenesis. Zoologicheskii Zhurnal 49 (10), 1573–1574.*

which is maximal at molting. In *Lathonura*, the gland surrounds the inner surface of the hindgut, whereas in *Alonopsis* it is situated in the dorsal surface of the trunk, inside the brood pouch.

Ephippia formation in *Daphnia* has been shown to depend on illumination, not, however, on its intensity or polarization (Buikema, 1968), but rather on a short-day photoperiod, in combination with the population density (Stross, 1969a,b, 1987; Stross and Hill, 1968). It has also been observed that *D. magna* does not form dormant eggs in complete darkness (Ślusarczyk et al., 2005). In *C. quadrangula*, the highest resting-egg production was at the photoperiod 6L:6D (Farhadian et al., 2013). The maximum percentage of ephippia is formed at the low concentration of P in food in *M. irrasa* (0.05−1 mg/L) and at higher P concentrations (5−10 mg/L) in *D. similoides* (Meng et al., 2014).

The number of resting eggs per unit area of the bottom of a water body may be very high, but is rarely actually counted. The number (per m^2) of diapause eggs may reach in *Daphnia* 25,000 (*D. pulicaria* in Oneida Lake, New York), 80,000 in *Daphnia galeata mendotae* (Cáceres, 1998), and in *M. macrocopa* 600,000 (data from Japan) (Murakami, 1961). In the author's experience, floating ephippia of *Moina* that were driven by wind to one side of a pond (in the park of the Agricultural Academy, Moscow) could be collected in unlimited quantities (using 8-L pails). Kaya et al. (2014) report tons of ephippia naturally produced in a dammed lake. Egg banks of representatives of zooplankton are discussed by Brendonck and De Meester (2003).

The respiration rate of the resting eggs (i.e., of the developing embryos within resting eggs) was determined to be c. 2.5 μg O_2/mg DW/h in *Leptodora kindtii* at 4°C and increased to c. 6 μg O_2/mg DW/h at 6°C; in *Bythotrephes longimanus*, the respiration was c. 0.5 μg O_2/mg DW/h and did not change within a temperature range of 2.4−6°C (Andrew and Herzig, 1984).

Resting eggs were reported to remain viable for long periods, producing living specimens after 4.5 years (*Daphnia* resting eggs) (De Meester and De Jager, 1993), 13 years (Crispim and Watanabe, 2001), over 1 century (*Daphnia*) (Cáceres, 1998; Kawasaki et al., 2004b), and even in some populations for longer than 200 years (Cáceres, 1998).

Resting eggs remain viable after periods of drying, freezing, and exposure to the digestive system of predators (Kawasaki et al., 2004b). For example, latent *Alona guttata* eggs remained viable after passing through the digestive tract of a duck (Proctor, 1964). Hatching rate was higher in 2-year-old eggs than that of younger eggs (less than 5 months) (De Mester and De Jager, 1993).

Freshwater Cladocera ephippia can also withstand seawater, but under such conditions do not hatch and their resting period is extended (Meijering, 1975). Exposure of diapausing eggs of *Acantholeberis, Diaphanosoma* sp., *Daphnia* sp., and *Leptodora* to open-ocean water (32‰) until they hatched demonstrated that they remain viable under such conditions (Gray et al., 2005).

Drying

Resting eggs may survive desiccation; it may be recalled that this fact was used by Sent-Iler (1860) and by Sars (1886, 1888) for rearing living specimens from dry lake sediment. This efficient method remains in current use (Van Damme and Dumont, 2010). Living Cladocera were obtained from sediments that had been dry for 13 years (Crispim and Watanabe, 2001).

After long periods of desiccation, the percentage of hatching eggs was observed to decrease, as was shown in *D. longispina* (Wood and Banta, 1937), *D. pulex* (Midzuno et al., 1960), and *Moina* (Murakami, 1961). Hatchability of resting eggs of *Ceriodaphnia pulchella* decreased if they were collected deeper than 4 cm of bottom sediments (corresponding to c. 14 years) (Moritz, 1987).

Dried and rehydrated ephippial eggs of *D. pulex* start developing when illuminated with light of wavelength 350−475 μm and energy of 2×10^6 ergs/cm^2 (2 kJ/m^2), with a wavelength of 410 μm being the most efficient

(Davison, 1969). For 100% activation, resting eggs had to remain in the dark for at least 5 days.

Latent eggs of *Bosmina obtusirostris* do not withstand drying, as Makrushin (1989) demonstrated experimentally, therefore they do not live in temporary water bodies.

Drying turned out to be unnecessary for the development of resting eggs of *D. longispina* (Wood and Banta, 1937), and *Streblocerus serricaudatus* (Fryer, 1972). Makrushin (1978, 1980) attributes the ability of resting eggs to withstand desiccation to several properties of the yolk: its homogeneity, fine structure, and absence of vacuoles. Because living progeny may emerge from latent eggs of various ages, generations at different periods within a biological community may be mixed.

Freezing

Under European temperate conditions, latent eggs hatch after ice thawing within a rather brief period (Cáceres, 1998). Diapausing eggs may withstand long-term freezing. Such is the case, e.g., in Diaphanosoma (Vekhov, 1987). Latent daphnid eggs can withstand freezing for 2 years and those of *Bo. obtusirostris* can withstand repeated freezing (Makrushin et al., 1990).

Resting eggs, at least those of midlatitude Palearctic Cladocera, can withstand freezing down to about −50°C, judging by observations made in an area with this average minimum winter temperature (Yakutia in Siberia, Russia); the diversity of such fauna in this region is no lower than that of warmer European areas. In addition, mud samples with diapausing eggs collected in West Greenland and kept frozen at −18°C for 18 years produced *Chydorus*, *Alona*, *Macrothrix*, and *Daphnia* (Meijering, 2003). However, latent eggs of *Limnosida* and *Leptodora* cannot withstand freezing and this delimits their distribution to the north, at least to shallow lakes fully freezing in winter (Vekhov, 1987).

A certain period of freezing is thought necessary before the start of ephippia development. Illumination is also necessary for this process. Both factors seem different for different species and clones (Stross, 1966). The hatching rate of ephippial eggs of *Moina mongolica* increased with incubation for up to 62 days under conditions of alternative wet−dry cycles, and illumination was also necessary for hatching (Lu et al., 2000).

Cryoprotectants

Dormant eggs of *D. magna* contain more glycerol and more Hsp60 (a heat-shock protein) compared with subitaneous eggs (Pauwels et al., 2007). Glycerol is involved in energy storage and is also a cryoprotectant. The heat shock protein is thought to assist in maintaining structural integrity and inhibiting cellular metabolism. Hoshi (1957) noted that sexual eggs contain no Hb.

Dormant *D. magna* eggs contain approximately double the amount of trehalose than their asexual eggs, which increases their tolerance of freezing (Putman et al., 2011).

Hatching of a resting egg occurs due to rupture of the outer, inelastic membrane (chorion) by the increased size of the developing embryo and subsequent uptake of water through the embryonic membranes (as observed in *Daphnia* by Selvaraj, 1979), or due to the osmotic uptake of water, which stretches the elastic embryonic membranes and breaks the outer inelastic membrane (Davison, 1969; Fryer, 1996). The swelling is osmotic and can be retarded by sucrose solutions (Davison, 1969).

The hatching of resting eggs depends on environmental conditions; for example, illumination favors the hatching of dormant eggs. *P. denticulatus* ephippial eggs stored in water in the dark for 84 days were induced by fluorescent illumination to hatch within 6−15 days; earlier times occurred at longer photoperiods (Shan, 1970). The hatching yield decreases at higher light intensities: in the light, up to 50% of *Daphnia* ephippial eggs hatched within 100 days, and only 0−2% in the dark. Moreover, several weeks of storage at room temperature was necessary before latent eggs could respond to light (Pancella and Stross,

1963) and previous desiccation reduced the hatching rate. Light perception by resting eggs is thought to involve carotenoids (Davison, 1969).

Exposure to decreased temperature and 12 h: 12 h L:D cycles resulted in the best hatching rates in *Daphnia carinata* (Tsitsilas and Barry, 2002). Moreover, it was shown that more resting eggs of *Acroperus* and *Alona rectangula* hatch at 10−20°C than at higher temperatures; and a photoperiod of 16 h L:D is generally more favorable (Vandekerkhove et al., 2005). However, these authors found no such preference in other species, e.g., *C. reticulata* and *Daphnia parvula*.

Hatching of *P. denticulatus* resting eggs occurs earlier at a higher illumination levels and longer photoperiods; however, their hatching rate decreased under continuous illumination at >600 fc (foot-candles; approximately 6458 lx) (Kuo-cheng Shan, 1970). Resting eggs removed from dark ephippia hatched earlier. However, a period of dormancy and drying is not always necessary for the further development of resting eggs. In *M. macrocopa*, the majority of resting eggs started development without drying within 2−24 days of their formation (Wood, 1932). It was then shown experimentally that dormant (sexual) eggs of *D. longispina*, as well as those of *M. macrocopa*, do not need drying for further development (Wood and Banta, 1937). These authors believed that altered osmotic concentration of the medium is responsible for the initiation of development of dormant eggs in *D. longispina*.

Failure of dormant eggs of *Daphnia* to hatch might be caused by degradation of biochemical components that are critical for responding to hatching stimuli and renewal of their development (Vanvlasselaer and De Meester, 2008).

Xenobiotics (as has been shown with reference to the fire retardant FireTrol 934) decrease the hatchability of resting eggs of *D. curvirostris* (Angeler et al., 2005). Fenoxycarb (an insect growth regulator) penetrates dormant eggs of *D. magna*; final stages are more sensitive to pesticide exposure (Navis et al., 2015).

Principal aspects of dormancy are discussed by Lampert (2011).

11.6 THE PHYSIOLOGY OF MALES

Almost all facts summarized so far have concerned females. However, the size and behavior of males is different, which implies physiological differences. Unfortunately, data on the physiology of males are very scarce.

The life span of males is considerably shorter than that of females because, as shown with reference to *D. magna* (MacArthur and Baillie, 1929), they are more sensitive to temperature changes. Smaller males of *D. magna* live for c. 30 days on the average (19.1 mln heartbeats), have 10 instars, larger males live for 23 days (13.6 mln heartbeats), eight instars; the longest life duration was 57 days (Fritsch, 1959).

Data on the growth of males are comparatively limited; it was briefly summarized by Frey and Hann (1985). In chydorid males, there are two immature instars and one mature instar (Frey and Hann, 1985). After their third (mature) instar, *Eurycercus longirostris* males live for up to 3 weeks (Hann, 1980). In *D. magna*, males live almost as long as females—up to 45 days at 28°C, and longer at lower temperatures (MacArthur and Baillie, 1929). When exposed to 10−50 mM NaOH or 1−10 mM KCN, the survival time of *Daphnia* males is 75−85% that of females (MacArthur and Baillie, 1929).

Daphnia males have a higher beat frequency of their thoracic appendages than do females (Peñalva-Arana et al., 2007), a higher oxygen consumption (Vollenweider and Ravera, 1968), higher heart rates (Fig. 6.8) (MacArthur and Baillie, 1929; Maynard, 1960), and a higher Hb content (Kobayashi, 1970).

The phototactic behavior of adult males of *D. magna* may be similar to or different from adult females, the difference depending on the clone (De Meester, 1992).

Males have a strong drive for attachment to females, as described, e.g., by Van Damme and Dumont (2006). Male *D. pulicaria* had a maximum distance of detection of females 6.4 mm (Brewer, 1998). In the presence of females, *C. sphaericus* males attach not only to females but also to other males. Sometimes, males (in the presence of females) attach to several other males, making chains of two to three individuals (Smirnov, 1971). Brewer (1998) also observed that male *Daphnia* occasionally grasp males, but for a short time only.

It was also noted that males (*Daphnia obtusa*) pursuing females frequently contact each other ("fight") (La et al., 2014).

There are different ways by which males can recognize conspecific females.

By Touch. In *Moina*, Goulden (1966, 1968) reported that males recognize the gamogenetic female of the same species by contacting their antennules with the surface of the female ephippial shell, which has various different sculptural characteristics in different species. The grasping antennules of *Moina* males are also somewhat different in different species. The same explanation was suggested for *Bosmina* by Kerfoot and Peterson (1980). Primary mechanical examination of mates of *D. obtusa* before actual contact was supposed by La et al. (2014).

By Chemical Sensing. The attraction of males by glycoproteins was demonstrated in *D. obtusa* and *C. dubia* by Carmona and Snell (1995). The attractant (pheromone) was present in the area around the ovaries, where it exceeded the background concentration by two or more times. According to Carmona and Snell, after the male attaches to the female's valve, it then introduces its first antenna and touches the ovary's surface with its sensory papillae. More than one type of glycoprotein was found to be present at this site, thus indicating a potential mechanism for the recognition of conspecific females by males.

11.7 SEX HORMONES

Sex hormones are derivatives of steroids: these include the male hormones, testosterone and androsterone, and the female hormones, estron (progesterone) and estradiol. Both growth and reproduction are steroid hormone-dependent processes. Although the presence of sex hormones may be assumed from general biological considerations, the first papers to directly demonstrate the presence and role of sex hormones in daphnids were only published at the beginning of 1994, although some experimental work was done earlier by von Dehn (1950, 1955).

von Dehn (1955) was probably the first to draw attention to fat in food as a factor controlling cladoceran population dynamics. While experimenting with *Moina*, she found that higher quantities of fat, which occur in declining algal populations, stimulate the appearance of males. von Dehn (1950, 1955) also found that feeding *Moina* with whole or defatted yeast with an addition of an extract containing ergosterin resulted in the production of 30% males, as well as females containing resting eggs.

11.7.1 Female Hormones

The normal content of glucocorticoids in *D. magna* is 8.4–12.7 pmol/g hydrocortisone, that of corticosterone is 6.9–10.1 pmol/g (Polunina, 1999); when estradiol was added to the culture medium, these values changed to 8.3–22.1 pmol/g and 14–20 pmol/g, respectively.

Fecundity of *D. magna* is increased by progesterone (Kudikin, 2001) and by enriching of algal diet with arachidoic acid (Ginjupalli et al., 2014).

Estrogenic effects on *D. magna* were determined using the model estrogen diethylstilbestrol (DES; a nonsteroid vertebrate estrogen) (Baldwin et al., 1995). Chronic exposure to 0.50 mg/L DES reduced molting frequency and the fecundity of second-generation daphnids. Over a period of

21 days, 4-nonylphenol treatment decreased the number of offspring in the first and second generations, whereas DES had a similar effect on the second generation only. When tested on *D. magna*, estrogens (i.e., 17β-estradiol, DES, and 4-nonylphenol) also inhibited molts, whereas bisphenol A did not (Baldwin et al., 1995).

Exposure of female *D. magna* to DES and to methoprene (an insect juvenile-hormone analog) stimulated development of the abdominal process (a characteristic of females) (Olmstead and LeBlanc, 2000). The number of young produced by *Daphnia* doubled in a solution containing gonadotropin [i.e., the urine of nongravid cows, at 0.3–0.7 International Units (IU)/mL in the culture medium] in comparison with medium containing gonadotropin inactivated by heating at 68 °C for 1 h (Čehović, 1954a,b).

11.7.2 Male Hormones

The biotransformation of testosterone in *D. magna* was investigated by Baldwin and LeBlanc (1994a) (Fig. 11.3). Numerous metabolites are produced at phase I of the biotransformation: polar metabolites are preferentially excreted, whereas nonpolar ones are preferentially retained. Testosterone and phase I metabolites are excreted in glucose-conjugated forms and 2α-hydroxytestosterone as a sulfate conjugate. Nonpolar phase I metabolites of testosterone are not sulfate conjugated. These results demonstrate that *Daphnia* can convert polycyclic compounds into multiple polar and nonpolar metabolites.

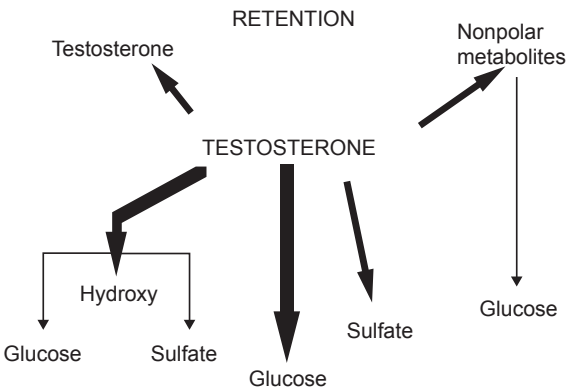

FIGURE 11.3 Testosterone metabolism by *Daphnia magna*. *Baldwin, W.C., LeBlanc, G.A., 1994b. In vivo transformation of testosterone by phase I and II detoxification enzymes and their modification by 20-hydroxyecdysone in* Daphnia magna. *Aquatic Toxicology 29, 103–117.*

Under conditions of chronic exposure to 0.50 mg/L diethylstylbestrol the rate of elimination of hydroxylated metabolites of testosterone in *D. magna* is significantly reduced and that of testosterone glucose conjugates is elevated, thus indicating an alteration to their steroid metabolism (Baldwin et al., 1995).

In *D. magna*, the elimination of several of the glucose-conjugated testosterone metabolites also decreased in proportion to the pentachlorophenol concentration; sulfate-conjugated metabolites also decreased (Parks and Le Blanc, 1996). Mu and LeBlanc (2002a) found that testosterone stops embryonic development in *D. magna* due to its ability to interfere with ecdysteroid control of development.

Testosterone metabolism is enhanced by tributyltin at concentrations approaching lethal levels (c. 2.5 μg/L); this was estimated by the elevated production of hydroxylated, reduced/dehydrogenated, and glucose-conjugated testosterone metabolites (Oberdörster et al., 1998). Exposure of *D. magna* to 1.3 μg/L tributyltin increases the rate of elimination of redox, hydroxylated, and glucose-conjugated testosterone derivatives (LeBlanc and McLachlan, 2000). These authors showed that tributyltin causes modifications to the metabolism (Fig. 11.4) that result in an increased production of oxido-reduced derivatives that are preferentially retained *D. magna* tissues.

It has been shown experimentally that oxytocin increases the fecundity of *D. magna* (Polunina, 1999; Nikitina and Polunina, 2000). Quantitative differences in the concentrations of hydrocortisone and corticosterone in the body of *D. magna* were determined to be seasonal and caused by exogenous oxytocin, prednisolone, testosterone,

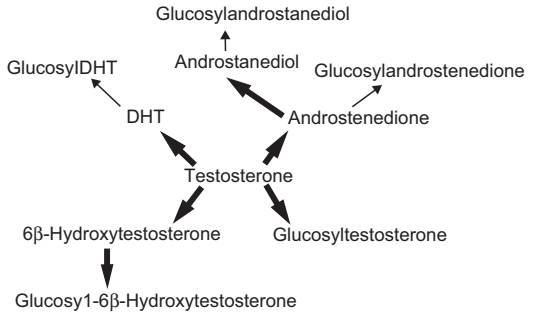

FIGURE 11.4 Metabolic pathways of testosterone in *D. magna* are stimulated by tributyltin exposure (shown in bold). *DHT*, dihydrotestosterone. *LeBlanc, G.A., McLachlan, J.B., 2000. Changes in the metabolic elimination profile of testosterone following exposure of the crustacean* Daphnia magna *to tributyltin. Ecotoxicology and Environmental Safety 45 (3), 296–303.*

and estradiol (Nikitina and Polunina, 2000). According to Palma et al. (2009b), fenoxycarb mimics the action of methyl farnesoate (MF) and thus causes in *D. magna* at 1 μg/L production of 95% of males. Females of *M. macrocopa* at 800 nM MF formed resting eggs (58%) and males (Volkova and Zadereev, 2012).

The sensitive period in ovarian egg development in *D. magna* occurs after 12 h when exposure to 400 nM MF results in the production of all-male broods (Olmstead and LeBlanc, 2002).

The development of embryos in *D. magna* takes two molt cycles: during the first cycle, an ovicell develops in the ovary; following molting, the eggs are transferred to the brood chamber, where they develop into embryos; and these are released just prior to the next molt. The beginning of the intermolt period in adult females is indicated by the presence of a molted exoskeleton. Ecdysteroid-regulated aspects of embryo development in *D. magna* are disrupted by MF, piryproxyfen, and fenoxycarb (antiecdysteroids) (Mu and LeBlanc, 2004).

11.7.3 Xenobiotics Imitating Male Hormones

The production of males may be also influenced by various substances: 20-hydroxyecdysone (a crustacean hormone) at concentrations of 1 or 10 μg/L in the medium increased production of all-male broods in *D. pulex*, but a concentration of 100 μg/L reversed the effect (Peterson et al., 2001). Similar to MF, the exposure of *D. magna* to methoprene (Olmstead and LeBlanc, 2003) and to pyriproxyfen (a juvenile-hormone agonist) (Olmstead and LeBlanc, 2003) causes oocytes to develop into males. Pyriproxyfen increases male production in a concentration-dependent fashion. Arachidonic acid represses pyriproxyfen-induced sex shifts and increases female production in *D. magna* (Ginjupalli et al., 2014).

According to Banta and Brown (1924), in *M. macrocopa* the critical period in which eggs within the ovary may be induced to develop into either females or males is confined to 2–4 h before the eggs leave the ovary. Agents that reduce the maternal metabolism induce the production of males. The critical time mentioned earlier is the period when the spindle is formed during embryogenesis.

The exposure of sensitive ovarian eggs of *Daphnia* to 400 nM MF (an endogenous crustacean terpenoid hormone) results in the production of 100% males, whereas exposure to 52 nM MF produces mixed broods (Olmstead and LeBlanc, 2003), in *M. macrocopa* the concentration 200 nM resulted in formation of 98% of males (Volkova and Zadereev, 2012). In the latter case the general fecundity decreased. Excessive male production was observed at exposure to MF in *D. parvula* (Stoeckel et al., 2008).

Males in *Daphnia* and *Moina* were obtained by addition of juvenile hormone analogs (insect growth regulators, e.g., fenoxycarb, pypiroxyfen, epofenonane) to the cultural liquid. Juvenile hormones are defined (Randall et al., 2002) as a class of hormones which promote retention of juvenile characteristics in insects [but not in Cladocera], fatty acid derivatives produced by neurosecretory cells.

In *D. magna* treatment with methoprene (juvenile hormone analog) resulted in the production of males and inhibited the production of

dormant eggs (Olmstead and LeBlanc, 2001b). Formation of males in *D. magna* was caused by exposure to solutions of MF or diofenolan (an insect growth regulator) (Abe et al., 2015). In *D. magna*, males were produced at concentrations of MF greater than 30 nM (Olmstead and LeBlanc, 2002), in *M. macrocopa*—greater than 50 nM (Volkova and Zadereev, 2012).

A short photoperiod belongs to factors inducing formation of males (Hobæk and Larsson, 1990). Keeping this in mind and taking into consideration that MF induction of males was previously made under long-day conditions, Lampert et al. (2012) applied MF to culture of *D. pulex* under a short photoperiod. Unexpectedly, the clones with a high tendency of male production produced fewer males and the clones commonly producing few males produced up to 40% male at 700 nm MF. The authors conclude that the endocrine disruptive effect of MF may depend on the clonal composition and on the photoperiod. Galimov et al. (2011) found that depending on the population up to 40% of females of *D. magna* exposed to MF do not produce males.

D. magna females were exposed to added to the cultural liquid MF, methoprene, two phenoxyphenoxy derivatives (pyriproxyfen and fenoxycarb), epofenonane, and juvenile hormone III (Tatarazako et al., 2003; Oda et al., 2006); all resulted in the progeny being dominated by males (Fig. 11.5). Exposure to pyripoxyfen and fenoxycarb at a concentration of 330 ng/L resulted in the production of almost all males. Other substances cause the production of males only at higher concentrations (over 3.7 μg/L).

In *D. magna*, they appear when the mothers are exposed to juvenile hormones and the insecticides kinoprene, hydroprene, epofenonane, and fenoxycarb (Oda et al., 2005b,c). Exposure of *Daphnia* to MF also results in the formation of males and to an increased Hb content in the body (Rider et al., 2005). Clones that produced males also showed increased Hb production, whereas nonmale-producing clones produced no Hb in response to the hormone. The authors, therefore, concluded that both physiological processes are regulated by the same signaling pathway.

Kim et al. (2006) obtained males in *Daphnia* and *Bosmina* species following MF treatment, despite the fact that, in three of their species, males previously were unknown. Following this method, Sinev and La-Orsri Sanoamuang (2011) exposed females of tropical chydorids to 100 nM MF and obtained males, which are usually rare in nature.

The induction of male formation by externally added substances indicates the possibility that stimulation by substances that accumulate seasonally in natural water affects the appearance of males. Xenobiotics may also induce the formation of males. The production of males increased in *D. pulex* exposed to polychlorpinen

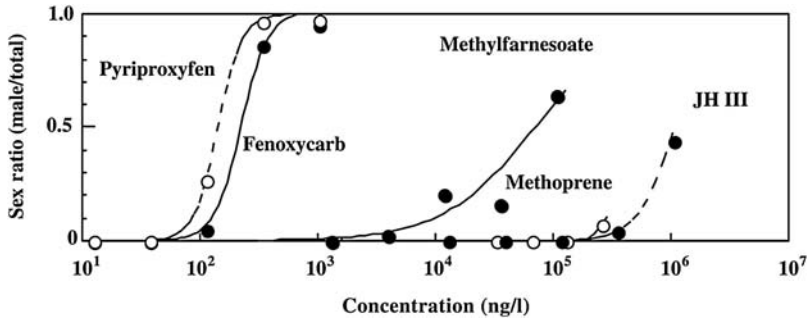

FIGURE 11.5 Increased proportion of males is caused by exposure to various hormonal substances. *JH*, juvenile hormone. *Tatarazako, N., Oda, S., Watanabe, H., Morita, M., Iguchi, T., 2003. Juvenile hormone agonists affect the occurrence of male* Daphnia. *Chemosphere 53 (8), 827–833.*

(at 0.01−0.05 mg/L) (Rivier and Flerov, 1973), in *D. pulicaria* in the presence of atrazine (at 2−2.3 ppb; Fig. 11.1) (Dodson et al., 1999), and in *Moina* and *Ceriodaphnia* in the presence of fenoxycarb (Shigeto Oda et al., 2005a,b,c).

11.7.4 Formation of Males Following Exposure to Chemical Compounds

In early studies Woltereck (1911) determined with reference to *Daphnia* that there are critical periods in egg development for sex determination by outer influences. Banta and Brown (1930) found that chloreton, phenylurethane, and potassium cyanide (KCN) retard the development of *M. macrocopa* and increase the percentage of males in their progeny (observed in the first broods). Substances that disrupt the endocrine system have been shown to also perturb sex determination. Due to these effects, an increased ratio of males was produced in *D. magna* exposed to solutions of dichlorodiphenyltrichloroethane (DDT), methoxychlor, and 4-nonylphenol (Baer and Owens, 1999), in *Moina* sp. and *Ceriodaphnia* spp., exposed to fenoxycarb (Oda et al., 2005a), in *M. micrura* exposed to carbendazim (Miracle et al., 2011). A combination of food deprivation and crowding resulted in the production of males and ephippia by *D. magna* (Olmstead and LeBlanc, 2001b), whereas treatment with methoprene resulted in the production of males and inhibited the production of dormant (resting) eggs.

Addition of acetone (0.1 mL/L) at 16 h light day and low food increased formation of males in the culture of *D. magna* (Zhang and Baer, 2000); however, the effect depended on culture conditions.

The development of morphological traits that are characteristic of a particular sex was shown dependent on biologically active substances; for example, the exposure of males to androsterone (a steroid vertebrate androgen) stimulated development of the first antennae (Olmstead and LeBlanc, 2000).

11.8 IMPACT OF ANTHROPOGENIC FACTORS ON REPRODUCTION

11.8.1 Inhibitory Effects

Generally, xenobiotics inhibit reproduction and reduce the number of progeny in Cladocera. For example, it was shown that at copper concentrations over 0.05 mg/L the number of neonates of *D. magna* decreases (Khangarot and Rathore, 2003; Shilova et al., 2010). Long-term exposure to increasing concentrations of nitrite (NO_2−N) delay maturation, inhibit reproduction, growth, molting, and cause mortality in *D. obtusa* (Xiang et al., 2012). Makrushin (1995) observed that in the zone of industrial pollution in *Bythotrephes* cells of the "placenta" became much smaller than normal ones and thus could not provide for normal development of embryos.

Exposure to acetylcholine esterase-inhibiting pesticides (dimethoate and pirimicarb) caused reproductive damage to *D. magna*, thus decreasing the number of offspring (Andersen et al., 2006).

Dodson and Hanazato (1995) drew attention to decreasing frequency of males of Cladocera in nature supposed to be caused by discharges of estrogen-mimicking substances and to far-reaching consequences of such disbalance.

Testosterone metabolism in *D. magna* is also modified by xenobiotics. Nonylphenyl-polyethylene glycol (NPPG) at 5.0 mg/L inhibits the metabolic elimination of testosterone (ecdysteroid receptor anatagonist) in *D. magna* (Baldwin et al., 1998) as glucose and sulfate conjugates but little affects the rate of elimination of its oxido-reduced and hydroxylated derivatives. Interference with testosterone metabolism in *D. magna* by 4-nonylphenol (product of NPPG degradation) was also studied by Baldwin et al. (1997, 1998). The presence of 25 μg/L or 100 μg/L 4-nonylphenol in solution disrupted the testosterone metabolic pathway, resulting in the elimination of testosterone and the accumulation of androgenic derivatives. These disturbances cause reproductive toxicity to *Daphnia*.

The formation of secondary morphological characteristics in *D. magna* was shown to be modified by 100 μg/L 4-nonylphenol in solution (Gibble and Baer, 2003).

Cadmium (as cadmium chloride; $CdCl_2$) levels as low as 0.01 mg/L negatively influence the growth of *M. macrocopa* and *Macrothrix triserialis* populations (Garcia et al., 2004). In *M. mongolica*, algae (*Chlorella*) preexposed to Cd caused a reduction in the number of neonates, which increased in subsequent broods; this effect was also caused by the lowest Cd concentration tested, 8.5×10^{-21} g Cd per *Chlorella* cell (Wang et al., 2010a,b).

In contrast, Copper incorporation into food *Chlorella* did not cause a decreased brood size for the first brood of *M. mongolica*; however, a decrease was observed in all subsequent broods (Wang et al., 2007). Dietary copper caused inhibition of egg production in *Ceriodaphnia silvestrii* (Rodher et al., 2009). N reduced the reproduction rate of *D. magna* (a decrease of 33% in the total number of offspring per female) and reduced the number of juveniles in the first brood by 21–33% (Evens et al., 2009). Moreover, *D. carinata* maturation was delayed by the presence of lanthanum (La) at levels below the lethal concentration (Barry and Meehan, 2000). The number of *D. pulex* progeny decreased in the presence of cobalt (at 5 μg Co/L; provided in algal food) but was restored by the addition of vitamin B_{12} (Keating, 1985). The highest number of progeny was reached at 0.75–1.00 μg/L vitamin B_{12}.

At concentrations of 0.04–2.66 g/L, banlen (a herbicide, consisting of 2-methyl-4-chlorophenoxyacetic acid and 2-methoxy-3,6-dichlorobenzoic acid) generally increased the number of eggs per brood in *D. magna*, but led to a high percentage of aborted eggs in subsequent generations (Trofimova, 1979). At a concentration of 0.66 g/L, this process was not accompanied by the production of deformed juveniles.

The presence of 2 μg/L endosulfan (an insecticide) reduced fecundity of *M. macrocopa* by 97% in (Chuah et al., 2007). Glyphosate isopropylaminate decreases fecundity of *Ceriodaphnia affinis*, the effect of the same concentration increasing at higher temperatures; some eggs in the brood pouch disintegrate (Melnichuk et al., 2007).

D. magna withstood exposure to 0.1–1000 pg/L of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), but its reproduction rate decreased after 8 days (Wu et al., 2001).

Anionic surfactants (alkyl benzosulfonate, alkyl monosulfate, and sulfanole chloride) in low concentrations caused abortion of eggs and embryos in *D. magna*, and sometimes resorption of their gonads (Shcherban, 1979). These surfactants are more toxic than are surfactants based on polymers.

Steroidogenic pathways are modified by exposure to ibuprofen, because it increases production of 17 β–estradiol, activity of aromatase, and testosterone decreases in a dose-dependent manner (Han et al., 2010).

Electromagnetic field with frequency of 500 Hz inhibits maturation of *D. magna*, decreases fecundity, and increases a part of nonviable progeny and a part of resorbing progeny (Krylov, 2007).

Curiously, all tested concentrations of benomile (a fungicide) suppressed reproduction not decreasing survival of *C. reticulata* (Mangas-Ramirez et al., 2007).

Impact on embryos. At superphosphate concentrations of >0.5 g/L, *Daphnia* embryos develop but cannot break their egg integuments and therefore perish (Sklayrova, 1938). Exposed to a juvenile hormone analog (Altosid ZR-515) at a concentration 1.6×10^{-6} M the embryos of *D. magna* stopped development at early stages, a concentration 3.2×10^{-5} M was lethal for *D. magna*, but a concentration 3.2×10^{-8} M produced no effect (Templeton and Laufer, 1983). Antiecdysteroids (e.g., fenarimol) lower ecdysone level in *D. magna* and result in abnormalities of embryos (incomplete development of antennae and shell spines) (Mu and LeBlanc, 2002b).

Abnormalities in embryos of *D. magna* are caused by the pesticides atrazine and endosulfan. Chlorofos causes abnormalities in embryos and in later stages of *D. magna*, probably inhibiting ChE (Palma et al., 2009c). Endosulfan sulfate increases the level of embryo deformities in *D. magna* and at 91.7 g/L leads to formation of more numerous males (Palma et al., 2009b). Palma et al. (2009a) found that the effect on embryonic development and delayed molting caused by endosulfan sulfate (but not those caused by atrazine) may be reversed by coadministration of 20-hydroxyecdysone. Thus atrazine promotes its toxicity without interfering in the exdysteroid activity.

11.8.2 Stimulatory Effects

In contrast to their effects at high concentrations, low concentrations of various substances may stimulate vital functions or increase life span. This phenomenon is known as *hormesis*. Low concentrations of triazofos or chlorpyrifos increased activity of cholinesterase in *D. magna* (Li and Tan, 2011). With reference to *C. affinis*, it was shown that its life span and fecundity increases at low concentrations of potassium dichromate ($K_2Cr_2O_7$), ethanol, or the antioxidant SkQ1 [(10-(2,5-dihydroxy-3,4-dimethylphenyl)decyl)triphenylphosphonium] (Gershkovich et al., 2012), tested at 1 μg/L Cr, 0.02−0.002 mg/L, and 0.03−3 μg/L, respectively. At 1 μg/L Cr, the average life span decreased compared with control. In addition, ethanol concentrations of 0.125% increased the reproduction rate of *Chydorus ovalis* compared with controls without alcohol (Yatsenko, 1928).

It was also shown that 25 μg/L $ZnCl_2$ increased the number of *M. irrasa* progeny over six broods (Zou, 1997) and chronic exposure to 36 g/L fluoxetine increased fecundity in *D. magna*.

Saponin is lethal for *D. magna* at 5 mg/L [100% lethal dose (LD_{100}) in 2 h] (Naberezhnyi and Gorbatenkiy, 1973). At lower concentrations, it leads to a decrease in life span. At 0.2 mg/L and 2 mg/L, it initially has a stimulatory effect, i.e., there are more numerous progeny than in the control.

A hormetic response (increase in number of neonates) in *D. magna* was observed at exposure to 0.002−0.22 mg/L trinitrotoluene for 21 days at 0.002−0.22 mg/L, whereas at 0.97 mg/L the effect was toxic (Stanley et al., 2013) and at exposure to 1−16 μg triclosan/L, whereas at 64−128 μg/L the effect was inhibitory (Peng et al., 2013). In case of trinitrotoluene this response was explained by its effects on lipid metabolism.

At sublethal concentrations of vertebrate hormones (17β-estradiol, nonylphenol) *Diaphanosoma celebensis* produced more neonates and this effect continued in two subsequent generations (Marcial and Hagiwara, 2007).

Polyhydroxy fullerenes (a kind of carbon nanomaterial) at 1−5 mg/L stimulate the reproduction and prolong the life span of *D. magna* (Gao et al., 2011).

Although brief, this section describes how pollutants that are initially toxic and inhibitory may be diluted and can then stimulate various vital processes (see also Chapter 10 concerning *Ceriodaphnia*).

Parasites and Parasitic Castration. Cladocera are attacked by both predators and parasites (extensively discussed, e.g., by Green, 1974; Lampert, 2011; and by Auld in Chapter 17). Epibionts are shed with each molting. Cladoceran parasites were noted by numerous early authors, such as Leydig (1860). Frequent and diverse *Daphnia* diseases caused by bacteria, sporozoa, fungi, and *Saprolegnia* were noted by Metchnikoff (1892). Metchnikoff (1888) described *Daphnia* that perished due to infestation by the bacterium *Pasteuria ramosa*. Sometimes, phagocytes ingest spores of this bacterium; this was described in detail, and it was confirmed that *Daphnia* egg production discontinued after infestation (Ebert et al., 1996; Auld et al., 2010).

Heavy infestations of both epibionts and endoparasites decrease Cladocera egg production.

Microsporidia that are developing inside the body, especially in the ovary, decrease fecundity in Cladocera (Green, 1974; Voronin, 1986; Voronin and Makrushin, 2006), resulting in a general decline in the population. They have been found in *Alonella*, *Bosmina*, *Chydorus*, *Daphnia*, *Ilyocryptus*, *Moina*, *P. truncatus*, *Scapholeberis*, *Sida*, and *Simocephalus*. In the summer of 1985, up to 97% of *B. longirostris* in the Volga Delta were infested with *Coelosporidium* (Gorbunov et al., 1995). A similar infestation of *Bosmina* was observed in the Rybinsk Reservoir (Russia) in the 1960s.

D. magna infestation with the microsporidium *Octosporea bayeri* induced a higher proportion of males in the progeny, and fewer sperm were produced in parasitized adult males (Roth et al., 2008).

Infestation of eggs by *Aphanomyces daphniae* (Saprolegniaceae) in the brood chamber of *D. magna* has also been reported (Stazi et al., 1994).

11.8.3 Transgenerational Transfer of Xenobiotics

The transgenerational transfer of methylmercury was studied in *D. magna* by Tsui and Wang (2004a). It takes 2.5–3 days for methylmercury assimilated in the gut to be transferred to eggs in the brood chamber. *D. magna* quickly acclimates to Hg, but animals that recover from Hg stress are more vulnerable to Hg toxicity (Tsui and Wang, 2005).

For selenium (Se), it was found that about 19–24% of Se in the F_0 generation is transferred maternally via reproduction to the F_1 generation (Lam and Wen-Xiong Wang, 2006); for Cu, c. 6.5% of the mother's Cu is transferred to the offspring within 7 days (Zhao et al., 2009).

CHAPTER

12

Locomotion

12.1 ANATOMICAL BACKGROUND

Most littoral species crawl and only occasionally swim. The littoral cladocerans have diverse forms: globular, lenticular, and elongated which probably influence their hydrodynamic properties. In one case (*Graptoleberis*), lateral compression is combined with dorsoventral flattening and elongation. In contrast to littoral species, the pelagic species are laterally compressed. True planktonic species all through their life cycle swim in open water and never contact substrata, except their latent eggs that fall to the bottom.

Cladocera muscles do not form large compact masses and are mainly discernible individually. The general structure of the muscular system was studied in planktonic *Daphnia* by Binder (1929, 1931) (Fig. 1.3). Since these early reports, no other investigators have attempted detailed, general investigations in this critically important field. Abundant, but incomplete, data have been published by Fryer (1963, 1968) for some chydorids, macrothricids (Fryer, 1974), and daphnids (Fryer, 1991) (see Figs. 1.1, 1.2, and 1.4).

Muscle fibers comprise the cross-striated skeletal muscles, the dilatators of the esophagus and anus, and the constrictors of the rectum; the longitudinal muscles of the gut consist of smooth fibers (Binder, 1929).

Organs involved in locomotion, as well as in the movements of the inner organs, are set in motion by muscles. Exoskeletal structures, in combination with the muscles, form mechanical tools for the various vital actions of the animal and its component parts. The attachment of muscles to the integuments in *Daphnia pulex* was investigated histologically by Schultz and Kennedy (1977), who studied *Daphnia* tendons and showed that they are located within muscle cells and not within the epidermis.

The exoskeleton of crawling species is strongly chitinized. In littoral species, the cuticle is up to 12 μm thick (in *Pseudochydorus*) (Fryer, 1968). In planktonic forms, it is very thin. Muscles are anchored to the inside areas of the exoskeleton with one end and to working structures with the other end producing skeletomuscular systems (Fryer's term). These systems vary in representatives of different genera but were never specially studied. There are various anchoring structures within the body producing the endoskeleton. With reference predominantly to *Daphnia*, the endoskeleton is discussed by Fryer (1991), who pointed out morphological and functional significance of its sheets, tendons, and fibers.

The largest cladoceran muscles are the dorsal muscles and the three longitudinal ventral muscles that extend along each side of the body from the head to the postabdomen. The lowest ventral muscle rises at the posterior and is attached in the upper zone of the abdominal region in *Daphnia* (according to Binder, 1931; Green,

http://dx.doi.org/10.1016/B978-0-12-805194-8.00012-X

1956c) and *Eurycercus* (Green, 1956c), and to the postabdomen in chydorids; the uppermost ventral muscle descends and is attached in the postabdomen. The crossing over of these muscles allows the possibility of postabdominal movements in a medial plane. From the lateral viewpoint, three large antennal (paired) muscles can be seen in the daphnian head: the two anterior muscles are the antennal abductors, and the third muscle is the antennal levator (Binder, 1931). There is also a small flexor antennulae that is well represented, e.g., in chydorids but reduced in *Daphnia* females.

The labrum is set in motion by levator labri; there are also other small muscles within the labrum. In addition, the adductor muscle of the carapace extends from valve to valve under the gut.

By all evidence, initially the Cladocera were crawling animals (Smirnov, 1975a,b; Fryer, 1987; Kotov, 2013). In the process of morphological and physiological radiation, they produced various benthic and planktonic genera. Obviously, this took place in the very remote past as both benthic and planktonic forms co-occur since c. 145 years before present (Kotov, 2013).

A major agent of transformation of the initial morphological pattern was probably locomotion.

The system of locomotion clearly differs between crawling and swimming species. The former represent the ancestral muscular system, but the available data for crawling species are mostly fragmentary (Fryer, 1963, 1968, 1974). In crawling species, movement is principally achieved by the action of muscles of the thoracic limbs, postabdomen, and antennae, but in pelagic, free-swimming species, this is done by means of the antennae with antennal muscles.

The biomechanics of cladoceran movements comprise levers, joints, and methods of muscle attachment to the skeleton. Variations of the muscular system among different families have not been described; in general, this is rarely commented upon. No detailed comparative investigation has been made in differently specialized species. Investigations of transformations in the muscle system and relevant skeletal structures in different genera are therefore highly desirable. Such studies would contribute to our understanding of fundamental issues regarding the phylogeny and mode of life of Cladocera.

Hebert (1978b) seems to have been the first to relate the swimming ability of *Daphnia* to the size of the antennal muscles, and made special measurements. He calculated the ratio of the length of the first antennal abductor (which he terms the *adductor*) to the carapace length (m/C) in seven *Daphnia* species and found it to vary from 0.15 (in *Daphnia nivalis*) to 0.33 (in *Daphnia projecta*). He also noted that the force of the stroke depends on the cross section of the muscle. The next step in evaluating the swimming capacity involved measuring the stroke rate of the antennae. Hebert (1978b, p. 318) also correctly highlighted the usefulness of this measurement for understanding locomotion in Cladocera: "a thorough investigation of locomotion in *Daphnia*, with particular regard to the relationship between muscle structure and propulsion in media with varying viscosities, would be well worthwhile."

For estimation of the locomotory capacity the ratio of the length of the second antenna (AII) to the body length (A/C) and the ratio of the length of the postabdomen (measured from the base of claw to the base of the setae natatoriae) to the body length (P/C) may be suggested. A/C is especially demonstrative. Its value in crawling chydorids is 0.2—0.3. In planktonic cladocerans it is 0.4—0.5 in *Daphnia* ssp.,— 0.5—0.6 in *Ceriodaphnia* and *Moina* —, and especially high in ctenopods—0.6—1 (in *Diaphanosoma* ssp. it is —0.7—1). P/C in chydorids ranges from 0.3 to 0.5, in ctenopods from 0.1 to 0.2.

12.1.1 Joints

Movement of the Cladocera, as well as some aspects of their food handling, is related to the

structure of their joints. Generally, the following kinds of joints are present in the body:

1. cylindrical joints, in which freedom of movement is controlled by their mechanical potential (e.g., joints of the antennal branches);
2. concertina-like joints (such as those at the base of the antennae);
3. telescopic joints (as in segments of the abdominal part of the body); and
4. a lever joint (between the body and the postabdomen), which is more developed in bottom-living chydorids (Fig. 12.1).

12.1.2 Levers

It is obvious that the general body form of cladocerans is related to their locomotion. It is notable that the posterior part of the body is transformed into a special region, the postabdomen, which is bent at 90–180 degrees to the body axis. The postabdomen with its muscles is the principal lever that pushes the animal (thus liberating it from obstacles) and cleans its food-collecting apparatus; it is especially well developed in bottom-living cladocerans, particularly in chydorids. Concerning the major joint of *Eurycercus*, Fryer (1963, p. 341) noted that "energy losses at joints are reduced to the absolute minimum as only a single 'joint' is involved" (i.e., between the postabdomen and the rest of the body). To understand the action of these levers, both their exoskeletal structure and the muscles that control their action should be considered. The resulting effort depends on where the muscles are attached and the length of the postabdomen. *Camptocercus lilljeborgi*, with its very long postabdomen, makes a unique, extreme case. Green (1956c) observed that its postabdomen may move outside the shell and liberate the animal from obstacles, including from adhesion to the surface film of the water. Green also discusses this problem with respect to his observations on the structure of the postabdomen and of postabdominal muscles in *Eurycercus* and *Daphnia magna*. These studies provide a starting point for further useful investigations. Little, if any, work has yet been done on this subject.

In swimming species, the levers are paired antennae with their muscles.

Chydorids crawl on substrata using thoracic limbs I, use their postabdomen for pushing, and occasionally swim by means of their antennae. Aloninae use their "swimming antennae" for walking and occasionally for swimming.

12.2 ENVIRONMENTAL BACKGROUND

Littoral Cladocera live among various substrata that offer all types of surfaces. Frequently, there are filamentous algae of various diameters commensurate with the crawling appendages of chydorids. There are also small and large clumps of organic debris and there may be both fine-grained and coarse-grained sediments of organic and mineral material on the bottom of water bodies. In bottom-living cladocerans, the form of the shell is usually streamlined, frequently containing teeth at a ventroposterior angle. This angle suggests that they serve to direct away the water flow formed at the posterior end of the animal, but the actual hydraulics have never been specifically studied.

Cladocera may stick to the surface film of water, from which they are not generally able to free themselves. The only reported exception was observed by Green (1956c): *C. lilljeborgi*, which possesses a unique system comprising a long, reinforced abdomen and a postabdomen containing specialized muscles, can rotate its postabdomen outside the shell and thus free itself from the surface film.

Pelagic Cladocera usually live their entire life cycle without making any connection to a substrate. In the aquatic medium, there are turbulent and laminar flows. The hydromechanics of lakes is extensively described by Hutchinson (1957).

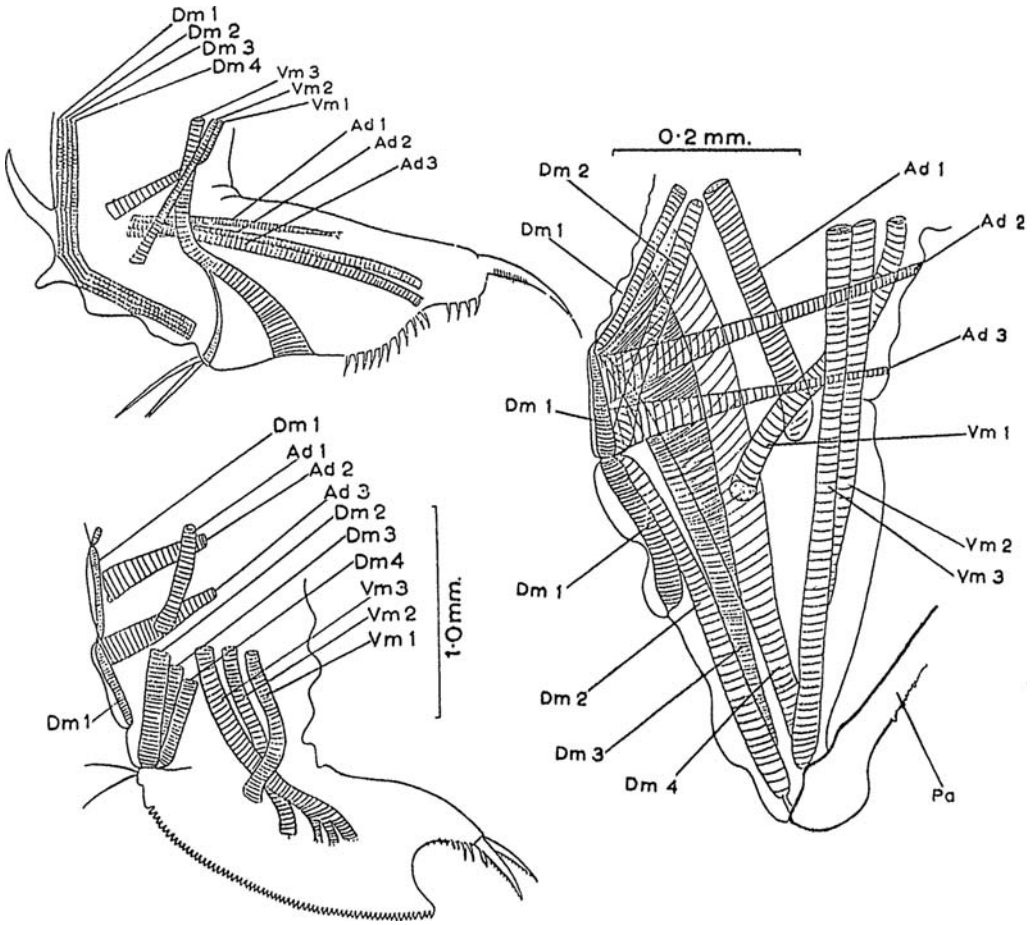

FIGURE 12.1 A lever joint between the abdomen and postabdomen (Pa) and its muscles. Upper left—*Daphnia*, bottom left—*Eurycercus*, right—*Camptocercus lilljeborgi*. *Ad*, abdominal muscle; *Dm*, dorsal muscle; *Vm*, ventral muscle; *Pa*, postabdomen. *From Green, J., 1956c. The structure and function of the post-abdomen of* Camptocercus *(Crustacea, Cladocera). Proceedings of the Zoological Society of London 126 (2), Figs. 4 and 5 on pages 287 and 288.*

The aquatic environment is viscid (Zaret, 1980) and may be characterized by the following parameters Eq. (12.1).

$$\text{Reynolds number } (Re) = \rho ua/\eta = ua/v \tag{12.1}$$

in which ρ is the water density (=1 g/cm^3); u is the flow rate in relation to the body; a is the length of a swimming animal; η is the water viscosity (= 0.010 ps at 20°C); and ν is kinematic viscosity (= η/ρ; for water, $\nu = \eta$ cm^2/s).

For Cladocera, length "a" may be assumed to be 0.1 cm. For small chydorids, "u" may be taken to be 5 mm/s.

Under these conditions, for most chydorids, $Re = 0.5$ cm/s × 0.1 cm/0.01 cm^2/s = 5 (which is much lower than for rapidly swimming and larger fish).

It may be assumed that for all Cladocera the *Re* ranges from 0.1 to 100. At slow swimming velocities, Cladocera movement depends on the viscosity of the aquatic environment, which in

its turn directly depends on temperature. Little is known about the hydrodynamics of small moving objects, such as Cladocera; this is therefore a promising field of investigation (as noted by Fryer, 1968, p. 347).

Issues concerning the specific weight of water and of Cladocera were considered by Wesenberg-Lund (1900), including the role of drops of oil in the body, obtained from food.

12.3 MOVEMENT TRAJECTORIES

Cladocera are slightly heavier than water and sink if they make no effort to swim. The sinking rate of immobilized chydorids is (mm/s at 17°C) 2–2.7, that of *Eurycercus* 2.8–13, of daphnids 2.5–8.6, of *Sida* 11.8 (Smirnov, 1971). It has been shown experimentally that living *Daphnia* sink more slowly than predicted by Stokes' law, and more slowly than anesthetized specimens (Dodson and Ramcharan, 1991; Gorski and Dodson, 1996). The difference may be due to their form and the feeding current produced by the *Daphnia*. A potential hydrostatic role for oil drops that are situated at certain places within the body is evident but has not been especially studied in Cladocera.

Pelagic Cladocera and most of the littoral species are always moving. Benthic species of the genus *Ilyocryptus*, on the other hand, either move slowly or do not crawl for long periods, and some species may be immobile for long periods, e.g., *Lathonura* (before suddenly jumping) and *Drepanothrix* (both belonging to the family Macrothricidae). *Scapholeberis* stays, at least for some of the time, attached to the surface film. The movement of Cladocera species is rarely unidirectional, and their tracks are mostly complicated and tortuous, especially in littoral species; however, until recently, methods for recording three-dimensional movement had not been developed (Uttieri et al., 2005).

12.3.1 Littoral Cladocera

The behavior of littoral Cladocera is extremely diverse; sluggish ones display prolonged immobility and rapid ones that run along various trajectories. Particular species may crawl and occasionally start swimming. Species living on the bottom may run rapidly or swim along tortuous trajectories, or they may be slow or even immobile for long periods. Fryer (1968) observed crawling (running on and between substrata) and scrambling (among clumps of debris and algal filaments) movements. The reasons for such tortuous movement have not been studied, although Scourfield (1905, p. 2) suggested that such movements may depend on the absence of landmarks from which the animal might take a bearing: "It is the difficulty of keeping a straight course owing to the want of fixed points or datum lines, for both horizontal and vertical directions, from which bearings may be taken."

Crawling is performed by means of thoracic limb I and in many chydorids is aided by the postabdomen. In most Aloninae (Chydoridae), their antennae also participate in crawling and are used when such species occasionally switch to swimming. In Chydorinae (Chydoridae), the antennae are pressed to the body while crawling and are only used while swimming. In crawling, *Lathonura* uses limbs I and limbs III, thus being a quadruped, unique among Cladocera. Crawling Cladocera only move forward (never backward). The gaits of crawling Cladocera have not been investigated.

Some Cladocera stay attached to substrata. *Simocephalus* anchors itself by hook-like ends of antennal setae and may withstand rather strong current. It may be mentioned that Meyer-Rochow (1979) suspected that its dorsal head pores of *Simocephalus* play a part in the attachment mechanism. *Sida* possesses a unique dorsal anchoring organ with special muscles and exists mostly attached to substrata (Günzl, 1978a,b; Korovchinsky, 2004).

The various trajectories of Cladocera, especially those of the littoral species, have been little studied and make a promising field of investigation.

12.3.2 Pelagic Cladocera

Pelagic Cladocera mostly exist without any connection with the bottom or any substrata, except their resting stages which may sink to the ground. They swim by means of the second antennae (AII) set in motion by their muscles.

Probably, the first attempts at investigation of *Daphnia* swimming with consideration of gravity were made by Scourfield (1900a,b). The trajectories of planktonic cladocerans consist of sinking and refloating ("hop-and-sink" behavior) as well as of a progressing vector (Lochhead, 1961). Changes in *Daphnia* swimming speed in response to light intensity was discussed by Ringelberg (1964). Parameters of horizontal swimming of *Daphnia* depend on development level of the helmet and the caudal spine (Jacobs, 1964).

However, swimming behavior may be variable and individualistic. Fryer (1991, p. 14) notes: "Even large, heavily built species, such as *Daphnia magna*, do not merely 'hop' essentially in the vertical plane, but can swim horizontally, dive steeply and rapidly head first, pursue a meandering course that can be changed with great rapidity, swim with the body inclined to one side, and orientate[sic] and navigate with great precision."

The observed swimming behavior of D*aphnia* spp. in three-dimensional (3D) space is strongly affected by the light level, food availability, and the volume of an observation chamber (Dodson et al., 1997). In the dark, *Daphnia* spp. swam slowly and exposure to infochemicals from fish changed neither their distribution pattern nor swimming speed (Larsson et al., 2000). Using video recording and computer analysis of their motion, O'Keefe et al. (1998) obtained digitized 3D video records of *Daphnia* movement. Large differences were observed in the swimming speeds of individuals belonging to different clones of the same species. Uttieri et al. (2004) noted that a random component is predominant in *D. pulex* movements.

Daphnia trajectories were recorded using a digital image analysis system (Baillieul and Blust, 1999) or a Critter-Spy, i.e., a high-resolution 3D recording system (Seurat et al., 2004), whereas a 2D representation of the movements of mating *Daphnia* was obtained by Brewer (1998) (see Fig. 12.2).

With reference to *Daphnia* progressive movement was demonstrated to accompany formation of a hydromechanical trail (a wake) (Brewer, 1998), described as a mushroom-shaped vortex by Gries et al. (1999). Characteristics of hydrodynamic trails produced by *D. magna* are studied by Wickramarathna et al. (2014). The volume of trails attains 500 times the volume of *Daphnia*. Gries et al. point out that the characteristics of the wake play a part in chemical communication (e.g., distributing pheromones). Escape response may be induced by kairomones released by predators, as was shown with reference to *D.magna* (Pijanowska et al., 2006).

Kerfoot (1978) filmed the trajectories and akinesis (dead-man response) of *Bosmina* when attacked by *Cyclops* (Fig. 14.1). In *Bosmina*, akinesis turned out to be brief, but long enough that a *Cyclops* would miss the prey. Contrasting changes between "quiet" trajectories of *Daphnia, Ceriodaphnia*, and *Bosmina* and those when attacked by a predatory *Acanthocyclops* are shown by Li and Li (1979). The swimming tracks of *Bosmina* and *Polyphemus*, and their turning rates, were compared in a study by Young and Taylor (1990). The tracks of *Polyphemus* were mostly oriented orthogonally to those of *Bosmina*, thus providing them with a greater probability of encountering prey.

12.3.3 Swimming Parameters

Movement may be measured as absolute units or in body lengths.

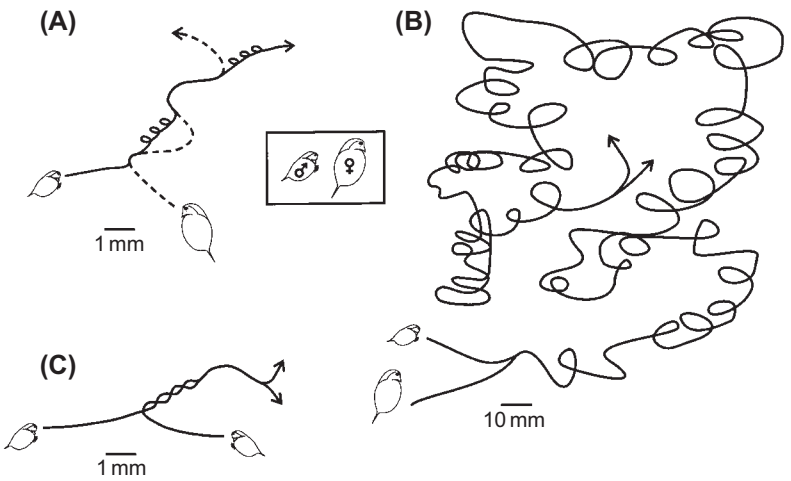

FIGURE 12.2 Trajectories of mating *Daphnia*. (A) male unsuccessfully pursuing female. (B) male successfully grasping female. (C) two males pursuing one female. *Brewer, M.C., 1998. Mating behaviours of* Daphnia pulicaria*, a cyclic parthenogen: comparisons with copepods. Philosophical Transactions of the Royal Society of London. Series B. 353, 805–815.*

Littoral Species

The swimming rate of small chydorids is 2.4–7 mm/s and is 17 mm/s for 2-mm long *Eurycercus lamellatus* (Smirnov, 1971, 1975a,b). As expressed in the number of body lengths per second, it is slowest in *Paralona pigra* (syn. *Chydorus piger*) (= 4.5) and fastest in *Alonella exigua* (= 47) (in *Alonella excisa* it is much slower) (= 19) (Fryer, 1968). In most littoral chydorids (with a body length of 0.3–1 mm), the swimming speed is from 10 to 20 body lengths/s (Fryer, 1968); the rate of antennal strokes in chydorids is 252–406/min (Smirnov, 1971).

Discussing crawling of *Lathonura*, Sergeev (1971) assumes that its center of gravity is situated rather closely to the ventral part of its body.

Pelagic Species

The rate of antennal beats in *Daphnia* ranges from 75 to 250/min (McMahon and Rigler, 1963; Fryer, 1991) but is variable and is usually interrupted by periods of rest (Fryer, 1991, p. 12). In *Bosmina longirostris*, the stroke rate varies within 5–40 Hz (Zaret and Kerfoot, 1980). In swimming *Ceriodaphnia reticulata*, the beat rate of their antennae is c. 1 every 2 s (Seely and Lutnesky, 1998).

The speed of Cladocera varies from 0.01 to 1.0 cm/s (Kerfoot et al., 1980). The swimming speed of planktonic *Daphnia longispina* is 1.4 cm/s at a water current speed of 6 cm/s (Luferova, 1962). At a moderate current speed, most *Daphnia* swam against the current, with a swimming speed of 0.6 cm/s. Szlauer (1965) observed that the swimming speeds of adult and young *Daphnia* are different, which leads to their different distributions. In *Ceriodaphnia quadrangula*, the swimming speed in pale and red specimens was about 43 cm/min at a low oxygen concentration and 25°C; increasing oxygen concentration was followed by an increase in swimming distance. In red specimens, when the hemoglobin (Hb) was blocked by carbon monoxide, the swimming activity increased at increasing oxygen concentrations in almost the same way as occurred in pale specimens (Kobayashi and Yoshida, 1986). The swimming time of pale and red *C. quadrangula* was also studied in nitrogen-saturated water (Kobayashi and Ichikawa, 1987).

Under conditions of food scarcity, hungry *Daphnia* show a tendency to wander, compared to satiated ones; this reaction starts after a delay, when the intestine becomes empty (Szlauer,

1962a,b). It has also been observed that the movement of *Daphnia obtusa* grown on severely phosphorus-limited green algae (*Scenedesmus*) is comparatively sluggish (Sterner et al., 1993). For gravid *D. magna*, it is thought that the antennal rate increases to counterbalance both its increased weight and the displaced center of gravity (Fox and Mitchell, 1953; Lochhead, 1961).

12.4 MUSCLE PHYSIOLOGY

Information about the physiology of cladoceran muscles is scarce and restricted to *Daphnia* only. Normal activity of muscles obviously depends on supply of nutrients. If fed solely on starch, *D. longispina* consumes its fat resources during one to two ovarial cycles, the fat body is not seen any more, work of different heart fibers becomes uncoordinated as well as functioning of the muscles of the antennae and thoracic limbs, and *Daphnia* die in about 6 days (Flückiger and Flück, 1952). Addition of vitamin B_1 (aneurin) to the culture medium of *Daphnia* fed solely on starch restored a normal heart rate.

Exposure of *D. magna* to strychnine solution (1:20,000) (Viehoever and Cohen, 1937) is described as the increasing twitching of antennal muscles seen as a rapid darting motion instead of a usual "hop and sink" movement; abnormal twitching of eye muscles; violent convulsions beginning in about 2.7 min; this is followed by "looping-the-loop and somersaulting" and by longer rest periods. Finally, the animals lapse into stationary convulsions, swim head down, occasionally make feeble attempts at rising to higher levels of water. Then they die due to "the debility of internal organs." Sollman and Webb (1941, p. 266) also noted that strychnine "causes rapid, continuous beating of the antennae which results in rapid circling and looping."

Sollman and Webb (1941) exposed striated (i.e., voluntary) muscles of *D. magna* to mecholyl, pilocarpine, and physostigmine and observed little or no effect. Epinephrine and nicotine stimulated muscular activity in *Daphnia*, but curare first increased and eventually arrested all movements.

Sollman and Webb (1941) also applied electrodes directly to the shell of *Daphnia* and gave them a single electric shock. This resulted in a single contraction of their postabdomen and antennae, and a few beats of their thoracic limbs. A swarm of *Daphnia* emits a weak electric field, originating from their muscular activities, which can be perceived by fish (Freund et al., 2002).

Flückiger (1952) observed the action of the swimming antennae muscles of *Daphnia* to be retarded by L-adrenaline bitartrate, D-adrenaline bitartrate, L-noradrenaline bitartrate, L-ephedrine hydrochloride, dihydroergotamine methanesulfonate, and oxyphenylethanomethylamine tartrate. However, no clear activity was observed in the muscles of their thoracic limbs.

Schwerin et al. (2009) observed that the increased actin concentration in cold-acclimated animals may contribute to the preservation of their muscular performance.

By immobilizing *Daphnia* with D-tubocurarine chloride and measuring their oxygen consumption by means of a Cartesian diver, O'Connor (1950) found that muscle activity consumes 32% of their total metabolism and during intensive activity may take up most of their oxygen consumption. See also Section 5.6.

12.5 IMMOBILIZATION

Now, we will discuss more about immobilization. Both littoral and pelagic Cladocera are very mobile. Their investigation often requires either retarding their movements or complete immobilization, as practiced by various authors. There are two ways to immobilize Cladocera: *physical* (e.g., attaching them to something, as mentioned in Chapter 2) and chemical immobilization.

12.5.1 Chemical Immobilization (Anesthesia or Narcotization)

Immobilization may be caused by low temperatures or by exposure to carbon dioxide. In a solution saturated with carbon dioxide, *Alona*, *Bosmina*, *Chydorus sphaericus*, *Scapholeberis*, and *Simocephalus* lost mobility within c. 0.1−1 min, and *Daphnia* in 14 s (Nikitinsky and Mudrezowa-Wyss, 1930; Mudrezowa-Wyss, 1933). Carbon dioxide produced general depression of *D. magna*, including decreased movement, and the *Daphnia* sank to the bottom (Sollman and Webb, 1941). Of practical importance is narcotization with carbon dioxide (using a saturated liquid) prior to preservation (e.g., with formalin) (Infante, 1978). Under these conditions, the animal, e.g., *Diaphanosoma*, is preserved in a dilated state. Rapid narcotics for Cladocera were carbonated water, chloroform, and methyl alcohol; quinaldine (in acetone and water, 1:16:32) and methyl pentynol caused immediate erratic movements (Gannon and Gannon, 1975).

In experimental practice, *Daphnia* have been immobilized with a chloretone solution (Anderson, 1933), propylene phenoxetol (1% aqueous solution) (Young and Downing, 1976), or 1% urethane (Postnov and Philippova, 1988); and *Moina* has been immobilized with urethane (Hubareva and Svetlichnyi, 1998; Svetlichny and Hubareva, 2002a,b, 2004). Anesthetization of *Simocephalus* and *Daphnia* with 1% urethane was used by Philippova and Postnov (1988) to measure the energy required for movement.

D. magna was efficiently anesthetized by bubbling halothane, isoflurane, and enflurane through the culture medium (McKenzie et al., 1992); this was confirmed by a lack of movement in response to stimulation with strong light. A mixture of chloroform and 96% ethanol at a 1: 10 (v/v) ratio efficiently immobilizes Cladocera (*Daphnia* and *Bosmina*), whereas rotifers are unaffected (Straskraba, 1964). Thus, planktonic crustaceans can be separated from rotifers, allowing quantitative measurements to be recorded separately. Gliwicz (1968) immobilized the pelagic *Bosmina*, *Chydorus*, *Daphnia*, and *Diaphanosoma* using physostigmine salicylate (eserinum). This chemical caused complete paralysis of their appendages at a concentration of 50 mg/L within 3−5 min; it acts by depolarizing and thus inhibiting neuromuscular synapse activity by inhibition of cholinesterases (responsible for acetylcholine breakdown). The author noted that this substance rapidly degraded in water. *D. obtusa* (10% solution) and *Ceriodaphnia dubia* have been anesthetized with tricaine methanesulfonate solution (Carmona and Snell, 1995).

Cyclophosphane also causes paralysis in *D. magna*, which develops as a result of acute energy deficiency (Ivnitskii et al., 1998). These authors found a high protective activity in transporting forms of succinic acid, a sensitizing activity in amino acids, and alleviation by glucose of the protective activity of nicotinamide.

Immobilization is caused by various toxicants, e.g., propanil (a herbicide). For *D. magna* its 48 h half-effective concentration (EC_{50}) value is 3.55 mg/L (Pereira et al., 2007). The time to immobilization of daphnid species in response to DDT (Anderson, 1945), to various pesticides (Munn et al., 2006) or the levels of immobilization in 48 h by metals (Deleebeeck et al., 2008) have been used as measures of toxicity.

Melittin (the main component of bee venom) immobilizes 55% of *D. magna* after 48 h (at 50 μg/mL) and is detrimental for its reproduction (Galdiero et al., 2015).

12.6 FATIGUE AND STRESS

Fatigue was observed by Clarke (1930) in experiments with reactions of *Daphnia* to dimming of light. Having clearly reacted to the first three stimuli the experimental animals then demonstrated a response of about one-third magnitude and needed a 3-h period of rest (complete darkness in these experiments), before a normal response was produced.

Heat-shock proteins (*HSPs*). In response to environmental stress, heat-shock proteins are formed. HSPs were investigated in *D. magna* by Bond et al. (1993). In *D. magna* exposed to 34°C, both HSPs and glutathione *S*-transferase were detected (Bond and Bradley, 1995).

Stress induced in *D. magna* by exposure to predators was demonstrated by increased HSP levels (Pauwels et al., 2005). After exposure to predators, the level of HSP60 increased; then returned to normal levels within 24 h. Mikulski et al. (2009) demonstrated that HSPs of *Daphnia* are involved both in abiotic stress (thermal) and in biotic stress (the threat of predation).

Males of *D. magna*, according to Mikulski et al. (2011), reduce production of HSPs comparing with females, which is supposedly related to their different behavior.

12.7 IMPACT OF TOXICITY ON LOCOMOTION

12.7.1 Natural Toxicity

With increasing salinity, there is an immediate decrease in the swimming velocity of *D. magna*, followed by acclimation and a return to the normal swimming velocity (Baillieul et al., 1998). Frequently, populations of cladocerans exist in blooms of blue-greens (blue-green algae). Using an automated movement tracking system, Ferrão-Filho et al. (2014a,b) studied swimming of *Daphnia* ssp. at abundant development of *Cylindrospermopsis* producing saxitoxin. Swimming time, resting time, distance traveled, and mean velocity were affected by neurotoxin of these blue-greens.

12.7.2 Impact of Xenobiotics

Effects of anesthetics, hypnotics, alkaloids, and toxins to *Daphnia* were briefly reported by Viehoever (1936). Inter alia, podophyllotoxin caused paralysis of *Daphnia*. Phenol caused convulsions of limbs in *Daphnia* followed by paralysis (Viehoever, 1936).

The swimming activity of *D. magna* is also decreased under conditions of acute, sublethal cadmium (Cd) stress (3.5–5.0 g/L) (Wolf et al., 1998), and exposure to 10–100 nM Cd^{2+} (Baillieul and Blust, 1999), tributyltin chloride, or polychlorinated biphenyls (Schmidt et al., 2006). Modification of trajectories (cumulative distance and angular change) in *D. pulex* was successfully used for measuring toxicity of insecticides (Zein et a., 2014).

A high sensitivity (immobilization) to heterocyclic compounds (benzofuran, dibenzofuran, 2-methylbenzofuran, 2,3-dimethylbenzofuran) is shown in *D. magna* (Eisentraeger et al., 2008).

Baillieul and Blust (1999) also demonstrated in *D. magna* that the frequency of antennal beats decreases from c. 4.6 to 3.7 beats/s at a Cd^{2+} concentration of 50 nM. A decreased average swimming velocity has also been demonstrated in *D. magna* exposed to above 5 ppb copper (Cu) using image analysis (Untersteiner et al., 2003).

Estimated by immobilization, toxicity of heavy metals to *D. magna* has different ranking in the following order: Hg (the highest toxicity), Ag, Cu, Zn, Cr, Cd, Pb, and Ni (Khangarot and Ray, 1987, 1989). Immobilization was measured as the number of immobilized organisms collected in the field after 48 h of exposure (Khangarot and Ray, 1987, 1989; Bossuyt and Janssen, 2005). Khangarot and Ray (1989) also found correlations between physicochemical properties of 23 metals and their toxicity to *D. magna*. Silver nanoparticles caused erratic swimming in *D. magna*, being adhered to appendages, external body surface, under the carapace, and ingested (Asghari et al., 2012). As well, they were toxic, the toxicity being dose dependent on the dose and kind of particles.

Toxicity was also different for different species. Immobilization by copper was lowest in *Scapholeberis mucronata* (5.3 μg/L Cu), *D. longispina*, and *B. longirostris*, and highest in *Disparalona rostrata* (c. 71 μg/L Cu), *Peracantha*

truncata (syn. *Pleuroxus truncatus*), and *D. magna* (Bossuyt and Janssen, 2005). Intraspecies differences were observed only in *Ctenodaphnia*.

Saxitoxins (neurotoxins) produced by bluegreen *Cylindrospermopsis raciborskii* immobilized *Daphnia laevis* (Restani and Fonseca, 2014).

Compared with the effect of single compounds, there was an approximately additive effect of exposure to a mixture of polychlorinated biphenyls and tributyltin chloride on *D. magna* swimming behavior, whereas there was a synergistic effect on their reproduction (Schmidt et al., 2005). Presence of amino acids in the medium decreased mortality of *D. magna* caused by copper (Khangarot et al., 1987). In the presence of organic exudates of *Anabaena* (cyanobacterium), the copper toxicity (estimated by immobilization) significantly decreased in *Ceriodaphnia cornuta* (Chouderi et al., 2009).

As known in *D. magna*, the neurotransmitter acetylcholine performing neurotransmitting in cholinergic synapses is hydrolyzed by acetylcholinesterase (AChE). AChE is inhibited by organophosphates (e.g., dichlorvos) causing hyperactivity, loss of coordination, convulsions, paralysis (Ren et al., 2015).

Of special interest is injection to *Daphnia* of the hydra extract, supposed to contain the toxin produced by hydra, performed by Viehoever (1936), which resulted in "brief initial cramps," then in paralysis and death. Unfortunately, his attempt is described in three lines, without details on this experiment.

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CHAPTER

13

Nervous System and Sense Organs

13.1 ANATOMICAL BACKGROUND: SENSE ORGANS

On the subject of Cladocera, Leydig (1860, p. 3, translated from German) remarked that "the nervous system, as the center of the animal organism, deserves, similar to in other animals, a complete and precise investigation."

Early data mainly dealt with the most discernible cephalic region. The first detailed general description of the nervous systems of *Daphnia* and *Moina* was made by Spangenberg (1877), who preferred to use young, recently molted specimens in his studies. He also dissected specimens that, according to his description, were placed in a weak wood vinegar solution for several days. The material was then macerated for 2 days in a mixture of alcohol, water, and glycerol, and then stained with osmic acid. This study was followed by an investigation of the nervous systems of *Bythotrephes, Leptodora, Sida*, and *Simocephalus* by Samassa (1891) (Fig. 13.1). This author preserved cladocerans in osmic acetic acid and used histological methods for their analysis. The principal traits of the nervous system in *Daphnia* were revealed by Fischel (1908) by means of intravital staining with alizarin (Fig. 13.1). The general structure of the nervous system of Cladocera was also discussed by Leder (1913). Referring to Leder, Sterba (1957a,b) distinguished in the brains of daphnids the following three principal parts: the protocerebrum, comprising frontal organ and eyespot with neuropiles I–III, optical ganglia, and neuropil IV; the deutocerebrum, comprising neuropil V belonging to antennule; and the tritocerebrum (situated outside of the brain on esophageal connectives), consisting of centers of antennae.

Weiss et al. (2012) used modern methods for a comparative investigation of the nervous systems of three species of *Daphnia*. These authors concluded that the function of the frontal filament (ventral frontal organ) is unclear and that the function of the dorsal frontal organ is probably secretory. They note that "however, it remains to be investigated which substances are secreted." By Nissl staining the head of *Daphnia* spp., they also revealed "bulged cells" in the dorsal area and near the antennule, whose function is thought to be secretory. These authors do not cite the study by Angel (1967), who indicated the presence of similar cells in *Daphnia magna*; the cell near to the antennule seems to correspond to that described by Weiss et al. (2012). Angel called them *storage cells* in the figure, but no comments were made in the text. The frontal organs have been examined by several authors, but their functional role is unclear.

The nervous system was studied in excellent detail in *Leptodora* (Kirsch and Richter, 2007) and in *Penilia* (Fritsch et al., 2013; who applied

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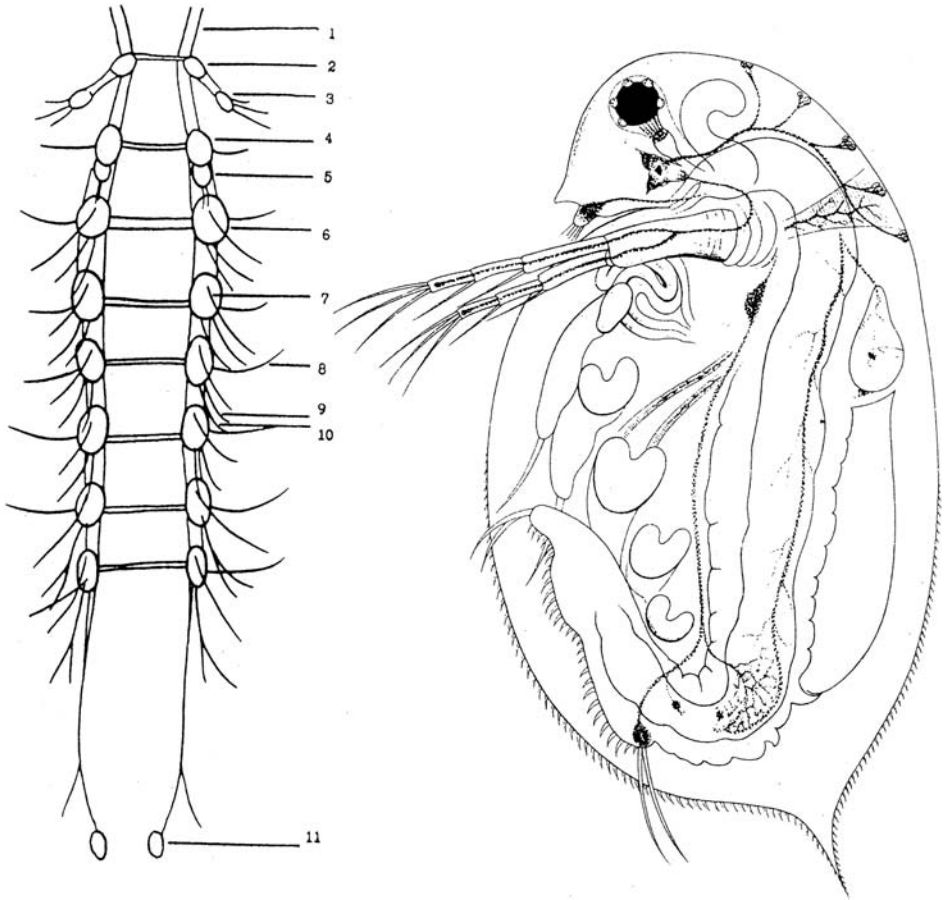

FIGURE 13.1 Nervous system. Left, *Sida crystallina*. Right, *Daphnia*. *Redrawn from Samassa, P., 1891. Untersuchungen über das centrale Nervensystem der Cladoceren. Archiv für Mikroskopische Anatomie 38, 100–141; right, Fischel, A., 1908. Untersuchungen über vitale Färbung an Süsswassertieren, insbesondere bei Cladoceren. Werner Klinkhardt, Leipzig, 60 p.*

confocal scanning microscopy combined with staining).

The ventral nerve cord consists, as shown, for example, with reference to *Sida* and *Leptodora*, of a double chain of ganglia joined by longitudinal connectives and transverse junctions, thus producing a ladder-like appearance. Each ganglion sends out nerves to the appendages. The largest anterior ganglion is the brain (the suprapharyngeal ganglion), which supplies the eye and the ocellus with nerves.

Central parts of the nervous system send nerves to various specific organs: the gut, heart, and sense organs. According to Fischel (1908), the brain supplies the ganglion opticum and the occipital organ with nerves. Fischel also demonstrated ganglion at the base of setae natatoriae (dorsal setae at the boundary between the abdomen and postabdomen) and nerves supplying the heart and thoracic limbs. The cephalic region of the nervous system is shown in Fig. 13.2. The brain and the adjacent optic ganglion were described by Lidvanov and L'vova (2003). These authors arrived at the conclusion that the nervous system of *Daphnia* is homologous to that of Anostraca. In *Daphnia*, most neurons of the

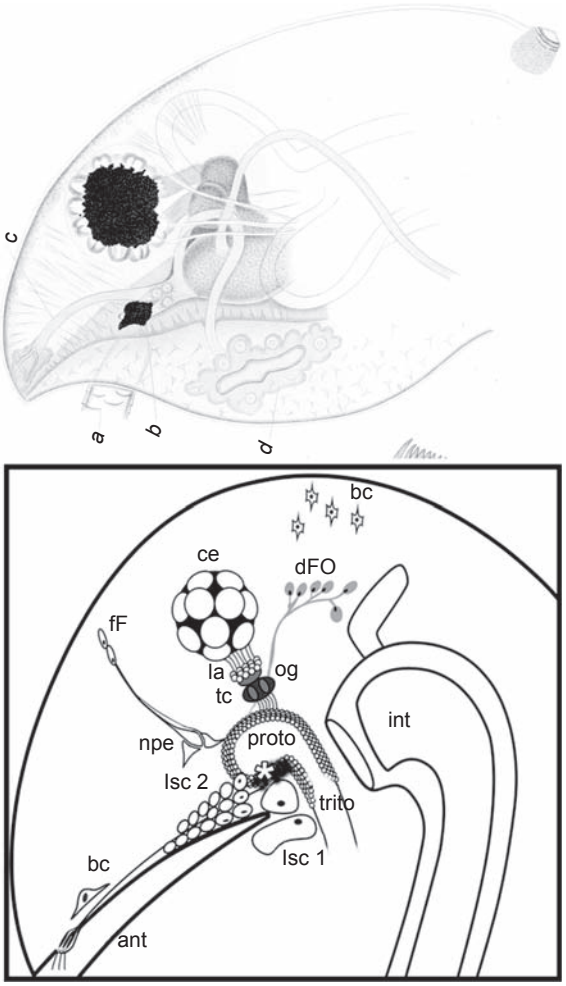

FIGURE 13.2 Cephalic region of nervous system. Above, *Eurycercus*, *a*, antennule; *b*, ocellus; *d*, lateral organ. Below, *Daphnia*; *ant*, antennule; *bc*, bulged cells; *ce*, compound eye; *dFO*, dorsal frontal organ; *fF*, frontal filament; *la*, lamina; *lsc*, labral secretory cells; *npe*, nauplius eye; *og*, optic ganglion; *proto*, protocerebrum; *tc*, tectum; *trito*, tritocerebrum; *, deuterocerebrum. *Above, Leydig, F., 1860. Naturgeschichte der Daphniden. Tuebingen, 252 p.; below, modified from Weiss, L.C., Tollrian, R., Herbert, Z., Laforsch, C., 2012. Morphology of the* Daphnia *nervous system: a comparative study on* Daphnia pulex, Daphnia lumholtzi, *and* Daphnia longicephala. *Journal of Morphology, 1–14.*

central nervous system are multifunctional, thus indicating that the system is primitive. They also arrived to the conclusion that there are no structures for the liberation of neurohormones into the hemolymph and that neurosecretions (the synthesized neuropeptides) are liberated via axons.

Responses of *Daphnia* to light are executed by less than 200 neurosecretory cells (Waterman, 1961). The optic ganglia and central brain of *D. magna* are recently described in detail by Kress et al. (2015). They identified 5 allostatin A−like neuron types, 13 FMRFamide-like neuron types, 5 tachykinin-like neuron types, and 6 histamin-immunoreactive neuron types. Strauss et al. (2011) identified a pigment-dispersing hormone (a peptide) restricted to interneurons in the brain and visual ganglia. Circadian rhythm is revealed in activities of a lateral pigment-dispersing hormone neuron.

Angel (1967) observed that many cells in the central nervous system exhibit a glycogen cycle.

Of special significance is the conclusion that cholinoreceptors of *Daphnia* are analogous to those in rats (Tonkopiy et al., 1994b).

Following on the ideas of Hutchinson (1953), it may be said that all environmental factors act together on all sense organs. Hutchinson (1953, p. 156) states that "there are an enormous number of interconnecting, internal things." This immensely complicates any estimation of the resultant effect by scientists; however, cladocerans themselves can do it, thus performing every function and movement in their own way.

13.2 NEUROSECRETION

The major functions of the nervous system comprise general neurosecretory regulation and general control of the activities of sense organs. As shown in daphnids, the cladoceran nervous system is cholinergic. Nervous impulses in Crustacea are transferred by acetylcholine (ACh) from nerve endings to particular organs. It is known [Prosser and Brown, 1967 (1962)] that ACh is synthesized in the presence of adenosine triphosphate and cholinesterase. Daphnid cholinesterase shows the characteristics of a pseudocholinesterase since it prefers

propionylthiocholine to ACh (Vesel et al., 2006). The characteristics of pseudocholinesterase (cholinesterase II) include inhibition by diisopropylfluorophosphate, prostigmine, physostigmine (eserine), tetraethyl pyrophosphate, and hexaethyl tetraphosphate [Prosser and Brown, 1967 (1962)].

13.2.1 Neurosecretory Cells of the Nervous System

Sterba (1957a,b) distinguished special cell groups in the cephalic part of the *Daphnia* nervous system that differ in their structures. He could clearly distinguish them from neighboring cells by their Gomori-positive staining and also distinguished different cell groups by their varying secretions; he identified cells in the protocerebrum, the deutocerebrum, a segment of antenna II, and a segment of the mandible (Fig. 13.3). Sterba found that secretions are liberated from these cells without accumulation in a special reservoir and that the amount of secretion is greater at the beginning of egg maturation and very low immediately before the eggs are transferred to the brood chamber. During these periods, molting takes place.

The gland on both sides of the labrum of *D. magna* and *Daphnia pulex* with a supposed endocrine function was indicated by Pyatakov (1955). This author published no drawings, and his description is rather vague. Probably, this gland may correspond to the labral secretory cells (lsc) in Weiss et al. (2012, Fig. 2A, lsc). Four distinct cell groups were identified by Angel (1967) in the supraesophageal ganglion of *Daphnia* (Fig. 13.3), and Halcrow (1969) revealed areas of presumed neurosecretory activity in the nervous system of *D. magna* (Fig. 13.4) after staining with paraldehyde fuchsin. Fuchsinophilic granules are found in the following regions of the nervous system: scattered throughout the optic ganglion, on the anterior and ventral surfaces of the brain, on circumenteric connectives, and at the junctions of the second ventral commissure with ventral nerve cords. The presence of these groups was confirmed and reinvestigated by Bosch de Aguilar (1969, 1972), who found the following groups of neurosecretory cells in the cephalic ganglion of *Daphnia*: frontal, ventral, a group of esophageal connectives, groups of postesophageal commissures, cells of the mandibular ganglion, neurosecretory cells at the site of connection of the ventral chain and of nerves of the second pair of thoracic limbs (Bosch de Aguilar, 1969), and the parapharyngeal group (Bosch de Aguilar, 1972). In *Podon intermedius*, neurosecretory cells were found in the protocerebrum, at the boundary between the protocerebrum and the deuterocerebrum, at the base of the antennal nerve, and in the tritocerebrum (Bosch de Aguilar, 1971).

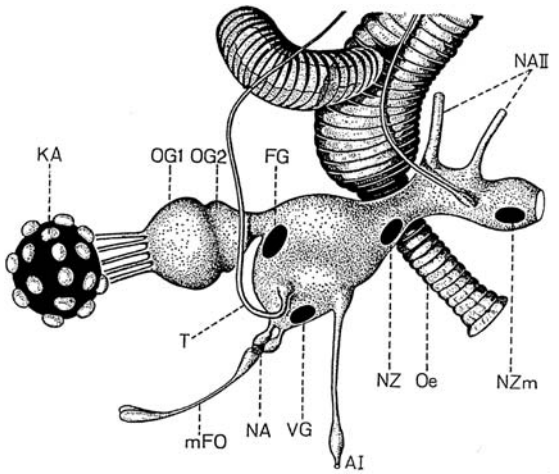

FIGURE 13.3 Neurosecretory areas in the anterior region of the *Daphnia* nervous system. *AI*, antenna I; *FG*, neurosecretory cells of "the frontal group"; *KA*, compound eye; *mFO*, median frontal organ; *NA*, eyespot; *NAII*, nerves of antenna II; *NZ*, neurosecretory cells on the circumenteric connective; *NZm*, neurosecretory cells on the mandibular ganglion; *Oe*, esophagus; *OG1*, optical ganglion 1; *OG2*, optical ganglion 2; *T*, tegmentarius, the nerve to lateral frontal organ; *VG*, neurosecretory cells of "the ventral group." *Sterba, G., 1957a. Riesenzellen der Daphnien-Oberlippe. Zeitschrift für Zellforschung 47, 198–213; Sterba, G., 1957b. Die Neurosekretorischen Zellgruppen einiger Cladoceren (*Daphnia pulex *und* D. magna, Simocephalus vetulus*). Zoologischer Jahrbücher, Abteilung Anatomie 76, 303–310.*

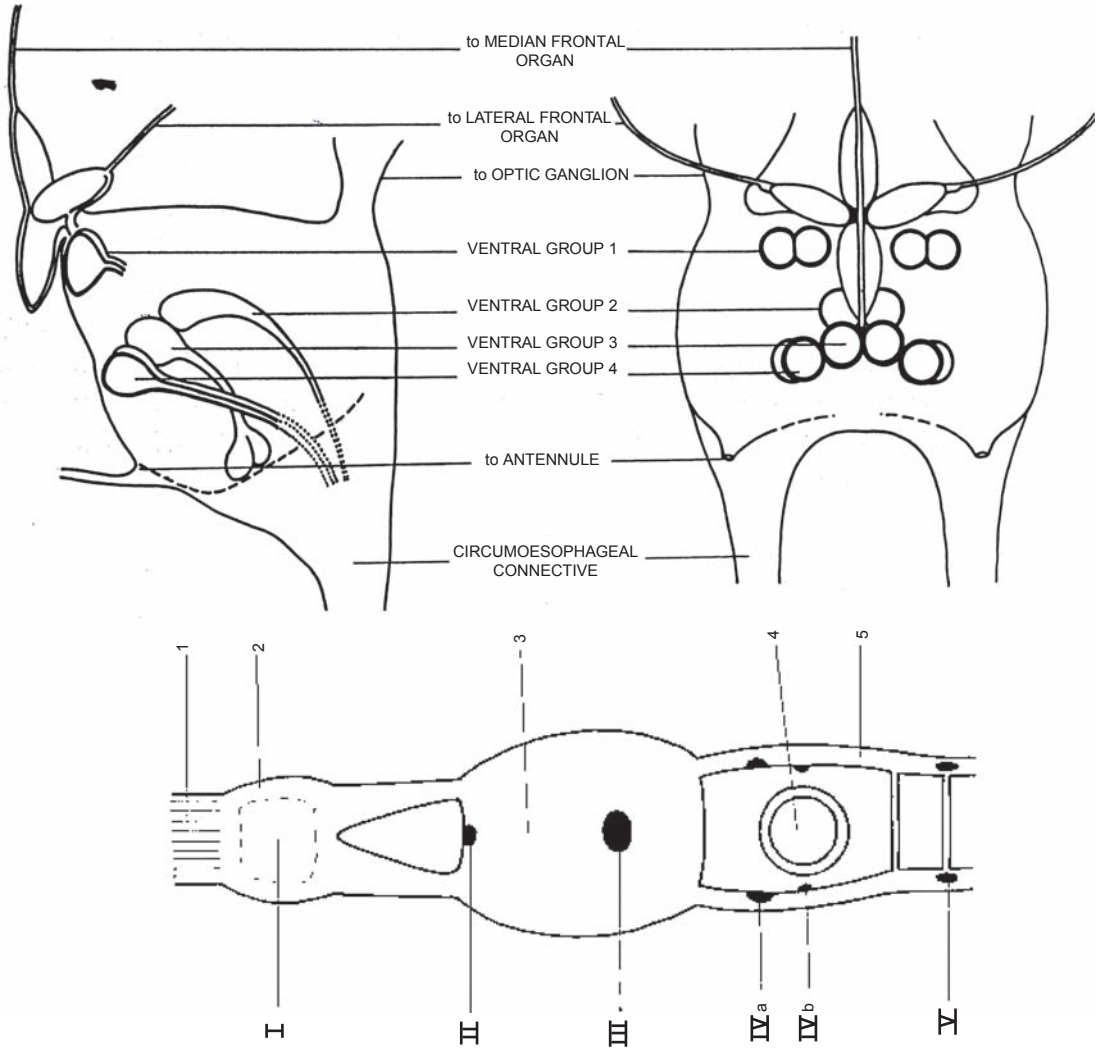

FIGURE 13.4 Neurosecretory areas in the anterior region of the *Daphnia* nervous system. *1*, nerves to compound eye; *2*, optic ganglion; *3*, brain; *4*, lumen of gut; *5*, circumenteric connective. *Upper, Angel, M.V., 1967. A histological experimental approach to neurosecretion in* Daphnia magna. *In: Stutinsky, F. (Ed.), Neurosecretion, Springer, Berlin, Heidelberg, pp. 229–237; lower, Halcrow, K., 1969. Sites of presumed neurosecretory activity in* Daphnia magna *Straus. Canadian Journal of Zoology 47 (4), 575–577.*

Neurosecretory cells have also been found in the brain of *Simocephalus* (Zahid et al., 1980).

In the nervous system of *D. magna*, a hyperglycemic hormone and immunoreactive neurons are found (Zhang et al., 1997). Their wide distribution is shown in Fig. 13.5.

A well-developed histaminergic system was identified in *D. magna* and *D. pulex*, including in the visual system, by McCoole et al. (2011) (Fig. 13.6); histamine takes part in the negative response of *Daphnia* to ultraviolet radiation (UVR), but exposure to cimetidine (a blocking

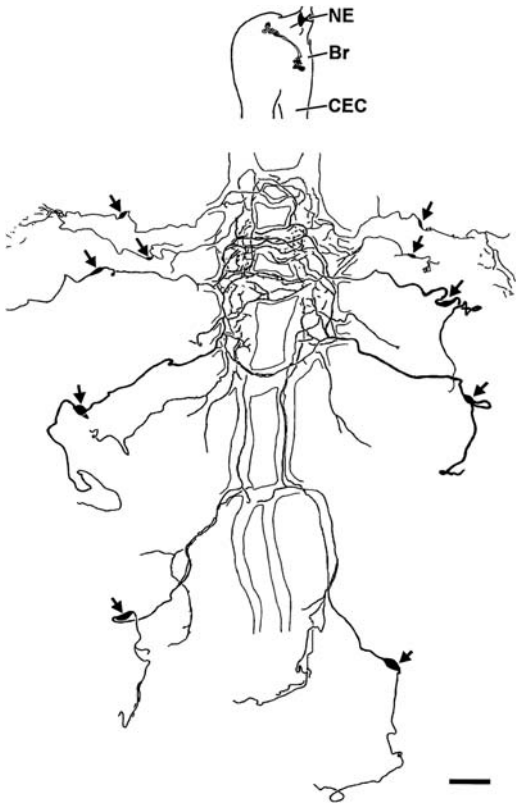

FIGURE 13.5 Distribution of hyperglycemic hormone-reactive neurons originating in the thoracic ganglia of *Daphnia magna*. *Br*, brain; *CEC*, circumesophageal connective; *NE*, naupius eye. Scale bar, 50 μm. *Zhang, Q., Keller, R., Dircksen, H., 1997. Crustacean hyperglycaemic hormone in the nervous system of the primitive crustacean species* Daphnia magna *and* Artemia salina *(Crustacea: Branchiopoda). Cell and Tissue Research 287 (3), 565–576.*

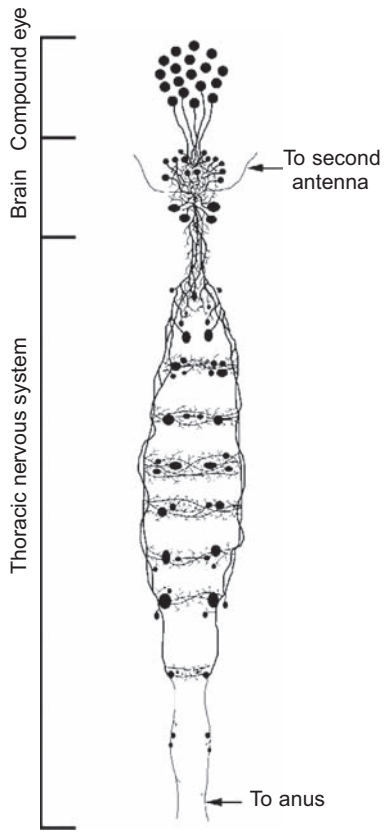

FIGURE 13.6 *Daphnia* nervous system showing histamine-like immunoreactivity sites (*filled circles*). *McCoole, M.D., Baer, K.N., Christie, A.E., 2011. Histaminergic signaling in the nervous system of* Daphnia *and a role for it in the control of phototactic behavior. Journal of Experimental Biology 214, 1773–1782.*

agent) inhibits this negative response. Further study led to the discovery in *D. pulex* of signaling dopamine, octopamine, and serotonin, as well as receptors and transporters for each amine (McCoole et al., 2012a). These studies demonstrate that *Daphnia* possess a nervous system that controls the functioning of various, distant structures. Continuing studies on neurochemistry, McCoole et al. (2012b) found signaling systems involved in nitric oxide, carbon monoxide, and small molecule transmitters (ACh, glutamate, and γ-aminobutyric acid); for each compound, the proteins responsible for its biosynthesis, packaging, reception, recycling, and degradation were identified.

13.2.2 Neurosecretory Substances

By observing the state of neurosecretory cells and the corresponding stages of molting and reproduction, Bosch de Aguilar (1969, 1972) concluded that the esophageal group produces

a factor that inhibits molting, the mandibular group controls development of eggs, and the ventral group produces a factor that inhibits formation of the ephippium.

The following classes of substances used for chemical communication in the nervous system of are now found in Cladocera, and their functions are supposed (Christie and McCoole, 2012): (1) Peptides are the largest class. Numerous peptides found in *Daphnia*, for example, a hyperglycemic hormone (Montagné et al., 2010), and their roles are listed by Christie and McCoole (2012): diuretic hormones, ecdysis-triggering hormone, insulin-related peptide, etc. (2) Specially, some proteins may be involved in diffusible gas transmitter signaling and in small molecule transmitter signaling (ACh, glutamate, and γ-aminobutyric acid systems). (3) Amines: dopamine, histamine, octopamine, and serotonin. As Christie and McCoole (2012) note, multiple proteins are involved in each aminergic signaling pathway.

Supposed neurotransmitters or neuromodulators (and their metabolites or precursors) were discovered in *D. magna* by Ehrenström and Berglind (1988). *D. magna* synthesizes and transforms these substances, which include 3,4-dihydroxyphenylalanine (L-DOPA), dopamine, noradrenaline, adrenaline, tyramine, epinine, 3-methoxyturamine, 3,4-dihydroxyphenylacetic acid (DOPAC), L-tryptophan, 5-hydroxytryptophan, 5-hydroxytryptamine, and 5-hydroxyindolacetic acid. The range of test concentrations for these substances was 0.01−32.8 ng/mg protein. The quantity of catechols L-DOPA, dopamine, and DOPAC in *Daphnia* showed diurnal variations, reaching a diurnal peak at 8 a.m., although other biogenic amines did not exhibit diurnal variations. A possible role as neurotransmitters or neuromodulators is ascribed to epinine, 3-methoxythyramine, DOPAC, L-tryptophan, 5-hydroxytryprophan, and 5-hydoxyindolacetic acid.

Acetylcholinesterase (AChE) hydrolyzes ACh and is inhibited by surfactants, as was shown in *D. magna* (Guilhermino et al., 2000). Different sensitivity of *D. magna* to enantiomers of organophosphates (insecticides profenofos, fonofos, and crotoxyphos) was shown by different inhibition of AChE (Nillos et al., 2007).

Tonkopiy et al. (1994a) demonstrated that M-cholinolytics decrease the toxicity of armine, aminostigmine, and arecoline for *D. magna*. On the basis of experiments with cholinomimetics, H-cholinolytics, and M-cholinolytics, it was concluded that *Daphnia* possess a well-developed cholinergic system, containing cholinoreceptors, that is analogous to that of mammals. In *D. magna*, Podosinovikova et al. (2001) inhibited the dopamine system by blocking the D_2 receptors with haloperidol. After this treatment, *Daphnia* were exposed to solutions of cholinoblockers (amedine, amizil, atropine, cyclodol, norakin, pentifin), and their antihaloperidol activity was estimated. The swimming behavior of *Daphnia* has been shown to become erratic at a dopamine concentration ca. 100 mM (Peñalva-Arana et al., 2007).

13.3 SENSE ORGANS

For a long time, only two kinds of sense organs were recognized in Cladocera: the eyes (Figs. 1.1, 1.2, 13.1, and 14.1) and the esthetascs (sensory papillae) on the antennules. Now, various other sense organs are also known to be present, or are suspected of being so, in cladocerans. While some sense organs, such as eyes, are anthropomorphically understandable, most of the sense organs of Cladocera have nothing in common with those of mammals, although they may provisionally be designated as organs of chemical sense (chemoreceptors) and tactile organs (mechanoreceptors). The latter may be involved in the perception of gravity and of equilibrium. The presence of sense organs that perceive vibration, for example, stridulation of aquatic organisms, is also suspected, and Dumont and Van de Velde (1976) suggested that one of the functions of head pores might be vibration detection.

The olfactory setae (esthetascs) were examined and compared by Scourfield (1896), who

also noted that there are nine in female anomopods, Sididae, and *Leptodora*; six in female *Holopedium*, and five in female Polyphemidae. Much later, Rieder (1987) described the esthetascs in detail (see Fig. 13.7).

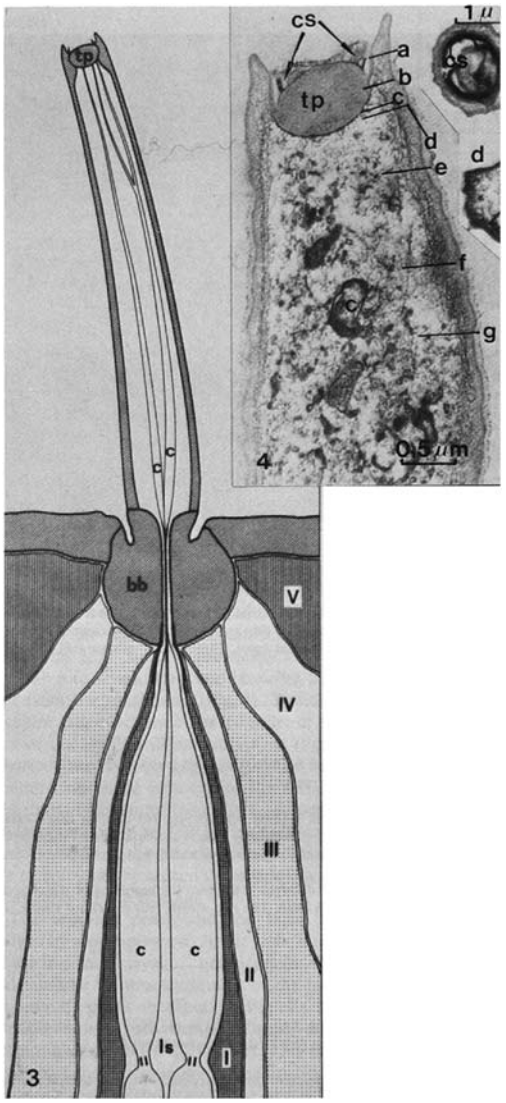

FIGURE 13.7 Structure of an esthetasc of *Daphnia magna* and of its terminal part. The inner space (Is) and the basal bead (bb) of each of nine olfactory setae are surrounded at its base by five sheath cells (I–V). The receptor cilia (c) extend to the terminal pellet, tp. a–g, cross sections at different levels. *Rieder, N., 1987. The ultrastructure of the so-called olfactory setae on the antennula of* Daphnia magna. *Hydrobiologia 145, 175–181.*

Sensilla were identified on the thoracic limbs of chydorids and macrothricids by Fryer (1963, 1974), Smirnov (1967) (Fig. 13.8), and Dumont and Silva-Briano (1997). No such sensilla are present on the limbs of daphnids. Generally, on the body (limbs included) of a cladoceran, there are numerous papilliform or setiform structures that are suspected to have a sensory function.

In the head, there is a lateral frontal (parietal) sense organ (Gicklhorn, 1931b) (Fig. 13.1), and each of its cell clusters is supplied by a nerve arising from the cerebral ganglion (Gicklhorn, 1931b; Fryer, 2004). Its function may be light perception or it may be a pressure gauge (Fryer, 2004). The median (ventral) frontal organ is similarly innervated (Fryer, 2004).

Cladocera can perceive a wide range of external stimuli, such as various kinds of irradiation, including daylight, colored light, and polarized light, plus various chemical, olfactory, and mechanical stimuli.

13.4 VISION

13.4.1 Anatomical Background

Most Cladocera possess an eye and an ocellus (a pigment spot), both of which are black. The eye consists of integumental cells, an intercellular

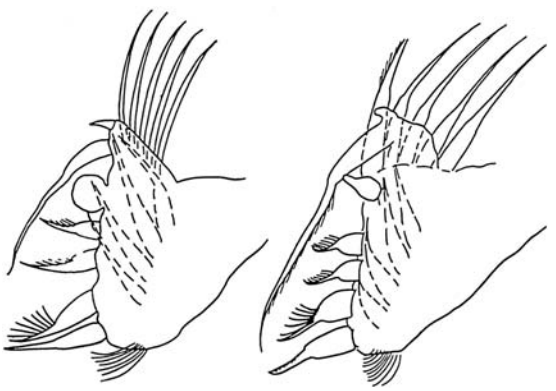

FIGURE 13.8 Esthetascs on thoracic limb IV. Thoracic limb IV of *Alona affinis* (bottle-like, right), of *Alona quadrangularis* (globular, left). *Smirnov, N.N., 1971. Chydoridae. Fauna SSSR, New Series, No. 101, Nauka, Leningrad, 531 p. (in Russian).*

substance, lenses, and receptor cells containing pigment (Figs. 1.1, 1.2, and 1.6) (Gueldner and Wolff, 1970). The structure of the eye is compound; a *Daphnia* ommatidium is shown in Fig. 13.9. The ocellus is a pigment spot.

In different species, both the eye and the ocellus may be developed to various extents or may be absent. The general trend is toward an increased ocellus in bottom-living forms, in contrast to pelagic species. The relative size of the eye is different in different species. It is very large in *Dadaya*. In polyphemids, it constitutes a large part of the total body volume. In some bottom-living species, which obviously live in the dark (in interstitial spaces), the eye may be small or absent, whereas the ocellus may be increased in size, or both may be absent. In contrast, in planktonic species, the ocellus is usually small or absent. The ocellus may be normally very small (as in *Daphnia* species) or absent (as in most *Moina* species). Both the eye and ocellus and their function have been studied using various experimental methods, including surgical removal (extirpation).

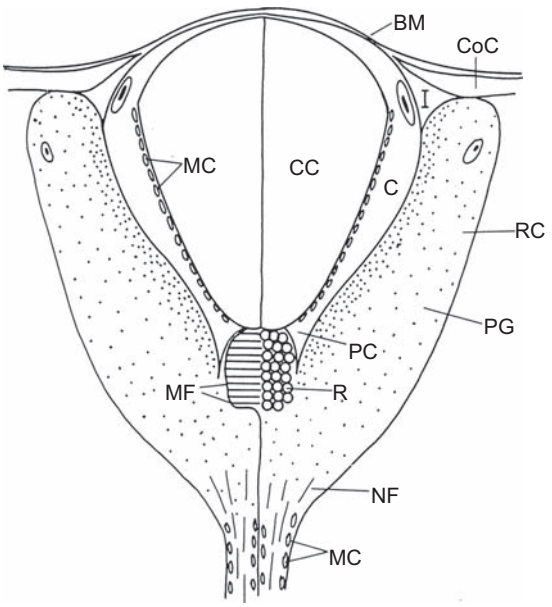

FIGURE 13.9 Structure of a *Daphnia* ommatidium. *BM*, basilar membrane; *C*, cone cell; *CC*, crystal cone; *CoC*, covering cell; *MC*, mitochondria; *MF*, microvilli; *NF*. Neurofilaments; *PC*, process of cone cell; *PG*, pigment granula; *R*, rhabdom; *RC*, retinula cell. *Ringelberg, J., 1987. Light induced behaviour in* Daphnia. *Memorie dell'Istituto Italiano di Idrobiologia 45, 285–323.*

In various chydorids, the eye is situated just on the cephalic ganglion. As direct observation shows, it is either immovable (as in females of *Alona affinis*, *Alonella nana*, *Camptocercus fennicus*, *Disparalona rostrata*, *Graptoleberis testudinaria*, *Picripleuroxus laevis*, and *Pleuroxus trigonellus*) or flutters within a very limited amplitude (as in *Acroperus*, *Chydorus sphaericus*, and *Pleuroxus truncatus*). The muscles of the eye seem to be short.

In *Eurycercus*, the eye is situated at some distance from the brain and is moved by thin muscles, as shown clearly by Leydig (1860). It flutters, and makes limited rotations, and is sometimes turned up by ca. 180 degrees.

As reported for daphnids, the eye is situated at some distance from the brain and is continuously rotated by means of thin muscles. Three pairs of muscles move the eye: the levator, the lateralis, and the depressor (Binder, 1931). These muscles are inserted ventrally, dorsally, and laterally onto the eye (Consi et al., 1987). It should also be mentioned, for comparison, that the eyes of vertebrates, for example, fish, are also supplied by six muscles: four rectus oculomotorius muscles and two oblique oculomotorius muscles. Downing (1974) discovered that the *Daphnia* eye is suspended in position by a membrane that forms a watertight seal between the eye and the hemocoel.

The eye in preserved specimens may be discolored by alkaline solution, and the number of lenses (ommatidia) may then be easily counted. This number may be up to 10 in *Penilia* and chydorids, 44–81 in other ctenopods (Korovchinsky, 2004), 28 in *Eurycercus lamellatus*, 22 in *Moina micrura* (Smirnov, 1975), and 22 in *Daphnia* (Heberdey and Kupka, 1942; Downing, 1974; Frost, 1975; Fryer, 2004).

Comparative investigations into the topography of the eye and its muscles, and of eye movements, although they are possible and would be very informative, have never been done in bottom-living Cladocera (Chydoridae, Macrothricidae, and Ilyocryptidae).

Bottom-Living Cladocera

Few studies have been made into the vision of bottom-living and littoral Cladocera. Occasional reports have been made on color vision (see the following) in the littoral species *P. truncatus* (syn. *Peracantha truncata*) and *Scapholeberis mucronata*, which are attracted by blue illumination (Peters, 1927), and on *Ceriodaphnia*, *Kurzia, Moina, Pseudochydorus, Sida*, and *Simocephalus* (Smith and Baylor, 1953).

Pelagic Cladocera

EXPERIMENTAL APPROACH AND DIRECT OBSERVATION

Most experimental evidence concerns planktonic species. The *Daphnia* eye rolls almost ceaselessly (i.e., is in a state of rotatory tremor) as it is pulled by special muscles, and is thought to scan visual information in this way. It was assumed that such eye movements are related to the mechanism of vision, that illumination is assessed by *Daphnia* in this way, and that the necessary angle in relation to the source of light is retained (Waterman, 1961). Frost (1975) determined four kinds of eye movement in *D. pulex*:

1. tremors at 16 Hz;
2. a scanning movement at 4 Hz;
3. large, fast eye movements; and
4. optokinetic nystagmus, produced by moving striped patterns around a daphnid.

The first two kinds of movement were observed in diffuse light and the third kind in the presence of spots of light crossing the visual field. Three other types of activity by the *D. magna* compound eye were discovered by Consi et al. (1990): a "flick" caused by a flash of light, "fixation" in response to a stationary light stimulus, and "tracking," i.e., following a moving stimulus (Fig. 13.10). When a light stimulus is situated at about 80 degrees dorsal to the eye's axis, there is no response (this is the null area).

13.4.2 Environmental Background

Solar light rapidly becomes extinct in water, especially long-wavelength (red) light (see, e.g., Hutchinson, 1957, Chapter 6), so the spectral composition changes with depth. Red light (720 nm) extinction is complete at ca. 4 m depth in pure water (Dodson, 2005). In water, polarized light has great ecological importance; it is reflected from various particles, including food algal cells.

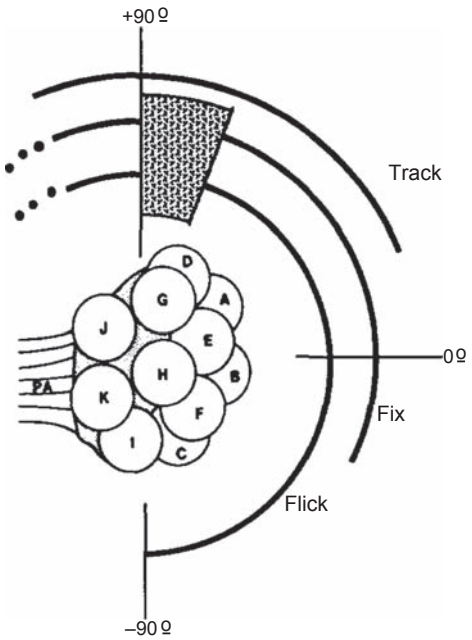

FIGURE 13.10 Behavioral regions on the compound eye of *Daphnia*. *A–K*, lenses; *fix*, fixation, *PA*, photoreceptor axons. *Consi, T.R., Passani, M.B., Macagno, E.R., 1990. Eye movements in* Daphnia magna. *Journal of Comparative Physiology A 166, 411–420.*

Information on illumination and day length is supplied by melatonin (indoleamine, a hormone). It is present in animals and plants, and was determined in *D. magna* for the first time by Markowska et al. (2009). In *D. pulex*, melatonin attenuated the response to predator stress (formation of neckteeth) and decreased production of resting eggs (Schwarzenberger et al., 2014). The peak of melatonin production occurs at midnight; its synthesis is controlled by arylakylamine N-transferase (Schwarzenberger and Wacker , 2015).

13.4.3 Surgical Methods of Investigation

Eyeless daphnids were used in some investigations into the effects of light, despite the fact that such specimens (*Daphnia* and *Simocephalus*) very rarely occur in nature.

Extirpation of the Eye and Ocellus

For investigating the reactions of eyeless *Daphnia*, Schulz (1928) removed the eye through a puncture hole made by a fine needle. For this procedure, the daphnid was placed upon a slide, and excess water was removed so that the daphnid's head was outside the water. When the integument above the eye was punctured, the eye emerged above the integument and could be separated out directly or was shed with the carapace at the next molting. This was confirmed by Downing (1974), who repeated this method. The percentage of successful operations was satisfactory, and, after two molts, all damage caused by the operation was healed and the animals could be used for experimentation.

Extirpation of the complex eye for experimental purposes was also described by Harris and Mason (1956), who copied the methods of Schulz (1928). In their experiments, a *Daphnia* was placed into a moistened groove made in paraffin, on its back and slightly tilted to the side. The carapace was then punctured above the eye with a fine pin. If the hole was successfully made, the eye emerged above the puncture. It was then removed, and the animal was returned to the culture medium. After some time, it became evident whether the animal had survived the operation; if so, such animals were used for experimentation. Generally, these animals survived for several weeks and reproduced even more intensively than normal animals. In a similar fashion, Schulz (1928) separated and removed the ocellus. Several animals survived the operation, molted, and were used in experiments into the effect of light on various processes. Later, Consi and Macagno (1985) also removed the ocellus from *D. magna* immobilized with carbonated water using a microbeam from a dye laser (Candela SLl-500; 450 nm) coupled to a Zeiss microscope. Several laser pulses at 26−27 kV were used to delete the ocellus. The animals recovered after 2−4 days and were used in experiments. Such animals had the same spectral sensitivity as controls.

After extirpation of the eye and the ocellus, the reactions of daphnids to light remain essentially the same due to their general light sensitivity (Schulz, 1928; Harris and Mason, 1956; Fryer, 2004). In Consi and Macagno's (1985) experiments, animals that had the ocellus removed had the same spectral sensitivity as normal animals. Schulz (1928, p. 543) concluded that "the ocellus of daphnias as a rudimentary organ does not play a noticeable part any more in the life of these animals." However, completely eyeless *Daphnia* do not exhibit a dorsal light reflex (Schulz, 1928). These blinded *Daphnia* are also more sensitive to vibration (Harris and Mason, 1956). In summary, *Daphnia* generally do well without eyes.

Surgical removal of eyes and ocelli in *D. magna* confirmed that they do not mediate accumulation of carotenoid pigments (Herring, 1968).

The effect of darkness. Cultivating *Simocephalus* in the dark, Jermakoff and Ermakov (1927) found that only 3−7% of specimens lost pigment in 25−20 days, and extreme starvation resulted in decomposition of the eye and ocellus, but

that happened in a short time before the animals perished. *Simocephalus* (Smyly, 1967) and *C. sphaericus* (Smirnov, 1971) successfully propagated in the dark. The latter demonstrated no change of eyes, comparing with controls kept in the light (Smirnov, 1971).

The effect of change in the spectral composition. When *Daphnia schoedleri* were cultivated in blue light, no pigment sensitive to red light was formed (over a period of 44 days); however, if returned to white light, the normal level of this pigment was reached after 90 days (McNaught, 1971).

The effect of food limitation. Scarce feeding resulted in reduction of the eye diameter in *Daphnia* sp. (Brandon and Dudycha, 2014).

13.4.4 Abnormal Eyes

Normally, Cladocera have one eye and one eyespot (ocellus). Ocellus is normally absent in some species (e.g., *Moina* sp.). There are species that normally have no eye (e.g., *Monosplilus*). Also, there are blind chydorids having neither eye nor ocellus (Brancelj, 1997). *Alona hercegovinae* has no eye, and its ocellus is represented by a single transparent cell (Brancelj, 1990a,b).

Though very rare, two-eyed specimens occur either in nature or are obtained experimentally (presence of the eye separated into two halves in late embryos is not meant here). There is only one case of two eyes in *Sida* from an unpolluted lake reported by Korovchinsky (1978). Short-term exposure of the initial culture of *D. magna* to potassium dichromate (1 mg/L) induces morphological abnormalities in the following generations (Isakova and Kolomenskaya, 2002): in the generation F2, small-sized juveniles, juveniles with deformed rostrum, and a unique specimen with two eyes were present; the progeny of the latter (198 specimens) had no morphological deviations.

Eyeless specimens of normally eyed species are also extremely rare, e.g., eyeless *Moina* and *Simocephalus* have been reported (Banta, 1921; Banta and Brown, 1922). Fryer (1953) identified a specimen of *D. pulex* that had no complex eye, although eyes were present in its embryos. Eyeless specimens were obtained in the progeny of *Daphnia* exposed to prometrin (a triazine) at concentrations 3–4 mg/L (F1–F3) (Pushkar and Usacheva, 1977).

Mutant *Daphnia* have been reported that lack black pigment in the eye (Young and Downing, 1976). With reference to *Simocephalus*, it was shown that the form of the ocellus is different in different individuals, and it may be even split into two parts (Jermakoff, 1924). The ocellus was smaller in *Simocephalus exspinosus* from ponds populated with predators than from fishless ponds (Konechny et al., 1982).

13.4.5 Taxes

Reaction to Light: Phototaxis

Daphnia have been found to be primarily negatively phototropic and positively geotropic, but a reduction in light intensity reversed the signs of tropism (Clarke, 1930). This reaction persisted for a few minutes, showing that *Daphnia* exhibit labile behavior in relation to illumination. If illuminated from the side, daphnids turn toward the more strongly illuminated side.

Wavelength peaks of light sensitivity are different in different species; in *D. magna*, they occur at 440, 470, 520, and 640 nm (Wechsler and St. John, 1960), and in *Daphnia retrocurva*, they occur at 370, 435, and 570 nm, and less so at 685 nm (McNaught, 1966; McNaught and Hasler, 1966).

The threshold of visual sensitivity in *Daphnia* is 10^{-4}–10^{-5} lx (McNaught and Hasler, 1964).

The response of *Daphnia* to illumination is dynamic. These reactions are not rigidly fixed and vary, depending on changes in the environment and on the condition of the animal (Loeb, 1924).

A reduction in light intensity reverses these reactions for a short time (Clarke, 1930), and periodic changes of phototactic signs have also

been observed under constant low illumination (Clarke, 1932). This may be understood as exploratory behavior. Experimental reversal of the reaction of *D. magna* to illumination was also observed by Chernykh and Panasyuk (1964). The basic observations by Ringelberg (1964, 1987) were that under conditions of decreasing intensity of illumination, *D. magna* move toward the light source; at increasing intensity, they move away from it. A continuous decrease in light intensity led to upward swimming.

Initially, photopositive *D. pulex* became more positive under the action of prolan (Skadovskiy, 1939b).

De Meester and Dumont (1988) identified three phenotypes of *D. magna* with respect to light: positive phototaxis, negative phototaxis, and random wandering between areas of low and high light intensity. They consider these phenotypes to be genetically determined. Thus, reactions to light may be individual; some specimens may be strongly negatively phototactic and others strongly positively phototactic, even within a single, natural *D. magna* population (Dumont et al., 1985). *Daphnia* specimens may deviate by up to 30 degrees from the direction perpendicular to the polarization plane (Waterman, 1960, 1961). However, shoaling *Daphnia* congregate at the water surface, i.e., contrary to the aforementioned tendency (Fryer, 2004). *Scapholeberis* also attach to the surface film of water under bright illumination and *Holopedium* ascends to the water surface by day and moves to deeper water by night (Fryer, 2004).

Starved *Daphnia* tend to move, whereas satiated ones stay at the same level. Starved *D. pulex* swim upward, but starved *D. magna* sink (Szlauer, 1962b).

The orientation of a daphnid depends on the direction of light. When illuminated from above, daphnids maintain a position with their dorsal surface turned toward the light (Schulz, 1928; Harris and Mason, 1956), an orientation known as the *dorsal light reflex*. When illuminated from below, the daphnids swim upside down, i.e., with their dorsal side turned toward the source of light (Schulz, 1928; Harris and Mason, 1956). These results deserve further investigation.

As a result of being attracted by light, some Cladocera can also be collected in light traps, especially *Bosmina coregoni*, *Bythotrephes*, *Eurycercus*, and *Leptodora* (Szlauer, 1971).

It is thought that the muscles that move the eye also transfer the stimulus to muscles of the antennae; if daphnid eye muscles are strained differently at each side of the eye, then the antennal muscles act to change the position of the body (Harris, 1953; Jander, 1959).

Movement of the *D. magna* complex eye may also be stimulated by its intermittent illumination (but not by the illumination of any other part of the body) (Young, 1974). If the complex eye is illuminated from above, then maximum movement is caused by the short-wavelength range of the spectrum; if illuminated from the side, then maximum movement is caused by the yellow—green range.

Genostrychnine intensifies the photokinetic activity of *D. pulex*, and chlorpromazine renders its occasional movements less frequent (Rimet, 1965).

Extraocular light sensitivity also exists (Ringelberg, 1987). Light is sensed through integuments, as well as the eyes, as shown for *D. pulex* by Scheffer et al. (1958). However, light perception and phototactic movements occur in *Daphnia* when both the compound eye and the eyespot are extirpated (Ringelberg, 1987). Fryer (2004, p. 46) also noted that "eyeless individuals manage their affairs reasonably well!"

Reversal of Phototaxis

The reaction of *Daphnia* to light may be reversed if some of the large constellation of factors that affect their behavior change (Loeb, 1924). For example, reversal of normal negative phototaxis in *C. sphaericus* and *Chydorus ovalis*

by acids or CO_2 was shown by Bryukhatova (1928, 1937). Clarke (1932) also demonstrated that phototaxis in *Daphnia* may be reversed by changes in external or internal factors.

Skadovskiy (1939a,b, 1955) noted that the reaction of *D. pulex* to light may be different depending on the environmental circumstances. In *Daphnia* settling in summer to lower water levels in a eutrophic water body, the positive reaction to light becomes more intensive and they move to warmer and better aerated layers, whereas with insufficient alimentation, their negative reaction to light increases and they settle into colder water layers with more abundant food.

In the presence of a sufficient quantity of undissociated carbonic acid molecules, of NaCN (0.0001−0.00001 m), and under anoxic conditions, phototaxis of *D. pulex* becomes positive (Skadovskiy, 1939a,b, 1955). Positive phototaxis also occurs in a situation where there is insufficient oxygen. In negatively phototactic *Daphnia*, the oxygen consumption rate is considerably higher than in positively phototactic ones.

At heart rate of 150 beats/min, *D. pulex* are totally positively phototactic, at 350 and higher, mostly negatively phototactic; in the same culture, positive specimens have a lower heart rate than negative specimens (Skadovslkiy, 1939a).

Various factors may be responsible for the reversal of phototactic reactions in *Daphnia*. The positive phototactic response in *D. magna* decreased as food became limited, and this response was also dependent on the particular clone of this species being investigated (De Meester and Dumont, 1989). The *D. magna* photoreaction also differs, depending on their previous adaptation to temperature (Lobashev and Ivanova, 1947).

The phototactic reaction (both positive or negative) also changed in *D. magna*, *Evadne*, and *Podon* following the addition of $CdCl_2$ ($1:10^2$−$1:10^5$ w/v) (Smirnova, 1960) to their environment; because it binds sulfhydryl groups, phototaxis could be restored by the addition of cysteine ($1:10^2$−$1:10^4$ w/v), a sulfhydryl group donor. The positive phototactic reaction was changed to negative in *Evadne* and *Podon* if $CdCl_2$ was added to the water (Smirnova, 1960), in *D. pulex* if illumination was increased from 1300 to 5274 lx (Rimet, 1961), and in *Daphnia carinata* in the field of electric direct current (Wang et al., 2013).

Initially positive phototactic reaction in *D. pulex* becomes negative in 0.05% caffeine solution and is retained during 1 h (Bryukhatova, 1937).

13.4.6 Perception and Effect of Polarized Light

Cladocera can detect polarized light, which influences their orientation and direction of movement, i.e., they exhibit polarotaxis (Waterman, 1961; Young and Taylor, 1990). In the aquatic environment, a source of polarized light is the light reflected by algal cells and other particles, which are potential food items. Thus, sensitivity to polarized light plays an important role in obtaining food. *D. magna* and *D. pulex* collected much more food in a zone of polarized light than in a zone of nonpolarized light of the same intensity (Verkhovskaya, 1940). If a tray with compartments filled with water or algal suspension is placed over a vessel with daphnids, they collect under the compartment with algae (Baylor and Smith, 1957).

It has been shown that several species (both littoral *Kurzia*, *Pseudochydorus, Moina, Ceriodaphnia*, *Simocephalus* and pelagic *Bosmina*, *Daphnia*, *Leptodora*) of Cladocera illuminated by a vertical beam of polarized light swim perpendicular to the polarization plane (Baylor and Smith, 1953; Waterman, 1960, 1961; Hazen and Baylor, 1962). Gromov (1992) also observed that *Daphnia* can distinguish between vertical and horizontal beams of polarized light; in the vertical beam, *Daphnia* movements are reduced by half. Following the addition of sodium bromide (NaBr), the response of *Daphnia pulicaria* to

linearly polarized white light (90 degrees e-vector orientation) became more random (Goksen and McNaught, 1995). These authors suggest that this is due to a disturbance in the transmission of nerve impulses, as Br^- ion blocks chloride (Cl^-) channels.

13.4.7 Perception of Colored Light

Ewald (1914) found *D. pulex* to be sensitive to colored light, with two maxima: in the green–yellow and blue–violet regions. Lumer (1932) exposed *D. pulex*, *D. magna*, *Moina*, and *Leptodora* to a graded series of light levels of different wavelengths but equal intensity. All of the tested animals moved toward the orange light (620–640 nm). *Moina* was also attracted by green light (540 nm) with approximately the same efficiency; its secondary maximum was in the blue range (440 nm). Each species tested showed its own characteristic reaction to light. The littoral species *P. truncatus* and *S. mucronata* aggregated in the zone of blue light (Peters, 1927).

Sensitivity of the *Daphnia* complex eye was shown to be highest to green light; it also perceives light through its integuments, but the maximum sensitivity in this case is to blue–violet wavelengths (Scheffer et al., 1958). *D. magna* showed peaks of light sensitivity at wavelengths 440, 470, 520 (blue), and 640 (red) nm (Wechsler and St. John, 1960).

In most cladocerans, four visual pigments have been found, with maximum sensitivity to light of wavelengths 370, 430, 560, and 670 nm (red) (McNaught, 1971). In oligotrophic lakes, the environment is predominantly blue, but during eutrophication, a redder environment is formed. In the ommatidia of *D. magna*, Smith and Macagno (1990) identified four spectral classes of photoreceptors with peak sensitivities at 348, 434, 525, and 608 nm for the dorsal ommatidia; in contrast, there was only a small difference in peak sensitivities for the ventral ommatidia.

Both the normal and the eyeless *Daphnia* were particularly attracted by yellow and green light in Schulz's experiment (1928). Color preference was also studied in *D. carinata*. For this, animals were placed in a vessel surrounded by black paper, with four openings illuminated by lights of different color (Maity and Saxena, 1979). After illumination for 15 min, the distribution of *Daphnia* was recorded: *D. carinata* preferred light of the following colors, in decreasing order: yellow, orange, red, violet, blue, and green.

Gromov (1992) also observed the influence of light wavelength (or color) on the locomotory activity of *Daphnia*, as seen by their attraction to light. The locomotory activity of mature *Daphnia* decreased within the range 400–525 nm (violet–green).

Daphnia tend to move vertically between 440 nm (blue) and 735 nm (red), and horizontally at 440 nm (violet) and under white light (Stearns, 1975).

When Cladocera were illuminated with a vertical beam of light, for which the wavelength was controlled using colored filters, they responded by swimming upward when the light changed from blue to white and downward when the light changed from yellow to white. This response to color change was observed in both littoral *Ceriodaphnia*, *Kurzia*, *Moina*, *Pseudochydorus*, *Sida*, and *Simocephalus*, and pelagic *Daphnia*, *Bosmina*, and *Leptodora* spp. (Smith and Baylor, 1953). Responses in *Ceriodaphnia* were preceded by a considerable time lag.

"Color dances" maintain a cladoceran "within a useful range of its food once such food is found" (Baylor and Smith, 1957, p. 24). When illuminated from above with red light (about 600 nm) at a uniform intensity over the aquarium surface, *Bosmina*, *Ceriodaphnia*, *Daphnia*, and *Moina* and were observed to generally dance upright, with a small horizontal vector in their movements, and thus stayed in the same area; under blue light, a large horizontal vector was manifested (Smith and Baylor, 1953).

If light falls on the *D. magna* eye through the top of the head, the action spectrum peaks at low wavelengths, but if it falls on the eye through the side of the head, the action spectrum peaks in the yellow–green region (Young, 1974).

The effect of radiation generated by the light-emitting diode (650 nm, 0.9 mW/cm^2) on *D. magna* was studied by Vorobyova (2013a,b). Exposure to this radiation induced some mortality and morphological abnormalities in subsequent generations: reduction of the number of antennal segments, of setae, or of a branch; complete absence of antennal setae; complete absence of antennae; ventralward incurvation of shell spine. Such specimens perished during 1–3 days.

A negative effect of blue light has also been observed. *Sida* show a negative reaction to blue light (Peters, 1927). Under blue light (about 500 nm), *Bosmina*, *Ceriodaphnia*, *Daphnia*, and *Moina* were distinctly agitated, leaning forward with a large horizontal vector, and thus swimming from one place to another; *Moina* was almost paralyzed by a sudden exposure to blue illumination (Smith and Baylor, 1953). Prolonged illumination with blue light (of about 500 nm) killed cladocerans (Smith and Baylor, 1953).

13.4.8 Photoperiod

The photoperiod, i.e., length of the day (i.e., light) in relation to the duration of darkness, plays an important role in controlling the life cycle and onset of gamogenesis (bisexual reproduction) in Cladocera. In culture, *Pleuroxus denticulatus* switched to gamogenesis under the influence of photoperiod; it was more prominent with a long day length in populations originating from the southern US and at a short day length in northern populations (Shan, 1974). *Pleuroxus procurvus* and *P. truncatus* started gamogenesis only under the short day conditions. It has also been shown in *Daphnia* that the formation of males may be induced by a decrease in day length to 12 h (Stross and Hill, 1965). The effect of the photoperiod is highly dynamic and depends on other factors. It was shown, for example, for *D. carinata*, that it depends on temperature, food concentration, and population density (Jiang et al., 2014).

13.4.9 Effect and Perception of Ultraviolet Radiation

The influence of UVR (i.e., radiation with wavelengths of <400 nm) on Cladocera has been studied by many researchers. Merker (1930–31) found that *Daphnia* perceive UV light. Later, it has been shown that UVR seriously endangers Cladocera. At naturally occurring intensities, it has lethal effects, for example, on *Daphnia middendorffiana* (Luecke and O'Brien, 1983) and *D. pulex* (Wübben and Vareschi, 2001). Hurtubise et al. (1998) determined the median lethal dose (LD_{50}) of UVR for *Ceriodaphnia reticulata*, *D. magna*, and *Scapholeberis kingii* using a solar simulator: the LD_{50} at 96 h, ranged from 4.2 to 84 $\mu W/cm^2$. *S. kingii* turned out to be highly sensitive, and *D. magna* and *C. reticulata* moderately so. *Bosmina meridionalis* showed no change in mortality over the whole range of UVR tested in comparison with the controls and in contrast to both *Ceriodaphnia dubia* and *D. carinata* (Wübben et al., 2001).

Two ranges may be differentiated: UVR-B (280–320 nm) and UV-A (320–400 nm). The valves of *D. magna* stop ca. 35% of damaging UVR-B radiation (Van Den Broecke et al., 2012). Lethal doses of UVR-B were found to be 51 $kJ\,m^{-2}$ for *D. magna* and 15 $kJ\,m^{-2}$ for *D. pulex* (Van Den Broecke et al., 2012).

The lethal effect was increased when *Daphnia longispina* were exposed to UVR-B rather than to UV-A; exposure to UV-A caused significant oxidative stress, as detected by increased malonaldehyde, whereas exposure to UVR-B was followed by increases in both malonaldehyde and catalase (Vega and Pizarro, 2000).

The addition of ascorbic acid reduced mortality caused by UV-A, but not by UVR-B.

Therefore, a protective mechanism against photooxidative stress is presumed to exist. UVR is also deleterious to newborn *Daphnia galeata* at the water surface, and this effect decreases with increasing depth (Winder and Spaak, 2002).

Under UVR illumination, *D. pulex* is negatively phototactic (Peters, 1927). *D. magna* illuminated by UV light also exhibit negative phototaxis (at a maximum spectral sensitivity of 349 nm), whereas phototaxis to visible light (120−600 nm) is positive (Storz and Paul, 1998).

It has been shown experimentally that downward migration provides effective protection for *D. pulex* (Vareschi and Wübben, 2001) and *D. galeata* (Winder and Spaak, 2002). Siebeck (1978) compared the effect of UVR on *D. pulex* (light brown) and *D. galeata* (uncolored), and found that the former was 1.5 times better protected from UV. Melanic clones of *D. pulex* and *D. middendorffiana* also survived exposure to 20 W/m^2 near UV light for twice as long as unpigmented ones (Hebert and Emery, 1990). The melanic morph of *D. longispina* survived better than the hyaline morph under solar UVR (De Lange et al., 2000).

Many species living at the water surface and at high altitudes develop melanistic coloration. Melanin in the carapace reduces UV penetration. UVR-B (280−320 nm) reduced survivorship and reproduction in nonmelanized *D. pulex*, contrary to heavily melanized specimens (Zellmer, 1995). It has also been shown in *D. pulex* and *Daphnia tenebrosa* that melanin plays a major role in protection against UVR (Hessen, 1999).

In the absence of blue light and UV, *Daphnia* do not reconstitute their carapace melanization after molting. The concentration of protective melanin in *Daphnia* increased after the ice breakup in North Finland, i.e., in the period of maximum underwater UV intensity (Rautio and Korhola, 2002). It was shown that the prophenoloxidase activating system takes part in melanization of *D. magna* (Mucklow and Ebert, 2003).

The damage and repair processes induced by UVR in Cladocera may be interconnected. Following exposure to damaging UVR-B (280−320 nm), *Daphnia*, in the presence of both long wavelengths and visible radiation, exhibited a large increase in survival due to stimulation of photoenzymatic repair (the induction of which is favored by UV-A radiation, 320−400 nm) (Williamson et al., 2001).

In the presence of UVR, a large proportion of *D. pulicaria* migrate downward; compared with that are specimens shielded from UVR (Leech and Williamson, 2001; Leech et al., 2005). UV is therefore a factor that induces downward migration of *Daphnia* spp., whereas species with a more highly pigmented carapace stay closer to the water surface when exposed to UVR (Rhode et al., 2001). The detrimental effects of UVR on *D. magna* and *D. tenebrosa* increased in the presence of a reduced calcium content in the solution (Hessen and Rukke, 2000b).

In *Daphnia catawba* exposed to UVR, the respiration rates increased by 31.8% (at sublethal irradiation of 2.08 kJ/m^2 UVB + visible photorepair radiation) and decreased by 70.3% (at 4.16 kJ/m^2) (Fischer et al., 2006). The enhanced respiration rates were attributed to energetic costs associated with the repair of damaged cell components.

It was found that repair of UV damage is only possible in the dark (Mucklow and Ebert, 2003). With increasing temperature, UV-induced DNA damage is increased to *D. pulicaria* at the water surface, as well as DNA repair rates; thus, net DNA damage is greater at lower temperatures, where survival may also be lower (Macfadyen et al., 2004). Photoprotection may therefore be more effective than photoenzymatic repair and more effective under low temperatures.

D. magna exposure to UVR did not induce a change in catalase activity and caused a slight increase in glutathione transferase activity; however, no changes in enzyme activity were recorded at different oxygen levels, although survival increased at lower temperatures (Borgeraas and Hessen, 2000). The activity of antioxidants (catalase, glutathione transferase, and

superoxide dismutase) was studied in *Daphnia* spp. in order to assess their role in UV photoprotection (Borgeraas and Hessen, 2002a). In alpine populations of *D. longispina*, there was a positive correlation between the water absorbance and catalase activity, which could be related to photoinduced hydrogen peroxide production. The activity of superoxide dismutase was high in *D. longispina* from a lowland humic pond. The activity of glutathione dismutase was low in a melanic *D. pulex* group. No difference in antioxidant activity was found between the alpine melanic and nonpigmented *D. longispina* populations. Following UVR exposure, catalase was found to be more active in *Daphnia* in the presence of organic matter, whereas the opposite was shown for glutathione transferase (both involved in protection and repair of UVR-caused damage) (Hessen et al., 2002). The activities of glutathione S-transferases and catalase in *Daphnia* were found to be proportional to food phosphorus-to-carbon ratio (Balseiro et al., 2008).

See also Section 3.2 on melanin.

A positive reaction to UVR (i.e., radiation with wavelengths of <400 nm) was reported for littoral *P. truncatus* (syn. *P. truncata*) and *Scapholeberis*. However, *Daphnia* had the highest eicosapentaenoic acid content in ponds with the highest UVR exposure, and sublethal damage of the gut preceded UVR-induced mortality (Zellmer et al., 2004).

Photoinduced toxicity in *D. magna* (estimated by immobilization) to various chemicals liberated by the pulp and paper industry was ranked as pyrene > anthracene > retene (Huovinen et al., 2001). The toxicity of these chemicals is thought to result from internal photosensitization reactions caused by UVR.

UV-damaged cells are thought to release histamine. Indeed, small doses of antihistamines (α-benzhydrylether-β-piperidoniethane, 2-phenylbenzylaminoethyl-imidazolin, or α-phenylamino-β-aminoethane) do suppress negative phototaxis (Poupa, 1948). The minimum effective dose for the first substance is 2.5 μg/mL. At higher doses, phototaxis became positive. Histamine takes part in the negative response of *Daphnia* to UVR (McCoole et al., 2011), and exposure to cimetidine (a blocking agent) inhibited the negative response of *Daphnia* to UV exposure.

Interestingly, completely eyeless *Daphnia* are repelled by UV illumination (Schulz, 1928).

UVR also affects food algae, thus influencing their consumers indirectly. It has been shown (Leu et al., 2006) that UVR exposure modifies the lipid content of green algae; the content of 18:1ω9 decreased, C18 polyunsaturated fatty acids (PUFAs) increased, and the ratios C:P and N:P decreased. In *D. magna* fed with these algae, the content of 18:1ω9 decreased, but the essential PUFA content did not.

The median effective concentration of nano-sized TiO_2 to *D. magna* estimated by immobilization was 1.2−3.4 mg/L under conditions of 0.56 mW/cm^2 UV-A radiation (Amiano et al., 2012), lower than that determined without consideration of illumination.

UVR increases the damaging effect of insecticides (e.g., fenoxycarb or pirimicarb) (Beketov et al., 2011) and of retene (Kumari and Kumari, 2011) to *D. magna*.

Exposure of *D. magna* containing embryos to low-frequency laser radiation of 633 nm for 60 s resulted in the production of larger juveniles (Osipova et al., 2010).

13.4.10 Perception and Effect of X-Rays

Irradiation with X-rays has a negative effect on Cladocera. In *Simocephalus*, it decreases the respiration rate and inhibits growth, possibly through changing cell permeability (Obreshkove and King, 1932a). In young *Simocephalus*, it also induces invagination of the brood pouch, which becomes worse at each molting (Obreshkove and King, 1932b). Baylor and Smith (1958) showed that *D. magna* irradiated by X-rays in red light

swim downward. These authors assumed that this action is a result of X-ray—induced free radicals having different actions on visual pigments in the compound eye and the nauplius eye. In *Moina macrocopa*, X-ray irradiation is reported to be destructive to the facets of the compound eye (Fuchikawa et al., 1995).

13.5 EFFECTS OF ELECTROMAGNETIC FIELDS

Cladocera are sensitive to electric fields. Electric fields are a normal environmental constituent, and Skadovskiy (1955) showed that in the bottom layers of water bodies, the electric potential may change by 0.6—0.8 V over 1 cm.

Daphnia swam to the anode in an electric field with an electric current density of 0.5—1 ma/cm^2 (Clarke, 1932; Clarke and Wolf, 1933). In the field of electric direct current, the positive phototaxis of *D. carinata* changed to the negative phototaxis; the threshold was 0.01 mA for 5 min (Wang et al., 2013).

Magnetic fields belong to permanent natural factors. In addition to the normal level (about 50 μT in temperate latitudes), there are magnetic storms (about 100—500 μT) related to fluctuations of solar activity and anthropogenic modifications. Magnetic fields influence size and quantity of progeny in *D. magna* (Krylov, 2011). Exposure to low-frequency magnetic field for 3 months resulted in larger and more viable progeny in *D. magna* (Krylov and Osipova, 2013).

Exposure to electromagnetic radiation of 50 Hz for 8 h over a period of 30 days delayed *D. magna* maturation and decreased fecundity, but was not lethal (Somov and Malinina, 2003). However, a low-frequency electromagnetic field (with a frequency of 50 Hz) accelerated *D. magna* maturation, whereas an electric field of 500 Hz (at 75 μT, imitating a magnetic storm) negatively affected both survival and maturation. It also increased the proportion of nonviable progeny (Krylov, 2008) and reduced both the number and size of the offspring (Krylov, 2010). Magnetic fields also accelerate heart rate of *D. magna* (Usanov et al., 2001a,b, 2003).

Exposure to electric direct current increased sensitivity of *D. carinata* to Cr^{6+} and Hg^{2+} (Wang et al., 2013). A low-frequency magnetic field decreased the toxic effect of phenol to *D. magna* (Rzyabiba and Usanov, 2012).

Electromagnetic fields of low frequency characteristic of mobile radio communication were tested on *Daphnia*. Exposure to mm-length radiation of 65 GHz noticeably increased filtration activity in *D. magna*, both in absence and in presence of heavy metals (Shilova and Rogachyova, 2011b). Cultivation in the low-intensity field of 1 GHz at 10 mW/cm^2 resulted in decreased survival and in decreased fecundity in the first nonirradiated generation (Paukova et al., 2012; Paukova and Sarapultseva, 2013).

In a weak strychnine solution (i.e., a saturated strychnine solution diluted 1:2000), *Daphnia* became positively phototactic and also swam to the cathode (Clarke and Wolf, 1933).

13.6 CHEMORECEPTION

13.6.1 Anatomical Background

Chemoreception in Cladocera is ascribed to the sensory papillae (olfactory setae and esthetascs) on the antennules. Scourfield (1896) found that females of most Cladocera possess nine olfactory setae on each antennule, though Holopedium has six and polyphemids have five setae. His data on males are preliminary, but nine setae are indicated for males of most chydorids. Scourfield (1896, p. 287) notes, "Unfortunately it must be admitted that we really do not know that they are olfactory setae at all." After some considerations, he concludes, "It appears tolerably certain, therefore, that unless the setae under consideration minister to some sense unknown to us, they must be olfactory organs." Esthetascs are more numerous in males; they are especially

numerous in males of *Leptodora* (up to 90) and of *Eurycercus*. Weismann (1918) clearly noted that numerous esthetascs in males of Cladocera increase their chances to find females.

The chemoreception function of these sensory papillae has been discussed by Gicklhorn and Keller (1926). A detailed description of the structure of olfactory setae was provided by Rieder (1987) (Fig. 13.7). The inner space (is) and the basal bead (bb) of each of the nine esthetascs of *D. magna* are surrounded at the base by five sheath cells (I–V), and the receptor cilia (c) extend to the very end of the seta (to the terminal pellet). The input from the neurons in antennular papillae is received directly by the neuropil in the deutocerebrum (Kress et al., 2015).

Sensilla were originally discovered on the thoracic limbs of *E. lamellatus* (Fryer, 1963) and later identified in many chydorid and macrothicid species (Smirnov, 1967, 1971). Sensilla on the thoracic limbs may be finger-like, globular, or bottle-like (Fig. 13.8). Dumont and Silva-Briano (1997) described the distribution of sensory structures on the trunk limbs of chydorids in detail. In crawling chydorids, esthetascs on the antennules face outward and contact the substratum (shown, e.g., in Fig. 1.3); thus, they perceive the substrate quality.

13.6.2 Chemoreception Process

This is a little investigated field. Chemoreception obviously combines taste and olfaction. The notion of taste has been rarely applied to Cladocera. DeMott (1985) specially investigated taste in Cladocera, offering flavored and nonflavored spheres. Flavoring of spheres was achieved by incubating them with algal culture; during incubation, the spheres adsorbed algal exometabolites. *Bosmina* ingested flavored spheres electively, while *Daphnia, Diaphanosoma*, and *Chydorus* did not respond to flavored spheres. *Bosmina* also preferred algae over unflavored spheres. Kerfoot and Kirk (1991) also reported that *Bosmina* demonstrate some ability to discriminate between food particles by means of taste, in contrast to *Chydorus* and larger cladocerans.

According to Hardy and McDougall (1894, p. 3), swallowing "starts at the mouth as a result of the stimulation of the sensory surfaces there"; however, these sensory surfaces were not commented on further or named.

Having developed a special method to record electroantennograms in fixed (stationary) *Daphnia*, Simbeya et al. (2012) investigated sensitivity of *Daphnia magna* and *D. pulex* to offered amino acids. The recording electrode was applied to the base of the antenna. The strongest concentration-dependent olfactory response was found to L-arginine. Presence of copper (7.5 μg/L) impaired the observed function.

Polyphemus was found to excrete 1-heptadecene, responsible to formation of swarms (Wendel and Jüttner, 1997).

Polyunsaturated aldehydes produced by diatoms may act as repellents for *Daphnia* (Jüttner, 2005).

13.7 MECHANORECEPTION

The setae of various Cladocera seem to include a sense of touch in their function. In chydorids crawling on substrata, antennules with their esthetascs are in contact with the various clumps and filaments they encounter. The outer distal lobes of their thoracic limbs I "are capable of independent movements" (Fryer, 1963, p. 362), and one may observe how a moving specimen touches its surroundings with the setae of these lobes. Goulden (1966, 1968) suggested that copulating males of *Moina* species recognize gamogenetic females of their own species using the sensory papillae and setae on their grasping antennules, and by feeling the surface sculpture of the female carapace (future ephippium),

which is different in different species. One of the functions of head pores, and of the attached tissues, is also thought to involve mechanoreception (Dumont and Van de Velde, 1976).

In Cladocera, *hearing* was not completely excluded by Leydig (1860, pp. 41 and 42, translated from German): "Whether the organ of hearing belongs to sense organs of cladocerans is doubtful … it is quite possible that one day it will be shown that the antennules of daphnids form the organ of hearing."

13.7.1 Orientation in Space

Littoral and bottom-living chydorids are oriented in relation to their substrata in various ways. They may crawl on the substrate, mainly at a right angle to the substrate's surface, although a cladoceran may also be located on the top or the side of a clump of debris. They may also swim over and between various littoral substrates. Of the macrothricids, *Lathonura rectirostris* remains immobile for rather long periods and then makes sudden jumps, whereas *Drepanothrix dentata* lies on the surface of bottom debris with its ventral side up and only its thoracic limbs moving. The diverse methods of movement of littoral Cladocera and their different orientations to the substrata have been insufficiently studied and offer a wide scope for further observations. There are also specialists that mostly live attached to the substrate by their dorsal side, i.e., *Sida* and *Simocephalus*, the former by their dorsal organ and the latter by means of hooked terminal setae on their antennae.

Planktonic cladocerans are oriented with their longitudinal axis at some angle to the vertical, i.e., with the head upward and forward. Strokes of its antennae pull the body forward.

Gravitation is supposed to be the main factor controlling the orientation of movements of *D. magna* (Szlauer, 1962a) or generally of planktonic species (Wolfschoon Ribeiro et al., 2014).

In *Bosmina*, the movement phase lasts for ca. 25 ms (Zaret and Kerfoot, 1980). Swimming is controlled by a complex set of environmental factors, with illumination being a prominent influence. *D. magna* and *D. pulex* swimming is oriented by the predominant direction of light. If they are illuminated with light of the same intensity from all sides, they rotate (Ringelberg, 1963, 1964); when the light intensity is then increased from one direction, the animal resumes its normal position. Under more normal conditions, if the illumination intensity decreases, then the *D. magna* swimming rate increases (Ringelberg, 1964); however, below a certain light intensity, *Daphnia* swim normally, independent of the angular distribution of light.

13.7.2 Control of Body Posture

The body of planktonic cladocerans (*Bosmina* and *Daphnia*) is generally oriented with its dorsal side upward and backward. Due to gravity, the animals sink, and their position is frequently corrected by strokes of their antennae. There are alternating short periods of movement and rest. According to Jacobs (1964), the elongated head of some *Daphnia* counterbalances the part of the body that is behind the antennae.

The position of the *center of gravity*, as noted by Scourfield (1900b), is one factor influencing the position of a cladoceran body in space. He observed that the position of the body in space differs between *Daphnia* (head and the dorsal side obliquely upwards) and *Simocephalus* (head and the ventral side obliquely upwards). Scourfield also changed the center of gravity by occasionally attaching a drop of glue to the animal or introducing an air bubble under the shell. In such cases, he observed the animal to swim abnormally. Changes to the orientation of the body due to displacement of the center of gravity may be observed, for example, in gravid *Eurycercus* compared with juvenile specimens of the same species. Female

Eurycercus that contain numerous embryos in the brood pouch swim with their head upwards and move with their dorsal side forward (Smirnov, 1971, p. 80), whereas younger specimens are oriented with their back upwards. Discussing movement of *Lathonura*, Sergeev (1971) takes the center of gravity into consideration. For gravid *D. magna*, it is thought that the antennal rate increases due to both its increased weight and the necessity of counterbalancing the displaced center of gravity (Fox and Mitchell, 1953; Lochhead, 1961).

No special observations of this kind have been made in representatives of other cladoceran genera.

Buoyancy. Buoyancy also depends on the specific weight of cladocerans; it is modified by oil inclusions (hydrostatic effect) and the development of slime envelopes. Planktonic cladocerans also possess thin exoskeletons. The obvious hydrostatic role of oil drops in the body of cladocerans has not been investigated.

Immobilized, small Cladocera sink at a rate of 2−3 mm/sec, or as a percentage of body length, 350−550% for Chydoridae and 250−72% for *Sida* (Daphniidae) (Smirnov, 1971). The likely perception of gravity by the antennal setae of *Daphnia* was suggested by Bidder (1929) and Grosser et al. (1953). The position of the body in space may also be controlled by the tension receptors of the oculomotor muscles transferring stimuli to the antennal muscles (Harris and Mason, 1956). Harris (1953) also noted that a different type of muscle located on different sides of the eye causes strokes of the antennae that contribute to daphnid rotation.

The presence of mechanical wave receptors in *D. pulex* was reported by Szlauer (1964). Jander (1966, 1975) suggested a more complex system of support for orientation in *Daphnia*, comprising commands from the eyes, the antennae, and a gravity receptor (Figs. 13.11 and 13.12).

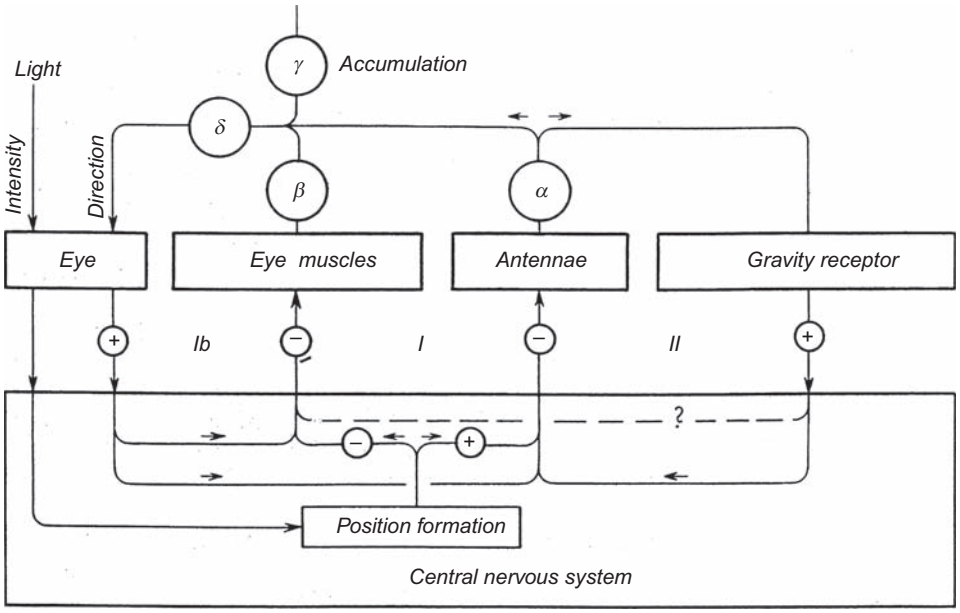

FIGURE 13.11 Scheme of the *Daphnia* orientation system. *I*, phototaxis; *II*, geotaxis; δ, direction of light falling on the eye depending on α, β, and γ. *Jander, R., 1966. Die Phylogenie von Orientierungsmechanismen der Arthropoden. Zoologischer Anzeiger 29, 266−306.*

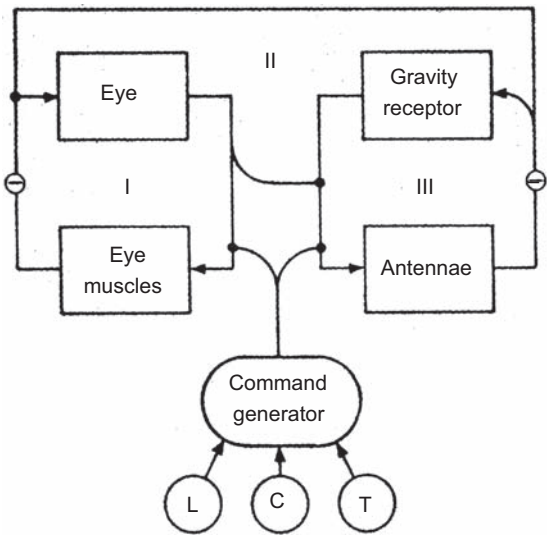

FIGURE 13.12 Later scheme of the *Daphnia* orientation, showing three negative feedback loops. *C*, chemical information; *L*, light intensity information; *T*, temperature information. *Jander, R., 1975. Interaction of light and gravity orientation in* Daphnia pulex. *Fortschitte der Zoologie 23 (1), 174–184.*

Later, Meyers (1985) studied in detail the setae at the base of the swimming antennae of *D. magna* and concluded that they are rheoreceptive and "mediate gravity perception" during the sinking phase of their movement. It should be noted that the paired setae situated on the external proximal surface of the coxopodite are known for various anomopods (Smirnov, 1971; Kotov, 2013). Similar setae are known for ctenopods.

Laforsch et al. (2014) found that the gravireceptive organ in Cladocera consists of setae natatoriae and associated structures at their base; in sinking *Daphnia*, the setae are deflated, and this signal is processed by inner structures. *Daphnia*, with these setae ablated, do not sense gravity. Similar gravisensing organs are supposed to be used by podonids (Wolfschoon Ribeiro et al., 2014). Special nerve ganglion at the base of setae natatoriae (Fig. 13.1) was indicated by Samassa (1891) and Fischel (1908).

In the dark, *C. ovalis* and *C. sphaericus* become homogeneously distributed in water; illumination is followed first by upward swimming, and then they become concentrated at the bottom (Bryukhatova, 1928). In complete darkness (as instantaneous photographs have demonstrated), *Daphnia* maintain a position corresponding to that adopted in the presence of illumination from above (Jander, 1966). In addition, after extirpation of both the eye and ocellus, the reactions of *Daphnia* remain essentially the same, owing to a general sensitivity to light (Schulz, 1928; Harris and Mason, 1956; Fryer, 2004).

Thus, orientation of the daphnid body obviously depends on a combination of several varying stimuli. It may also be noted that locomotory responses may differ in individuals of different sexes. Thus, *D. pulex* females run away from a thin rod, whereas males approach it and may even become attached to it (Szlauer, 1964). Movement related to predators principally involves the strategy of akinesis (mainly in littoral species; Chapter 14, Section 14.3.1) or the strategy of escape (mainly in pelagic species; Chapter 14, Section 14.3.2).

13.8 ENDOGENOUS RHYTHMS OF ACTIVITY

In continuous darkness, *D. magna* is least active at midnight and maximally active at noon (Harris, 1963), whereas under constant illumination, their activity period was revealed to be about 28 h. A circadian time of 27 h, 50 min in *D. magna* was also reported by Ringelberg and Servaas (1971). Under natural conditions, a similar circadian activity rhythm was found in *D. pulex*; its activity was low at night, maximum in the morning, and then declined thereafter (Stearns, 1975).

13.9 EFFECT OF XENOBIOTICS

Diphenhydramine (a pharmaceutical substance) released in the environment is toxic to *D. magna*, affecting ACh and histamine (Berninger et al., 2011). Lokhanskaya et al. (2008) estimate the action of pesticide norvel (containing chizalofol-P-ethyl, $C_{18}H_{17}Cl.N_2O_4$) on *D. magna* and *Ceriodaphnia affinis* as neuroparalytic and narcotic.

There are only a few examples of experiments related to the effect of xenobiotics on movement. In the presence of 0.02 mg/L Cu^{2+}, *D. magna* became less positively phototactic (Kien et al., 2001). Cr (as K_2Cr2O_7) inhibits phototaxis in *D. carinata*, as shown by a negative linear correlation between the phototactic index and Cr^{6+} concentration (Wu et al., 2005).

CHAPTER

14

Behavior

14.1 DIFFERENCES IN BEHAVIOR AMONG SPECIES

It is likely that each of the numerous littoral and pelagic exhibits species-specific reactions and behavior. Representatives of the same genus may be strikingly different, e.g., *Chydorus gibbus* is strictly bottom living, whereas other species of this genus tend to swim in open water. The well-known urge of *Sida crystallina* to attach itself to any surface was studied quantitatively by Szlauer (1973). Males show a strong drive for attaching to females, as reported, e.g., in *Chydorus sphaericus* (Smirnov, 1965b, 1971; Van Damme and Dumont, 2006).

14.1.1 Littoral and Bottom-Living Species

The modes of locomotion related to substrata include crawling, attachment to various substrates, and attachment to the surface film of water (Scourfield, 1894, 1900a, 1923, 1929). Crawling is characteristic of the littoral forms, is combined with occasional swimming over substrata, and is diverse, ranging from burying into the bottom mud to rapid running over the surface of various bottom substrata. The action of pushing through narrow passages was termed *scrambling* by Fryer (1968). Crawling over a flat substrate is characteristic of *Graptoleberis testudinaria*, which also attach themselves to the substrata by sucking (Fryer, 1968) and are thus able to glide along the surface. The ventral side of this chydorid is flat, the outline is densely supplied with plumose setae, and the suction force is achieved by pumping water from inside the valves.

Movements are notably slow in the bottom-living *Ilyocryptus*. After producing progeny, they remain in the same place surrounded by their young (Chirkova, 1972a,b). There are also some species that live on the surface of mud with their ventral side up (*Ilyocryptus* and *Drepanothrix*). Another species that lives on the mud surface, *Lathonura*, finds a convenient place, arranges itself ventral side up, and can then be observed to be immovable for hours, with only its thoracic limbs moving.

Cladocerans that crawl on the bottom substrata can stay in any position while feeding on the substrate. Stygobionts (inhabitants of interstitial, karstic waters and caves) make a special ecological group of Cladocera (Brancelj and Dumont, 2007). Interstitial species, obviously, have to move through whatever available narrow spaces.

Some chydorids occur in the moist terrestrial environment: *Bryospilus* crawls in moist, moss-like growths on the trunks of tropical trees (Frey, 1980). Despite having a morphology characteristic of other chydorids, it refuses to swim if placed in water.

http://dx.doi.org/10.1016/B978-0-12-805194-8.00014-3

14.1.2 Pelagic Cladocera

Pelagic Cladocera, on the other hand, may live all of their life cycle without making contact with the substrata. They support their body in the water by the strokes of their antennae (Fryer, 1991); there are two kinds of strokes: slow, sufficient for keeping the sinking body in place; and rapid, used to travel through space. Cladocera movement depends on various environmental factors; it is highly variable and dynamic but the physiological mechanisms that transform the stimuli into the responses are not always obvious. Hutchinson (1953) highlighted the random stimulation of all *Daphnia* sense organs by turbulence.

From fine flow of water from a narrow tube *Daphnia pulex* run away downward (Szlauer, 1964).

In rising temperatures, daphnids swim upward, whereas in falling temperatures they tend to sink (Gerritsen, 1982). The redox potential also influences the direction of movement. In the presence of catechol (E_h, otherwise termed E'_o, $= +0.33$ V), daphnids move upward, and in the presence of cysteine ($E'_o = -0.14$ V) they move downward (Baylor and Smith, 1957). The transitional level is about $E'_o = +0.045$ V.

The behavior of females and males of the same species is clearly different. For *D. pulex*, Szlauer (1964) observed that females fled from a thin rod whereas males swam toward it and sometimes attached to it. Female *Daphnia* avoided approaching individuals, whereas males moved toward them.

The mating behavior of *Daphnia pulicaria* was investigated by Brewer (1998) (Fig. 12.2), who showed that male swimming was faster than and orthogonal to that of females. Video recording of the swimming trajectories of females and males revealed that males pursued and contacted both females and males, the latter being contacted more often, although for a shorter time. *D. pulicaria* males can detect a female at a maximum distance of 6.4 mm (i.e., four body lengths), principally via mechanoreception of fluid disturbances created by the swimming females. The trails of swimming *Daphnia* were visualized and photographed by Brewer (1998) by using a Schlieren optical path and a smooth density gradient.

Surface Film

Some cladocerans may attach themselves to the surface film of water with their ventral side up (e.g., *Scapholeberis* and *Dadaya*) and thus feed for long periods in an "upside-down" position on particulate matter and probably also on the unimolecular film of organic matter. Both behavior and the structure of the ventral side of the valves in *Scapholeberis* were examined by Scourfield (1894, 1900b) and later reported in more detail by Dumont and Pensaert (1983). *Scapholeberis* sometimes swims within the water column, in a similar way to other Cladocera, but once below the surface film it is reluctant to leave it (Fryer, 1991). Fryer (2006) also noted that, despite this characteristic behavior, there is no sign of reshaping of any of its organs in response to such an inverted position.

14.2 MIGRATION AND SWARMING

14.2.1 Migration

Vertical migration is known to occur in both littoral species (as indicated for *Acroperus* and *Eurycercus* by Szlauer, 1962a, 1963a) and many pelagic daphnids, including *Daphnia* species (Bainbridge, 1961; McNaught and Hasler, 1964; Wright et al., 1980). Swimming *C. sphaericus* and *Pseudochydorus globosus* manifested a delayed ascent in response to decreasing illumination but, if attached to substrata, did not react at all (Meyers, 1980). Vertical migration in *Daphnia* is discussed in detail by Ringelberg (1993) and Lampert (2011).

Cladocera can withstand the changes of pressure experienced during vertical migration. Harris (1963) photographed a group of *Daphnia magna* kept in continuous darkness and found

that it revealed a 24-h cycle of locomotor activity, rising at noon, not determined by external stimuli but following the biological clock.

Under conditions of decreased illumination, *D. magna* and *Ceriodaphnia reticulata* demonstrate a rapid upward movement (Meyers, 1980). Vertical, but delayed, ascent was also shown in *C. sphaericus* and *P. globosus* (if they were not attached to a substratum), and their ascent was stimulated by decreased oxygen levels.

Changes in the eye of *Daphnia longispina*, such as an increased pigmented surface under laboratory conditions of constant illumination, were found to precede its ascent during vertical migration; this therefore forms the expression of an endogenous rhythm (circadian rhythm) (Cellier et al., 1998; Cellier-Michel and Berthon, 2003). Vertical migration may, however, occur without participation of the eye; this has been shown both in the dark and in specimens with an extirpated eye. Szlauer (1963a) demonstrated that daily vertical migrations of *D. magna* occur in the absence of changes in illumination or in the dark. *Daphnia* that have had their eyes removed can still migrate vertically in response to differences in light intensity (Harris and Mason, 1956).

Vertical migration may be different in different clones of the same species (De Meester, 1993; Műller and Seitz, 1993; Spaak and Hoekstra, 1993).

Gliwicz (1986) highlighted the dependence of Cladocera population density on lunar cycles, resulting from a combined effect on the vertical migration of Cladocera and the feeding intensity of the fish.

Daily vertical migrations in *D. magna* may also be modified by fish kairomones (Loose et al., 1993; von Elert and Pohnert, 2000).

Diel horizontal migration in *Daphnia* is known but less investigated (Burks et al., 2002).

14.2.2 Swarming

Swarming has been observed in both littoral (Lastochkin, 1930) and pelagic (Birge, 1898; Künne, 1926; Dumont, 1967; Johnson and Chua, 1973) cladocerans, including *Bosmina*, *Ceriodaphnia*, *Daphnia*, *Moina*, *Polyphemus*, and *Scapholeberis*. Swarms may range from a few centimeters to a few meters across (Young and Taylor, 1990). Sometimes such shoals consist of mixtures of various species. Kotov (2000) observed a shoal in Lake Glubokoe (Moscow region, Russia) that predominantly consisted of *Scapholeberis*, but in which about 10% was made up of *Bosmina*, *Ceriodaphnia*, *Pleuroxus truncatus*, and other chydorids. In experiments, *Bosmina* swarming behavior was observed only in the light and with abundant food (Jacobsen and Johnsen, 1988). Lampert (2011, p. 97) notes that "Causes of swarm formation in *Daphnia* other than the exploitation of food patches have been rarely analyzed."

A central question here is: what stimuli induce individual cladocerans to gather into swarms? The mechanism may involve visual landmarks or chemical stimuli (e.g., attractive odors), or it may simply be behavioral. It was also observed that *Bosmina* and *Polyphemus* are reluctant to leave a swarm and change their swimming behavior in the marginal zone of a swarm (Young and Taylor, 1990). *D. magna* has a tendency to form and maintain aggregates in response to chemical cues from fish and invertebrate predators (Pijanowska and Kowalczewski, 1997).

Within swarms of *Moina macrocopa*, the concentrations of various forms of N and P increase, protein and carbohydrate fractions appear, and various amino acids are present, as well as palmitic, tridecanoic, and stearic acids (Kalacheva et al., 2000). *Polyphemus pediculus* releases 1-heptadecene into the surrounding water; 7 ng/L was found within a swarm versus 1.5 ng/L some distance away; this substance may be involved in the formation, maintenance, and recognition of swarms (Wendel and Jüttner, 1997). Thus, swarms may create a special physiological environment that, in turn, influences the members of the swarm.

A swarm may also create its own general water movements, which would not otherwise take place. As they are uniformly oriented, the members of a swarm can create general water movements within the swarm. Individuals within a swarm are integrated into it, and the swarm may move as a single entity within a water body (Young and Taylor, 1990).

14.2.3 Hydrostatic Pressure

Cladocera have been found at depths of up to 150 m (as summarized by Smirnov, 1971). For example, *Eurycercus* and *Camptocercus* have been recorded at 150 m (Zschokke, 1911). In Lake Baikal, *Alona labrosa* was abundant at c. 70 m and *Alona. setosocaudata* was present at depths of up to 133 m (Vasilyeva and Smirnov, 1975). Thus, cladocerans can live at considerable hydrostatic pressures. A water depth of 10 m approximately corresponds to 1 bar (1 atm = 1.013 bar = 14.696 psi). Another characteristic is their ability to withstand changes in hydrostatic pressure during vertical migration. It has been demonstrated experimentally that during vertical migration (in response to light) *D. magna* is unaffected by high pressure (Lincoln, 1970).

14.3 EMOTIONAL REACTIONS

Cladocera have immediate reactions to various stimuli, shown as a temporary increase in heart rate by about 20% (Smirnov, 1965b) following, e.g., a slight mechanical irritation. In addition, upon seeing specimens of its own species, oxygen consumption in *P. pediculus* was shown to change: it decreases in parthenogenetic females containing eggs, and increases in females containing developed embryos, newborns, gamogenetic females, and males (Butorina, 1979, 1980). In newborns, the increase can reach 69%.

The mating behavior of *D. pulicaria* was investigated by Brewer (1998) (Fig. 12.2). Cladocera also exhibit an immediate reaction to disturbance: akinesis in most littoral species (i.e., they faint) or an escape reaction in planktonic species (they "flee in fright").

14.3.1 Akinesis

When disturbed, chydorids and macrothricids stop moving, fall to the bottom, and remain immovable, sometimes for a long time. If you shake a glass vessel containing littoral cladocerans, you will see that some of them stop their usual movements and sink to the bottom. This behavior is called *akinesis* (sham death or "dead-man response"), and is an important reaction in arthropods, related to the fact that predators will only attack moving prey (Kerfoot et al., 1980). It is manifested principally in littoral Cladocera (Smirnov, 1977), whereas planktonic species use a different method to avoid predators: they flee from danger (they manifest "the escape reaction").

When disturbed, chydorids, macrothricids *Drepanothrix*, *Lathonura* (Smirnov, 1977), and *Ilyocryptus* (Mordukhai-Boltovskoi and Chirkova, 1973; Fryer, 1974, p. 142; Smirnov, 1977) play dead for a few seconds. *Eurycercus lamellatus* and *Lathonura* remain immovable for more than 3 min, whereas this may continue in *Chydorus* for several minutes (Kerfoot, 1978). In these motionless specimens, the thoracic limbs may either stop or continue beating, e.g., in *Ilyocryptus* (Mordukhai-Boltovskoi and Chirkova, 1973). In *Lathonura*, either the thoracic limbs and heart both stop beating or only the thoracic limbs stop during akinesis. Akinesis may continue for rather a long time until the animal resumes its usual movement for no obvious reason.

In planktonic *Bosmina*, it was impossible to discern its brief akinesis by direct observation. However, using cinematographic photography, Kerfoot (1978) (Fig. 14.1) found that *Bosmina* use brief akinesis (sham death) when attacked by a predator, and passively sink by 2–4 times body length at 0.6–0.8 mm/s, just sufficient for

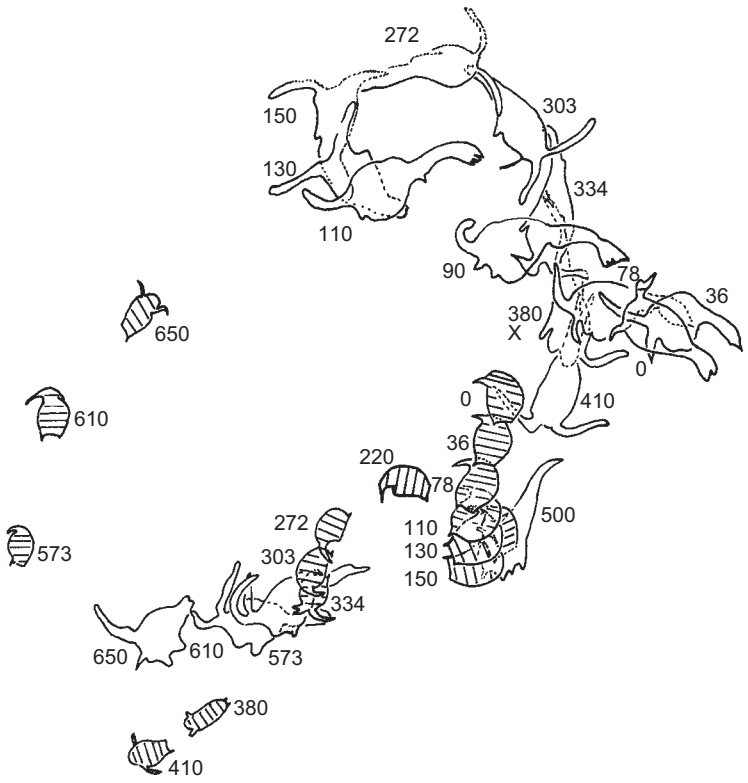

FIGURE 14.1 Trajectories and akinesis (dead-man response) in *Bosmina* (*shaded*) attacked by *Cyclops* (*not shaded*). Right bottom, an attacked *Bosmina* in the state of akinesis sinks by several its diameters. Numerals, frames in the film sequence; x, initial contact. *From Kerfoot, W.C., 1978. Combat between predatory copepods and their prey:* Cyclops, Epischura, *and* Bosmina. *Limnology and Oceanography 23 (6), 1089–1102.*

the attacking predatory cyclops to miss its prey (Kerfoot, 1978; Kerfoot et al., 1980).

I did not manage to find any references to investigations on the physiological background of akinesis in arthropods. It seems that attempts to provide a deeper explanation of this widely known fact have not been made. In addition, quantitative and qualitative ratings of the stimuli that cause akinesis have never been made.

14.3.2 Escape Behavior

Most pelagic cladocerans (*Ceriodaphnia*, *Daphnia*, *Diaphanosoma*, *Leptodora*, *Polyphemus*, *Sida*, and *Simocephalus*) escape predators by fleeing and do not manifest akinesis, as shown e.g., by Szlauer (1964). The escape capacity of sidids was discussed by Korovchinsky (2004). Szlauer (1964) observed that female *Daphnia* avoid a tube or glass wand of a certain diameter when it is used to model a predator by moving it through a *Daphnia* culture at a certain speed. Their escape ability was recorded as the inverse of the number of specimens sucked into a tube. Interestingly, males behaved differently: they approached the object and some even attached themselves to it. The highest escape ability was manifested by *Diaphanosoma*, and the lowest by *Bosmina* and *Chydorus* (Szlauer, 1965). By applying a similar method, Drenner et al. (1978) studied capture frequencies (by a siphon tube) for different cladocerans in relation to their distance from the tube. Capture frequency of cladocerans was greater than that of copepods.

Somersaulting has been observed in *Daphnia* both in response to a predator and to chemical cues from crushed conspecifics (Pijanowska and Kowalczewski, 1997). *D. magna* reacts to

these stimuli by escaping, sinking to the bottom, or making aggregates; specimens that experience these cues were more successful in avoiding predators (Pijanowska, 1997; Pijanowska and Kowalczewski, 1997). Kairomones tested in the light increased escape rate in *Daphnia* spp. (Brewer et al., 1999).

14.4 IMPACT OF XENOBIOTICS ON BEHAVIOR

At sublethal concentrations, xenobiotics disturb the normal behavior of Cladocera in various ways. Upon exposure to deltamethrin, *Daphnia spinulata* jerks as if prodded with a needle; its escape reaction became less intense and is followed by erratic swimming (Alberdi et al., 1990). Upon exposure to lindane, *D. magna* failed to migrate in a directed manner (Goodrich and Lech, 1990). Phototaxis was depressed in *D. magna* by bacitracin and lincomycin, whereas aminosidine increased the reaction to light and erythromycin did not alter phototaxis (Dojmi di Delupis et al., 1992). *D. magna* lost mobility either completely or partly on exposure to dimethoate or pirimicarb (Andersen et al., 2006). Their recovery following single-pulse exposures was also studied.

Hyperactivity was observed in *D. magna* exposed to pesticides, followed by reduced activity and death (Matthias and Puzicha, 1990).

Cladocera manifest avoidance of toxicants as shown, e.g., with reference to the gradient copper (copper sulfate) solutions and *D. longispina* (Lopes et al., 2004). Escape-like behavior increased in *D. pulex* and it exhibited extreme escape behavior at an acutely toxic level (40 ppb) of carbaryl (Dodson et al., 1995). *D. magna* avoids atrazine, thus it may escape its deleterious concentrations (Rose et al., 2012). Cu and Cr increased ability to escape (comparing with the control) in *D. magna*, *Pseudosida variabilis*, and *Ceriodaphnia*, whereas endosulfan reduced the ability to escape (Gutierrez et al., 2012a,b). It was shown that *D. magna* reacts by avoidance rather to concentrations than to specific qualities of toxicants (deltametrin, chlorothalonil, and nitrofen) (Ren et al., 2009).

CHAPTER

15

Ecophysiology

Cladocera live in natural water, which actually is a complex dynamic solution of inorganic and organic substances, as well as a colloidal solution of a wide range of minor particles. This solution comprises biologically active and toxic substances (the latter may be partly of natural origin, e.g., phenols). Dodson (2005, p. 176) summarizes the situation as thus: "Aquatic organisms live in an olfactory field of dozens, if not hundreds of biologically significant chemical signals.… For example, *Daphnia* can probably sense at least a dozen different chemical signals. These signals provide information about whether to accept or reject food and about the presence of macrophytes, specific predators, competitors, congeneric individuals, and males." The presence of complex and variable chemical factors should also be mentioned, as well as the fact that factors react and interact in solution, either alleviating or enhancing the action of particular components. Primary and secondary producers are sensitive to the presence of dissolved organic acids, vitamins, and so on, which are variously distributed in space and time. These are "complex chemical landscapes," according to Keller et al. (2001), and it should be added that such landscapes, both experimental (Bowles et al., 2002) and natural, are highly labile.

Cladocera obviously need this solution. In triple distilled water, *Chydorus ovalis* perished (Winberg, 1933), and the ordinary distilled water caused high mortality in *Daphnia magna* within 24 h (Stobbart et al., 1977).

All aquatic invertebrates are adapted to the natural dynamics of their environment. Investigation into the distribution of Cladocera species in relation to the ranges of environmental factors has revealed that at least some species are present within a limited range of factors (Mäemets, 1961; Røen, 1962; Fryer, 1993); their limited distribution may depend on physiological differences between the species. Therefore, physiological mechanisms must be examined to provide explanations for these phenomena.

The present-day global change of the aquatic environment comprises universal industrial and agricultural pollution, acidification by acid rains caused by industrial discharges, eutrophication due to discharge of fertilizers, damming of small and big rivers, amelioration (mostly draining), general decrease of environmental diversity, inter alia, due to deforestation, all leading to perturbation of the natural balance. Creation of water reservoirs and eutrophication result in formation of artificial communities of Cladocera and in outbreaks of superdominant species. Intensive pollution kills most kinds of ordinary life, including Cladocera. Low-level pollution is not less dangerous for the balance of life, especially being not immediately recognizable.

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15.1 PHYSIOLOGICAL BACKGROUND OF THE LIMITS OF ENVIRONMENTAL FACTORS

The population dynamics of Cladocera depends to a great extent directly or indirectly on environmental factors interacting with physiological potential of particular species. This aspect motivates the ecological physiology, i.e., the field attempting to explain why certain species withstand certain limits of environmental factors. Latitudinal distribution of Cladocera species is also involved here, though in this case, historical reasons are important too.

Their morphological and physiological potential enables Cladocera to live in a variety of situations. Physiological characteristics belong to reasons that control their spatial and temporal distribution, as well dominance or rarity of particular species. Unfortunately, the actual values of physiological characteristics are not known for most species. Various Cladocera species inhabit a wide variety of environments: lakes, ponds, microtelmata (accumulations of a few cubic centimeters of water in epiphytic plants) (Smirnov, 1988), various substrates, interstitial capillary water (Dumont, 1987, 1995; Sabater, 1987), surface films, and moist and moss-like growths on tree trunks in tropical forests (*Bryospilus*) (Frey, 1980).

Physical factors are also in a state of continuous change, and reactions may be different to constant and fluctuating factors. Thus, Rider et al. (2005, p. 15) formulated the dependence of physiological processes on the environmental conditions as: "Environmental signals regulate a variety of key processes in animal physiology. In many genera, environmental signals such as changes in day length or temperature signal the onset or termination of reproduction. These environmental signals typically stimulate the release of neuroendocrine signaling molecules that initiate endocrine cascades culminating in physiological responses to the environmental cue."

Cladocera species integrate various environmental factors, which results in a certain quantity of progeny. Depending on combination of numerous and not precisely discernible factors, the result may be a peak or a decline in abundance. In a particular water body, certain species produce peaks of abundance, although in different years, such peaks are different in height, and their timing may be significantly different. Cladocera species may differ in their sensitivity to xenobiotics.

Xenobiotics released to the aquatic environment mostly become highly diluted. Thus, two items deserve consideration: (1) the impact of low concentrations, and (2) agent toxicity in low concentrations. In addition to toxicity of silver in low concentrations, an extremely high toxicity in low concentration of the parasiticide invermectin may be mentioned (Garrick et al., 2008). This parasiticide is toxic in concentrations below the analytical level of detection.

15.1.1 Temperature

Cladocera have been observed to develop in temperate latitudes in warm seasons, i.e., from May until September in the northern hemisphere. Most (but not all) macrothricids prefer warm-water subtropical and tropical situations. Distribution of *Ephemeroporus* spp. (fam. Chydoridae) seems to be confined to warm latitudes. It was therefore inferred that they prefer warm waters.

In *Daphnia longispina*, the growth rate and maturation time remain constant within 14–20°C (Sarviro, 1985). In a temperature gradient, most *D. magna* have been shown to collect together at 18–20°C (Skadovskiy, 1955), at 20°C for adults and 25°C for juveniles (Chernykh and Panasyuk, 1964), or at about 24°C, depending on their previous acclimation (Verbitskii and Verbitskaya, 2011). It was shown, with reference to *D. magna* and *Daphnia pulex*, that Cladocera may survive sudden temperature

changes over the range of 5−30°C (Goss and Bunting, 1976). In *D. magna*, the temperature range is 11−30°C, and the thermal sensitivity for avoidance responses is about 1.5°C (Lagerspetz, 2000).

For *Simocephalus vetulus*, the final selected temperatures are 13.4−25.4°C, the temperatures of normal life activities are 7−29°C, pessimal temperatures, −4 to 7°C and 29−32°C (Verbitsky et al., 2014a), for *Ceriodaphnia quadrangula*: 17.4−26.5°C, 13−27°C, 6−13 and >27°C, respectively (Verbitsky et al., 2014b).

The upper limit of temperature for *Chydorus sphaericus* was determined to be 34°C (Mortimer, 1936) or about 32°C (Bogatova, 1962). For daphnids, the upper limit of temperature [100% lethal dose (or LD_{100})] has been determined as 32°C for *Daphnia cucullata*, 35°C for *D. magna*, 36°C for *D. pulex*, 31°C for *Scapholeberis mucronata* (Mortimer, 1936), 30°C for *Daphnia* and *Ceriodaphnia* (Mallin and Partin, 1989), and 36°C for *D. longispina*. *Moina macrocopa* is inhibited by a temperature of 36°C (Maksimova, 1969) and perishes at 39.5°C (Brown and Crozier, 1927) or 41°C (Maksimova, 1969). *D. magna* is eliminated from natural waters at this temperature, and *D. pulex* at slightly over 30°C (Brown and Crozier, 1927; Goss and Bunting, 1976), so there are clearly differences between species (LaBerge and Hann, 1990). For *Bosmina longirostris*, the tolerant zone for life activities is up to 36°C, but the zone of normal life activities is 11−23°C, according to Verbitsky and Verbitskaya (2002).

It is likely that tropical macrothricids can withstand somewhat higher temperatures, although this has not been investigated. *Macrothrix* spp. are abundant in Iraq in pools that are normally hot under the normal weather conditions of that country. *Latonopsis* (Sididae) and *Alona cambouei* (Chydoridae) have been found in hot springs in India at 34−36°C (Padhye and Kotov, 2010). Upper limits of temperature that immobilize Cladocera are indicated as 38.5°C (Bogatova, 1973).

A further increase in temperature eventually causes protein denaturation (Brown and Crozier, 1927). For *Daphnia middendorffiana*, the upper level of temperature was found to be 26°C, above which inactivation of respiratory enzymes started (Yurista, 1999).

Bownik et al. (2014) investigated the effects of ectoin (an amino acid) on *D. magna* subjected to heat stress within the range 23−42°C and exposed to different concentrations of ectoin, from 2.5 to 25 mg/L. Ectoin was found to be thermoprotective at biochemical and behavioral levels, inter alia, ectoin prolonged time to immobilization.

All of the evidence indicates that the winter minimum temperature is not disastrous for the cladoceran fauna, judging, for example, by the rich fauna in Yakutia, which is within the zone of global minimum temperatures. Egg latency protects the local species of Cladocera. The lower limit of temperature tolerance seems to be about 0°C, since *C. sphaericus* remained active and survived at this temperature, and did not avoid melting snow (Smirnov, 1971). The life span of *D. longispina* at 5°C is much longer than at 20°C (Munro and White, 1975). Some cladocerans stay active and reproduce at a temperature of about 0°C (Rivier, 1986, 1992). They are known to exist on Arctic islands and in tundra, where they are exposed, at least for some of the time, to very low temperatures. Rivier (1986) described winter communities from many ice-covered lakes and reservoirs in which *Daphnia* and *Bosmina* contained embryos. She also reported the presence in winter of *Daphnia* with numerous embryos at the bottom of ice-covered Lake Siverskoe, at a low level of oxygen (c. 2 mg/L O_2).

Some Physiological Events Occurring at Low Temperatures. The effect of environmental temperature is dual. Temperature either (1) controls the intensity of physiological processes, or (2) changes the qualitative aspects of metabolism. It was suggested by Farkas et al. (1984) that *D. magna* cannot overwinter in an active state due to a failure to adapt

their phospholipid composition and the physical state of their membranes to low temperatures. Docosapolyenoic acid is not present in *Daphnia* phospholipids, and the level of polyenoic acid does not increase when the temperature decreases from 20 to 10°C. Schlechtriem et al. (2006) cultivated *D. pulex* at 11 and 22°C for 1 month on a highly unsaturated fatty acid-free diet. Long-term exposure to cold temperature caused a significant increase in eicosapentaenoic acid (EPA; 20:5ω3), and conversion of C18 fatty acid precursors to EPA and arachidonic acid (20:4ω6) was observed. Schwerin et al. (2009) observed compensatory control of physiological processes in the cold: in *D. pulex*, most cold-repressed proteins comprise enzymes involved in protein digestion (trypsins, chymotrypsins, astacin, and carboxypeptidases), and cold-induced proteins include several vitellogenin and actin isoforms. The increased actin concentration in cold-acclimated animals may contribute to the preservation of their muscular performance.

Assuming that polyunsaturated fatty acids (PUFAs) and sterols are involved in adaptation of biological membranes to changing temperatures, Martin-Creuzburg et al. (2012) cultured *D. magna* at 10, 15, 20, and 25°C with food differing in PUFA and sterol composition. PUFAs improved growth at low temperature (10°C), while cholesterol improved growth at increasingly higher temperatures.

Generally, rising temperature is followed by a rising intensity of activity. For example, the heart rate of *D. magna* increased from 60 to 400 beats/min when the temperature rose from 5 to 28°C (MacArthur and Baillie, 1929) (Fig. 6.6). It has also been noted that daphnids swim upward in rising temperatures, whereas they tend to sink at falling temperatures (Gerritsen, 1982). Elevated temperature changes relative activity of proteolytic enzymes as was shown in *D. pulex* (Dölling et al., 2016).

Fluctuating temperature was shown to result in a lower population growth than at constant 25°C (by c. 50%) with reference to *M. macrocopa* (Gama-Flores et al., 2015).

Heating water from 15 to 25°C, with a subsequent reduction to 20°C, resulted in larger body sizes of *C. quadrangula* young, and shorter longevity and earlier maturation of exposed juveniles (Romanovskiy and Loganova, 2010). In *Simocephalus*, Verbitsky and Verbitskaya (2009) also identified the aftereffects of previous temperature changes, for example, increase of the population growth after previous cooling.

It was shown experimentally that the ability of *Daphnia* spp. to avoid fish predation by submerging to colder (deeper) water layers is suppressed by absence of EPA in their food (Brzeziński and von Elert, 2015).

Following UV-B irradiation, *Daphnia* spp. showed increased survival and removal rates of light-dependent DNA damage at 10°C than at 20°C (Connelly et al., 2009).

According to Gainutdinov et al. (2000), the thermostability of *D. magna* is modified by the addition of glutamate, glycine, or adenosine 3′,5′-cyclic monophosphate, with the changes being slow, strong, or fluctuating, depending on the concentration of substance added. The authors interpreted this process as "coding of the chemosensory information by the nervous system," although it is unknown which organs of Cladocera are involved in temperature perception.

15.1.2 Water Salinity

Most Cladocera are freshwater animals, but some are marine species or live in saline lakes. Their ability to live at different salinities depends on their capacity for osmotic regulation (discussed in Chapter 8). It should be noted that marine salinity and that of salt lakes differ in their salt composition.

15.1.3 Acidity–Alkalinity Ranges

Most Cladocera prefer a neutral pH. The upper limit of pH was determined for chydorids as 10.6 (Bogatova, 1962), while a lower limit of pH 3.7 has

been reported for *Acantholeberis curvirostris*, *Alonella excisa*, *C. sphaericus*, *Ceriodaphnia setosa*, and *Ophryoxus gracilis* (Kitaev, 2007). Havas and Likens (1985) showed that *Daphnia catawba* die below pH 5.0, but that *Holopedium* can survive even at pH 4.0.

There exist acidophilic species—the macrothricid genera Acantholeberis, *Streblocerus*, and some Australian endemic chydorids—that are confined to acid coastal dune lakes (Smirnov and Timms, 1983). Preference for an acid medium is especially clear in the macrothricids *Streblocerus serricaudatus* and *A. curvirostris*. The latter retains sodium (Na) better than does *D. magna*, which is improved in acidic medium; the rate of sodium loss at pH 3 is lower than at pH 7 (Potts and Fryer, 1979). *Bosmina obtusirostris* is an acidophilic species whose abundance increases at low pH (Lazareva, 1996).

D. pulex usually lives in circumneutral conditions, and Weber and Pirow (2009) investigated its response to acid stress. At pH 7.8 and normocapnia (i.e., a normal partial pressure of CO_2), its extracellular pH is 8.33 and P_{CO2} is 0.56 kPa, and bicarbonate concentration in its hemolymph is 20.9 mM, covering 93% of the total buffer value. Acidic conditions caused a slight acidosis (ΔpH was 0.16−0.23), a 30−65% bicarbonate loss, tachycardia, hyperventilation, and hypermetabolism. At pH 5.5, defense mechanisms were activated, extracellular P_{CO2} decreased to 0.33 kPa, and the animals tolerated only short-term exposure to this pH. These authors also suggested a model of whole-animal CO_2 transport.

For *C. sphaericus*, HCl was more toxic than H_2SO_4 in all concentrations (Tauson, 1924b).

On the other hand, there are sodic alkaline lakes (pH over 8) that are inhabited by a few specialized species of Cladocera (Dvihally and Ponyi, 1957; Metz and Forró, 1989; Forró, 1992).

15.1.4 Oxygen Concentration

Some Cladocera, for example, *Ceriodaphnia laticaudata*, can withstand low levels of oxygen (Fox, 1945; Burgis, 1967). Accordingly, this species developed in great abundance during the first year of existence of the Cherepovets Reservoir (in the Upper Volga basin) in response to a general oxygen deficit caused by decomposition of flooded vegetation and forest litter (Smirnov, 1966). In subsequent years, not a single specimen of *C. laticaudata* was found, although *Ceriodaphnia pulchella* and *C. quadrangula* became dominant instead.

15.1.5 Illumination

Photoperiod (see Section 13.4.7) and seasonal changes of illumination obviously influence the population dynamics of Cladocera. However, some Cladocera can live in the dark.

Ultraviolet Radiation

UV radiation (UVR) is thought to affect Cladocera that inhabit water bodies at high altitudes and high latitudes. These Cladocera develop black pigmentation, which is thought to be protective. Indeed, exposure to sublethal UV irradiation resulted in damage to the intestine in a large proportion of subarctic *Daphnia* (Zellmer and Arts, 2005). It was shown by Zellmer et al. (2006) that following exposure to sublethal solar UVR, *D. pulex* suffers damage to the intestinal system similar to that induced by fasting, although the function of its digestive enzymes (amylase and cellulase) is not much altered.

15.2 ADAPTATION

Species of the same genus may differ greatly in their reactions to environmental conditions and in their behavior. For example, different species of *Ceriodaphnia* are known to tolerate different oxygen limits (Burgis, 1967), different *Moina* species either inhabit freshwater or live in saline waters, *Daphnia* species are ecologically

different, and *Chydorus* species either swim or are confined to the bottom (e.g., *Chydorus gibbus*). It is remarkable that the reactions of Cladocera to external factors are so labile and that the values that characterize cladocerans' reactions to various factors are so variable. The numerical values of the physiological parameters discussed throughout this text are therefore not absolute. Such values depend on:

1. age and sex;
2. previous adaptation;
3. interaction with other factors (synergies and antagonistic interactions); and
4. the presence within a morphologically defined species of genotypes (clones) that have different tolerances to environmental factors.

This is why the characteristics of environmental factors are relevant to a discussion of the geographic distribution of a particular species. It should be noted that the values given for ranges of stability to certain factors, and any such figures, are not constant, but instead depend on other variables and on previous acclimation. This has been shown, for example, for copper (Cu) by Bossuyt et al. (2005), and for acclimation to different previous temperatures by Shkorbatov (1982): temperature shock in *Daphnia* acclimated to 28°C occurs at a higher temperature than that of *Daphnia* acclimated to 8°C. However, beyond a certain limit, physiological temperature adaptation may be replaced by a regular diapause (Mitchell et al., 2004).

Survival may also depend on the previous environment of the specimens being tested. Shkorbatov (1953) highlighted the effects of prolonged physiological adaptation of Cladocera, for example, *S. vetulus* taken from a pond survived in deoxygenated water much longer than did specimens taken from a river. In addition, Stroganov (1983) emphasized the fact that individuals also differ in their stability, as observed in *D. magna* in solutions of sodium pentachlorophenolate.

15.3 GENOTYPES

Green (1956a,b) found "racial and clonal differences" within the same *Daphnia* species in the ability of individuals to synthesize hemoglobin and thus of their adaptation to low oxygen levels in the habitat. Quantitative estimations of the response of *D. magna* to the impact of environmental factors may also vary, depending on genetic factors (Barata et al., 2000). In *D. pulex*, 92 genotypes have been found (among 227 specimens); in *S. vetulus*, 18 genotypes were identified (among 112 specimens), which showed physiological differences (LaBerge and Hann, 1990). In 25 tested clones of *D. magna*, thermoresistance of the heart, when tested at 38°C, was variable: some clones exhibited a high mean thermoresistance, whereas in others, it was low (Pashkova et al., 1998). *D. longispina* lineages have been shown to differ in their resistance to Cu (Martins et al., 2005). Thus, it is likely that the elimination of vulnerable lines may lead to a loss of genetic diversity.

15.4 ENVIRONMENTAL CONDITIONING BY CLADOCERA

15.4.1 Biofiltration (Clearance)

As a mass group, the Cladocera within a water body filter great volumes of water (see also Chapter 4, Section 4.1, "Feeding"). Their filtering rate has been determined to be 0.9–5.15 mL/individual (ind.)/24 h in 0.7–1.8 mm long *D. pulex*, increasing with size in a curvilinear fashion (Richman, 1958); in 2-mm long *D. pulex*, it is about 5 mL/ind./24 h in the daytime, increasing at night up to about 20 mL/ind./24 h (Haney, 1985). The clearance rate of *D. magna* was c. 4.2 mL/ind./h, and it decreased at high algal concentrations in the environment; that of *Bosmina* was determined as less than 10 μL/ind./h (Bogdan and Gilbert, 1982), ranging by the data of various authors within 6–300.

As summarized for ctenopods by Korovchinsky (2004), it is 1–248 mL/ind./24 h in *Diaphanosoma brachyurum* s.l., 10–58 mL/ind./24 h in *Holopedium*, 2–657 mL/ind./24 h in *Sida*, and 32–252 mL/ind./24 h in *Penilia*. In *D. magna*, it is 0.24–3.56 mL/ind./h, depending on the food concentration (Porter and Orcutt, 1980). In one example, the clearance rate (i.e., filtering rate) of a *Daphnia hyalina* population was about 50% of the lake volume per day during most of the growing season (Balayla and Moss, 2004). Cladocera thus clarify water, and their feces fall to the bottom.

15.4.2 Impact on the Gaseous Conditions of the Environment

Cladocera consume oxygen and release carbon dioxide. The extent of their influence may be assessed from their rate of oxygen consumption and the quantity of Cladocera per unit volume of water (see Chapter 5).

15.4.3 Enrichment of the Environment With Organic Matter

Cladocera release considerable quantities of nitrogen (N), phosphorus (P), and carbon (as dissolved organic C). While N is released as final products of protein metabolism, the source of liberated P seems to be digested compounds from lipids and phosphoric acid. The N:P ratio in the surrounding water is increased due to activities of *B. longirostris*, thus increasing phosphorus limitation (Balseiro et al., 1997). As a result of their abundance in the aquatic environment, Cladocera make a large contribution to the complex solution that is natural water. This contribution can be quantitatively estimated by consideration of the available data. For such quantitative estimations, see also Chapter 7, Section 7.2.

Cladocera also release a variety of organic and inorganic compounds into the water (as noted, e.g., by Fish and Morel, 1983), which may influence other invertebrates. In laboratory experiments, Matveev (1993) demonstrated that the liquid after cultivation of *Daphnia carinata* reduced the feeding rate of *Daphnia*, *Moina*, and *Diaphanosoma*. The mean release of urea by well-fed *Daphnia* was estimated to be 0.36 μg/mg/h and that of ammonia to be 0.76 μg/mg/h (Wiltshire and Lampert, 1999); in contrast, starved *Daphnia* liberated 0.06–0.1 μg/mg/h of urea and 0.45 μg/mg/h of ammonia. The released urea is presumed to influence the development and morphology of green algae.

D. magna release about 40 pmol/organism/h of organic Cu-binding compounds, thought to consist of molecules larger than amino acids (Fish and Morel, 1983). *D. magna* also release a chemical (an organic substance of small molecular mass) into the environment that induces colony formation in the green alga *Scenedesmus acutus* (Lampert et al., 1994). Yasumoto et al. (2005) also demonstrated that *D. pulex* releases aliphatic sulfates that increase the size of *Scenedesmus cenobia*.

Immediately after molting of a single *Daphnia pulicaria*, the activity of β-N-acetylglucosaminidase in the culture medium increased by 40–100 times (Vrba and Machaček, 1994); this molting enzyme sustained its activity for >2 days.

Alkaline phosphatase and a small amount of acid phosphatase are released by *D. magna* into the environment (Boavida and Heath, 1984; Zhao et al., 2006a,b). The liberation of enzymes by animals to their aquatic medium may be a common occurrence, as it has also been shown for fish (Kuzmina et al., 2010).

15.4.4 Infochemicals

The substances released by invertebrates, as far as they are involved in chemical communication in the aquatic environment, are known as infochemicals. The related processes are universally important. The quantitative scale of these

processes may be estimated by considering the aforementioned data. A particular example is modification of formation of cenobia in the green alga *Scenedesmus* influenced by a chemical released by *D. magna* during its nondigestive metabolism (von Elert and Franck, 1999). Infochemicals released by *Daphnia* influence functional traits of *Microcystis* (a blue–green) (Van Gremberghe et al., 2009) and of *Chlorella* (growth and esterase activity) (Yang and Kong, 2011).

The infochemical released by *D. magna* has been characterized as an olefinic low-molecular-weight carboxylic acid (von Elert and Franck, 1999).

Xenobiotics (anthropogenic pollutants) act as infodisruptors, disconnecting or modifying natural ecological interrelations, disturbing chemical communication between species (Gutierrez et al., 2012b).

15.5 CLADOCERA IN THE POLLUTED HYDROSPHERE

Worldwide water pollution has been recognized for a long time ago already. Anderson (1954) evidenced for an industrial economy (North America): "What has happened to our apparently limitless water resources? We have found that these resources are not limitless. Moreover, we have used much of what we have in ways that render it unfit for further use without extensive reprocessing" (p. 60). "Natural waters contain almost everything under the sun.… Polluted waters differ from natural waters in the relative quantities of the impurities present" (p. 61). Most species of Cladocera live now in the polluted environment.

15.5.1 Inorganic Substances

It was shown in *D. magna* exposed to tritiated water with an activity of 5×10^2–5×10^8 Bq/L that the number of broods, the size of each brood, life span, and growth rate decreased within five generations, embryos developed disproportionately, and their eggs decomposed (Gudkov and Kipnis, 1995).

The following sequence of toxicity (in decreasing order) was shown for *D. magna* (Shcherban, 1977): $Cd^{2+} > Cu^{2+} > Zn^{2+} > Ni^{2+} > Mn^{2+}$. Toxicity increased at higher temperatures. Some compounds are comparatively highly toxic. Thus, *C. sphaericus* is killed in 24 h by 0.00001% silver nitrate, 0.0005% potassium bichromate, and 0.003% potassium permanganate, while most of compounds of K and Na are lethal at 0.05–2% (Smirnov, 1971).

Aluminum (*Al*). *D. catawba* and *Holopedium* were not found to be particularly sensitive to aluminum (Havas and Likens, 1985).

Arsenic (*As*). The content in European natural waters of 2–3 mg/m^3 As was indicated by Hutchinson (1957). It has been shown that trivalent arsenic is more toxic than pentavalent arsenic to *D. carinata* (He et al., 2009).

Cadmium (*Cd*). It was determined experimentally that *Ceriodaphnia dubia* is more sensitive than *D. magna* to cadmium (as $CdCl_2$) (Suedel et al., 1997). The uptake rate and killing rate of Cd for *D. magna* increased with an increase in temperature from 10 to 35°C (Heugens et al., 2003). Cadmium chloride increased the total acid phosphatase activity in *D. magna*, although the activity of individual molecular forms of this enzyme either increased or decreased (Tsvetkov et al., 1997).

Chromium (Cr) was more toxic for *C. dubia* at higher densities of algal food (green alga *Pseudokirchneriella* grown in the medium with Cr) (Rodgher and Espindola, 2008). Seasonal changes in sensitivity of *D. magna* to potassium bichromate were recorded (estimated by fecundity); the sensitivity was lowest in February and highest in March–May (Isakova and Yukleevskikh, 1998). No further explanation is given by these authors.

Copper (*Cu*). The following data are supplied by Riley (1939). Its content for waters in the US was indicated as 0.009–0.28 mg/L. In the body

of *D. magna*, Cu is discovered after immersion in solution of diethyldithiocarbamate as brown coloration in the intestine, heart, and muscles. At toxic concentrations, Cu causes first a stimulation of movement, followed by the toxic effect "accompanied by degenerative morphological changes" (not further specified) (Riley, 1939, p. 87). The presence of copper in water decreases the oxygen consumption in *Daphnia* (Knyazeva, 1994). The effect of Cu depends on the clone, as shown for *D. longispina* (Lopes et al., 2005). Cu toxicity decreases in the presence of Na (De Schamphelaere et al., 2007) or organic matter in the medium, especially humic acids, as has been shown for *D. magna* (De Schamphelaere et al., 2004) and *C. dubia* (Kim et al., 1999). Observations in water bodies formed after copper ore excavation would be demonstrative.

Mercury (*Hg*). The scale of mercury contamination may be illustrated by the case of Lake Managua (Nicaragua), where about 40 tons of elemental Hg was introduced from a chloralkali industry during the first 12 years of its activity (Lacayo et al., 1991). One result was the complete disappearance of *Bosmina* from the plankton. In the aquatic situation, Hg is transformed into methylmercury, which is even more toxic. According to Tsui and Wang (2004b), the uptake of Hg and methylmercury by *D. magna* is proportional to their ambient concentration, and efflux rates are comparable; release takes place via excretion, egestion, molting, and neonate production. In bioaccumulation, methylmercury is predominant. In lakes in Wisconsin (US), the methylmercury concentrations were 1–211 ng/g in *D. pulex*, *Daphnia galeata mendotae*, and *Daphnia ambigua*, and 40–419 ng/g in *Holopedium* (Back and Watras, 1995). A low concentration of mercuric chloride ($HgCl_2$; a daily rate 0.01 μg Hg^{2+}) given with food to *Ceriodaphnia affinis* stimulated reproduction, but survival and longevity decreased (Gremiachikh and Tomilina, 2010).

Potassium (*K*). Juvenile *D. magna* exhibit a very varied sensitivity toward potassium dichromate ($K_2Cr_2O_7$) (Klein, 2000), as noted by their immobilization.

Silver (Ag) is one of the most toxic elements. *C. sphaericus* perish at 0.00001% of $AgNO_3$ in 24 h (Smirnov, 1971). *D. magna* and *C. dubia* have been shown to be highly sensitive to $AgNO_3$, but less so to AgCl (Rodgers et al., 1997). As shown for *D. magna*, nanosilver acts in a similar mechanism to ionic silver (Hoheisel et al., 2012); however, the uptake pathway of silver nanoparticles is different from that of $AgNO_3$, namely, nanosilver particles are ingested and c. 60% of them are found in the gut (Zhao and Wang, 2012). Ingestion rates was size-dependent and decreased in the following sequence: 20 nm > 50 nm > 100 nm (Zhao and Wang, 2012).

Titanium (*Ti*). TiO_2 as c. 10 nm powder reduced the growth, reproduction, and survival of *D. magna* at concentrations 4.5–7.5 mg/L (Das et al., 2013).

Uranium (*U*). It was determined that the chemical toxicity of uranium predominated over its radiotoxicity for *D. magna* as estimated by decreased somatic growth and reproduction (Zeman et al., 2008; Massarin et al., 2011). U affected C assimilation; consequences increased across generations (Massarin et al., 2011).

Zinc (*Zn*). Acclimation of *D. magna* to Zn was demonstrated, which influenced determinations of Zn toxicity (Muyssen et al., 2002).

15.5.2 Organic Substances

Natural Toxicity

Tannins. Taking into consideration natural toxicity due to tannins liberating to water from decaying natural vegetation, sensitivity of Cladocera to tannic acid was tested. Tannic acid was more deleterious to *C. sphaericus* than to *D. pulex* (Pautou et al., 2000). At 0.125–0.5 mM, degenerative changes occurred in the midgut epithelium.

Phenol. A part of phenol originates from natural sources. Of the Cladocera tested, *S. vetulus*

was the least sensitive to the presence of phenol, and *Sida crystallina* was the most sensitive (Luferova and Flerov, 1971b). These authors mainly attribute the latter effect to the higher (almost double) beating rate of the thoracic limbs in *Sida*. The motor activity of *D. longispina* increased at increasing phenol concentrations, but abruptly decreased at a concentration of 70 mg/L (Luferova and Flerov, 1971a). Na transport in *D. magna* is inhibited by 2,4 dinitrophenol (Stobbart et al., 1977).

Pollutants

Pollutants may be differently toxic for a particular species. The following sequence of toxicity (in decreasing order) was shown for *D. pulex* (Flerov, 1977): chlorophos—polychlorpinen—ammonium perchlorate—phenol.

Acetylsalicylic acid is more toxic to *D. longispina* than to *D. magna* in acute assays, but is more toxic to *D. magna* under chronic exposure (Marques et al., 2004). Its metabolites were also tested; these turned out to be most toxic to *D. longispina* (Marques et al., 2004). Nonviable neonates and aborted eggs were produced in addition to the direct toxicity observed.

Crude oil. Oil and its derivatives were shown (Ratushnyak et al., 2009) to be toxic for *D. magna* in the following sequence: diesel oil > crude oil > benzine A-76 > aqueous extract from benzine A-76. Crude oil was shown to be a most potent anticholinesterase agent for *M. macrocopa*, followed by its mixtures with lead or sodium dodecylbenzene sulfonate (Martinez-Tabche et al., 1997).

Fipronil (a phenylpyrazole insecticide) is more toxic for *C. dubia* than its optical isomers (enantiomers), racemate, or photodegradation product (fipronil-desulfinyl) (Konwick et al., 2005; Wilson et al., 2008). Enantiomers of the same compound (present in pesticides) were found to have drastically different toxicities when tested on *C. dubia* and *D. magna* (Liu et al., 2005).

Exposure to near-UV light makes nontoxic 2,3-dinitrotoluene, 3,4-dinitrotoluene, and 4-amino-2,6-dinitrotoluene toxic (phototoxicity) to *D. magna*; 2-amino-4,6-dinitrotoluene was toxic and phototoxic (Davenport et al., 1994).

The survival of cladocerans exposed to toxicants also depends on the period between repeated exposures (Naddy et al., 2000). *D. magna* can withstand the acutely lethal organophosphate insecticide chlorpyrifos (at c. 1 μg/L), provided there is adequate time for recovery between exposures (Naddy and Klaine, 2001).

The effects of different xenobiotics are also discussed in sections describing the particular functions.

15.6 SYNERGISM AND ANTAGONISM AMONG ENVIRONMENTAL FACTORS

Numerous dynamic factors can act "in concert," as Steinberg et al. (2010) noted, and the independent action of one factor may be modified by other factors. "Impacts of multiple stressors" are also discussed (Agatz and Brown, 2013), leading to polytoxicoses of vague origin. Antagonism (or synergism) between ions in solution modifies their individual actions in certain species.

Tauson (1924a,b) was probably one of the first to demonstrate this in *Daphnia* and *Simocephalus*. She observed a decrease in the death of various cladocerans in the presence of certain combinations of cations in comparison to treatment with single ions. Winberg (1933) demonstrated experimentally that the destructive action of the potassium ion (K^+) on *C. sphaericus* is decreased in the presence of the sodium ion (Na^+), but is unaffected by the presence of calcium ions (Ca^{2+}). In contrast, in the case of *C. ovalis*, Ca^{2+} also moderates the negative action of K^+.

Toxicity of Pb for *C. dubia* is increased at low pH, but humic acids protect it against toxicity (Mager et al., 2011).

Various aspects of synergism are studied on *D. magna*. Combinations of metal ions are more dangerous for *D. magna* than are isolated ions at the same concentration. It has also been shown by Dediu et al. (1995) that combinations of metals are more dangerous than separate metals at the same concentration, as shown for Cu + Zn, Cd + Zn, Hg + Zn, Cu + Cd, Cd + Hg, and Cu + Hg. Pairs of metal ions may be either antagonistic (as estimated by toxicity in *D. magna*), e.g., Al + Mo, Cr + Co, and Cr + Mg, or synergistic, e.g., Cr + Se, Cr + Zn, and Fe + Se, as demonstrated by Tomasik et al. (1995). The relationships between ions also depend on their concentration. Yakovlev et al. (2000) found that the combination of Zn + Cd is antagonistic toward *D. magna* survival, whereas Zn + Cu are synergistic. According to these authors, there are also still more complex interactions. Selenium uptake increased significantly as sulfate concentrations decreased (Ogle and Knight, 1996). In addition, Ca and Hg each inhibits the accumulation of the other (Teles et al., 2005). Elevated Na concentrations increased the uptake rates of Cd and Pb (Komjarova and Blust, 2009).

The zinc (Zn) toxicity toward *D. pulex* is alleviated by Ca^{2+}, whereas in *D. magna*, in the presence of Ca, the Cd, Ni, Pb, and Zn, uptake is suppressed (Clifford and McGeer, 2009).

Synergistic relationships are also true of pollutants of complex structure. Dodson and Hanazato (1995) drew attention to synergistic relationships of sublethal concentrations of carbaryl with natural chemicals with reference to *Daphnia*. The effects on *C. dubia* of mixed spinosad and R-11 (an adjuvant used with pesticides) (Deardorff and Stark, 2009); of R-11, nonylphenyl polyethoxylate, and imidacloprid (Chen et al., 2010); and of imidacloprid and thiacloprid (Pavlaki et al., 2011) were found to be synergistic (i.e., acted in a more than additive manner). There are two possible explanations for these observations: effects on the direct toxicity of particular ions or the more complex effect of deviation from the natural equilibrium of ions in fresh- or brackish water, to which certain species are adapted.

On the contrary, glyphosate reduces the acute toxicity of Ag, Cd, Cr, Cu, Ni, Pb, and Zn (but not of Hg and Se) toward *C. dubia* (Tsui et al., 2005). Mixtures of glyphosate and paraquate manifested decreased toxicity than their isolated action on *M. macrocopa* (Lui and Xi, 2012).

Humic substances variously influence toxicity of xenobiotics: toxicity of copper (Cu^{2+}) for *Ceriodaphnia silvestrii* is decreased by humic substances (Santos et al., 2008), and toxicity of Al for *Moinodaphnia macleayi* is decreased by fulvic acid (Trenfield et al., 2012). In humic water, the uptake rate of Cd by *D. magna* was twice higher than in humic-free water, the depuration rate was equal in both environments (Penttinen et al., 1995).

15.7 CLADOCERA AS TOOLS IN WATER QUALITY AND MEDICAL TESTING

There are three main applications of Cladocera to consider: their use in measuring pollution levels, drinking water quality, and in medical testing.

Historically, *Daphnia* was one of the first biological indicators and the first one among Cladocera (Naumann, 1929). It appeared early in toxicological studies (Beklemishev, 1923a,b, 1924; Tauson, 1924a,b; Anderson, 1944). In various medical investigations, it was used as a reagent by various authors (MacCallum, 1905; Billiard, 1925). Occasionally, it was rediscovered as such to great satisfaction of later authors: "Pioneering for 10 years with a new living reagent — *daphnia* — by the senior author and his students, or associates, has made it possible to solve the intricate problems of great scientific, medicinal and economic importance" (Viehoever and Cohen, 1937, p. 285).

First, *Cladocera* spp. have found a role in the system of indicators of the saprobic level of water (i.e., of pollution with organic substances) (Kolkwitz and Marsson, 1909) and in subsequent modifications of this system. According to these authors, they appear to decrease pollution due to self-purification in both β-mesosaprobic and oligosaprobic zones. Obviously, their distribution depends on hydrochemistry. Hrbácek and Hrbácková (1980) discussed the use of planktonic Cladocera for the estimation of water eutrophication. Andronnikova (1996) described mixtures of zooplankton characteristic of oligotrophic, eutrophic, and polyhumic lakes.

Second, *Daphnia* was the first organism used to determine the quality of drinking water (Naumann, 1929). Cladocera, principally *Daphnia* species, have frequently been used for this purpose (see, e.g., Anderson, 1944, 1948, 1954; Lesnikov, 1967). *C. dubia* has also been used for the biotesting of water (Flerov et al., 1988). In the wide field of aquatic toxicology, the Cladocera (mostly daphnids) are frequently used as test objects or sentinel species (Lesnikov, 1967; Isakova and Kolosova, 1988), and *Daphnia* has been described as an excellent model organism for studying multiple environmental stressors (Altshuler et al., 2011).

The characteristics and conditions for using daphnids in toxicity testing have been described by many authors (Anderson, 1944; Lesnikov, 1967; Adema, 1978; Leeuwangh, 1978; Ten Berge, 1978; Braudo, 1987; Zhmur, 2001; Pieters, 2007). The suitability of Cladocera as indicators of various environmental conditions has been discussed by Walseng and Schartau (2011). Lesnikov (1967) suggested estimation of the condition of daphnids by scoring their oogenesis, embryogenesis, coloration, and oil drop status, as well as fullness of the gut.

Daphnia was especially recommended for phenol assessment (Tumanov et al., 1988a,b). *D. magna* can also be used to evaluate the quality (toxicity) of bottom sediments (Terra et al., 2010; Romanenko et al., 2011).

The sensitivity of *D. magna*, *D. pulex*, and *D. cucullata* to various chemicals was found to be similar (Canton and Adema, 1978), although Lesnikov (1967) found that *D. pulex* is somewhat less sensitive, and different lines (or clones) of this species noticeably differ in their sensitivity. Of course, pollutants act as a mixture of interacting ingredients, the effect of which is unpredictable, as particular substances can react with each other, leading to partial neutralization or entering into synergistic relationships.

A wide range of salts was tested as to their concentration causing immobilization of *D. magna*. Toxicity of sodium salts (in ppm) was very different (Anderson, 1946): from NaBr 8200 and Na_2SO_4 5960 to Na_2CrO_7 and $Na_2Cr_2O_4$, c. 0.31. That of chlorides (Anderson, 1948) ranged (ppm) from NaCl 3680 to CdCl c. 0.002 and CuCl 0.027. The insecticides ranged in toxicity (immobilization) for *D. magna* from (ppb): EPN [O-ethyl O-(*p*-nitrophenyl)thiobenzenephosphate] 0.1, parathion 0.8 to chlorobenzilate 1400 (Anderson, 1960). The pesticides tested by Frear and Boyd (1967) ranged from extremely toxic [median lethal dose (LD_{50}) of carbophenothion = 9 ppt (parts per trillion)] to noticeably less toxic (LD_{50} of prometone = 35 ppm).

Tumanov et al. (1988a,b) suggested a quick method for measuring pollution using previously prepared calibration curves of *Daphnia* immobilization by particular organophosphates at different concentrations.

The use of *D. magna* as an ecotoxicological indicator is allowable if its genotype and previous adaptation status are taken into consideration, as discussed by Baird et al. (1989). A fast toxicity test using parthenogenetic *D. magna* eggs in vitro was also suggested by Sobral et al. (2001) and Krylov (2011). The rate of formation of males in *D. magna* was used to measure the presence of pollutants with hormone-disrupting effects (Tatarazako and Oda, 2007).

Of the littoral species, the use of *C. sphaericus* was suggested for assessing water quality

(Pieters et al., 2008) or sediment toxicity (Dekker et al., 2006). For *C. sphaericus*, LD_{50} at 96 h was determined by Dekker et al. (2006) to be 46 mg ammonia (NH_3)/L.

Third, an attempt has also been made to use *Daphnia* for testing the impact of human physiological liquids: urine, blood serum, hydroptic liquid, and pleural fluid, as well as various biologically active substances (Billiard, 1925). This author determined the average survival time of *Daphnia* in urine to be 7 min (Billiard, 1926). If the daphnids lived longer, the urine was considered hypotoxic; if shorter, it was considered to be hypertoxic. In patients with lung inflammation and a high temperature, the urine contained albumin and the daphnids lived for an abnormally long time (Billiard and Perrot, 1926).

As well, there had been pharmacological investigations into the effect of various drugs on Cladocera species, such as, for example, of purgatives (MacCallum, 1905), various drugs (Viehoever, 1936; Sollmann and Webb, 1941), sympathomimetics (Flűckiger, 1952, 1953), cholinomimetics (Tonkopiy et al., 1994a), and metformin (antidiabetic) (Sheng et al., 2012).

Viehoever (1936, p. 1116) was highly enthusiastic as to the application of *Daphnia* in pharmacological research: *Daphnia* "is truly a remarkable biological reagent. Its use opens a new world for experimentation. It proves a new tool for identification, differentiation and evaluation of physiologically active substances." A little bit later, Sollmann and Webb (1941, p. 267) were more reserved: "The pharmacologic reactions of *daphnia* differ qualitatively from those of vertebrates in many particulars, which limit the usefulness of this animal as a pharmacologic reagent." Recent information demonstrates specific physiological features of Cladocera and analogies with mammals (Tonkopiy et al., 1994a), thus outlining fields of their actual application for such purposes.

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CHAPTER

16

A Cytological Perspective

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Early cytological examinations of cladocerans focused on general body plans and embryological development in *Daphnia*, *Leptodora*, and *Moina*, and included valuable drawings of tissue and cellular structures (e.g., Weismann, 1874; Grobben, 1879; Lebedinsky, 1891). Grobben's (1879) survey of development in *Moina*, for example, contained detailed sketches of early cleavage, and was thought to be the impetus for later studies "on cell lineage and on formative substances" (Witschi, 1934). But as many of these contributions were published in German more than a century ago, complete translations are not readily available, limiting accessibility. Later investigations shifted from basic morphology, to cell size and details of specific structures such as the labrum and nervous system (e.g., see Sterba, 1957a,b). The first cytogenetic examinations were completed long before the links between chromatin, genes, DNA, and heredity were established and consisted of the gross organization of chromosomes.

Today, information on nuclear structure, nuclear membranes, Golgi apparatus, and so forth are rather limited in this group and perhaps continued search for these cytological details has been interrupted by the molecular revolution.

Currently, researchers apply a burgeoning assortment of microscopy and molecular tools to address questions of genome structure/organization and the pivotal roles that genes play in cellular growth and development. These studies contribute to our understanding of the genetic and developmental bases for an array of ecological and physiological traits in cladocerans. The ability to pinpoint the location of factors in cells as well as evaluate the upregulation of proteins, for example, will strengthen our understanding of intra- and intercellular processes, and by extension the physiology and biology of the organisms. The highly advertised release of the nuclear genome sequence for a specific clone of one of the recently diverged species of the *Daphnia pulex* lineage, *Daphnia arenata* (Colbourne et al., 2011; plus supporting online material) has revealed some remarkable findings. Like its body size, the genome of this model organism is diminutive, but it still manages to contain many more genes than are found in the human genome (>30,000, which is 5000−10,000 more) (Colbourne et al., 2011). The inflated gene count appears to be a result of duplications as well as the presence of novel genes; about one-third of the identified genes have no known homology with the proteome of any other species.

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Furthermore, using complementary DNA (cDNA) libraries and microarrays, Colbourne et al. (2011) found that the expression of much of the transcriptome changes with altered environmental (biotic and abiotic) stresses. This represents an enormous step forward in our quest to understand the links between genetics, ecology, and life history strategies of *Daphnia*. In addition, the project led to chromosome analyses that have provided us with further insights into their structure and organization (see later discussion).

Many cladoceran species make ideal ecological study organisms due to their ease of maintenance in culture and their parthenogenetic life cycle. However, in most species, the body carapace, which restricts growth except at ecdysis, combined with a small body and similarly small genome size, has generally hindered cytological research on the group. Members of the genus *Daphnia* (especially *Daphnia magna* and *D. pulex*) have been at the center of much of this research, so comprehensive comparisons are often lacking and coverage of some topics is quite limited. In this chapter, we aim to synthesize the available literature on cladoceran genome size variation, cytogenetics, endopolyploidy, and cytology of various tissues. Current gaps in our knowledge of the order will also be highlighted.

16.1 GENOME SIZE AND POLYPLOIDY

16.1.1 Background

Studies estimating the amount of chromatin present in a nucleus predate the discovery of the structure of chromosomes (for a brief review, see Gregory, 2002, 2005). For example, Swift (1950) found a constant and characteristic amount of DNA per haploid chromosome set for each of two plant species. He also demonstrated that cells in somatic tissues regularly contain a discrete multiple of the haploid amount. From Swift's work, the term *C-value* (meaning genome size) was defined as the quantity of DNA that is contained in a species' haploid chromosome complement. Mirsky and Ris (1951) provided the first two crustacean estimates (for a decapod and a barnacle), but the first cladoceran estimate was not published until much later (Rasch, 1985).

Genome size is thought to vary by more than 200,000-fold across eukaryotic species! This range shrinks at lower taxonomic levels (excluding incidents of polyploidy), so comparisons across members of the same order tend to show contracted differences. Gregory's (2016) animal genome-size database includes records from more than 1500 invertebrates, 330 of which are crustaceans (representing nearly 300 species). Such meager coverage for a group comprising nearly 70,000 extant species demonstrates our limited knowledge in this area. As estimates accumulate, patterns in the data that seem obvious today may become murky tomorrow and patterns may appear next week that were not obvious yesterday.

Among crustaceans, published C-values range from 0.14 to 64.62 picograms (pg), with a mean of ~5 pg (Gregory, 2016). This represents a greater than 400-fold range, the widest range of published estimates among invertebrate groups; members of the Platyhelminthes exhibit the second largest invertebrate range (less than 200-fold) (Gregory, 2016). To place the crustacean range in perspective, 64.62 and 0.02 pg represent the largest and smallest published invertebrate estimates (Gregory, 2016). So, where do cladoceran genomes fit within the crustacean range of DNA contents?

16.1.2 Overview of the Techniques

The genome size, or amount of DNA in the haploid cell, has been determined via several methods over the years, including biochemical separation, photometric estimates (typically using the

DNA-specific Feulgen reaction or flow cytometry), and even genome sequencing. The methods differ in their ease of use and cost, as well as the expertise, time, and equipment required.

Early eukaryotic measurements were produced via biochemical analysis. Genome size was predicted from the molecular weight for a large tissue sample divided by a cell number estimate; this provided imprecise but strong evidence of the constancy of DNA (Hardie et al., 2002). More recent methods have utilized the properties of the Feulgen reaction, which selectively stains nucleic acids, in concert with densitometry. During the staining protocol, mild acid hydrolysis (typically using fixed tissue) cleaves purine bases, exposing free aldehyde groups (Chieco and Derenzini, 1999). Subsequent staining with Schiff reagent allows aldehyde groups to bind the pararosaniline present in the reagent, turning it from colorless to magenta (see Fig. 16.1 for examples). The intensity of the stain is proportional to the number of aldehyde sites available and, therefore, to the amount of DNA present. A key step in the reaction is acid hydrolysis; extending the duration of this step beyond the tissue-specific optimized time frame leads to increased

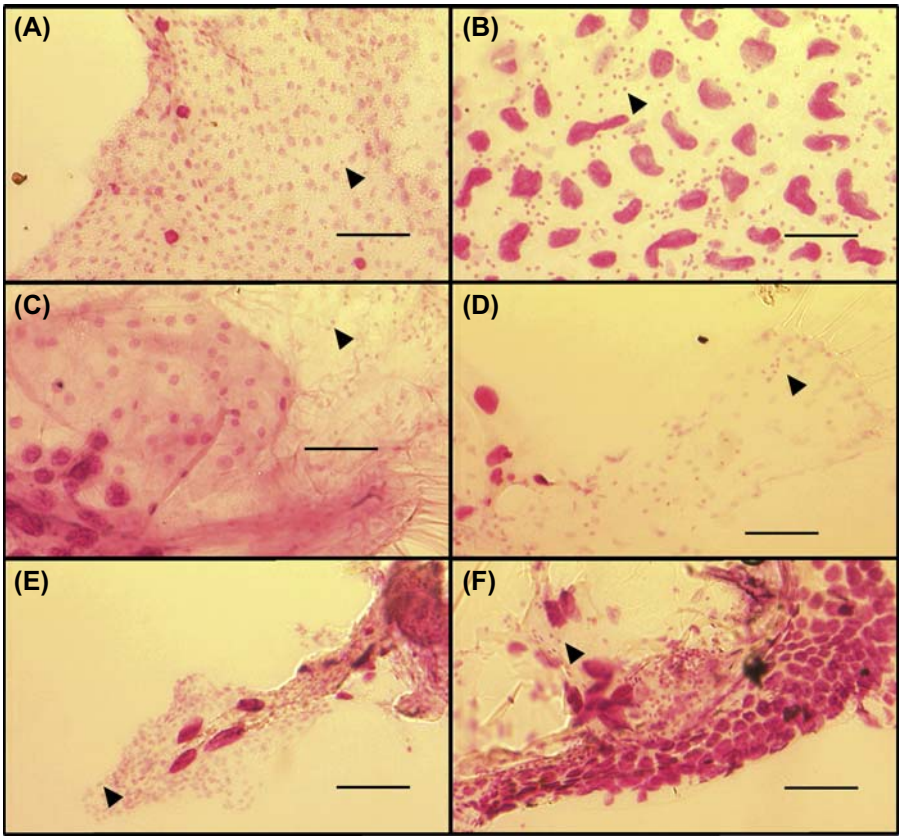

FIGURE 16.1 Feulgen-stained cladoceran tissues. For all images, scale bars represent 50 μm and arrowheads point out diploid nuclei. (A) *Simocephalus* sp. epidermis, (B) *Sida crystallina* shell gland, (C) *Daphnia longicephala* limb (distal tip to the right), (D) *S. crystallina* limb (distal tip to the right), (E) *Simocephalus* sp. labrum (distal tip to the left), (F) *S. crystallina* one-half of adhesive gland (anterior tip to the right).

breakage and loss of sections of the DNA strands, and thus decreasing the available staining sites and likewise the intensity of the stain.

Developments in densitometry techniques to measure staining intensity of Feulgen-treated nuclei have also progressed from flying spot to scanning stage, and most recently to image densitometry. The difference between the level of light able to pass through a nucleus compared to the background is a measure of its transmittance (T), which can be converted into optical density (OD) by the simple transformation, $OD = \log_{10}(1/T)$ (Hardie et al., 2002). Arbitrary OD levels can then be converted into to a DNA amount (typically in picograms, pg) with the inclusion of "standards" (cells for which the DNA content is known) in each staining procedure.

Flying-spot and scanning-stage densitometry both systematically measure OD across a nucleus, with the amount of light passing through a small aperture recorded at each location. Multiple measurements collected across a single nucleus can be integrated to produce an OD measure for that nucleus. The flying-spot and scanning-stage methods differ in their mechanics. The former method uses a beam of light that moves across the nucleus, whereas a stationary beam and a motorized stage gliding the slide (and thus the nucleus) across the beam's path is employed in the latter (Hardie et al., 2002). Image densitometry calculates the OD in the same manner but employs captured images. The benefit of this method is that each image may include dozens of nuclei, all of which can be processed simultaneously, thus tremendously accelerating the rate of acquisition of nuclear measurements.

Finally, flow cytometry has occasionally been employed to estimate the DNA content of cells. This technique involves creating a suspension of cells that have their nuclei marked with one of a number of DNA-specific fluorescent dyes [e.g., 4′,6-diamidino-2-phenylindole (DAPI) and propidium iodide]. The fluorescently tagged cells are passed through a laser beam of the appropriate wavelength for the chosen dye, and a detector records the brightness as the cells pass through the beam (Hardie et al., 2002). As with Feulgen densitometry, the inclusion of a suspension of cells of known DNA content allows the intensity of fluorescence to be converted to a measure of the DNA amount. Flow cytometry can process thousands of cells in a few moments, but a drawback of the method is that cells from a tissue must be isolated from each other.

16.1.3 Evaluation of Techniques

A close inspection of all cladoceran genome estimates reveals both perplexing and intriguing results. In total, 62 C-value estimates have been published, representing 49 species and including multiple estimates for five species of *Daphnia* (*D. arenata*, *D. magna*, *D. pulex*, *Daphnia pulicaria*, and *Daphnia tenebrosa*). First, substantial intraspecific variation is evident, which is probably due to the method of estimating genome size. Second, cladoceran genomes rank among the smallest among crustaceans, and indeed invertebrates. Of the 330 crustacean estimates listed in Gregory's (2016) database, 101 are less than 1.00 pg and include all of the cladocerans as well as a number of amphipod, barnacle, copepod, and ostracod estimates.

Although interspecific differences are routine, the genome size is generally predicted to be constant and stable for members of a species. Some differences reported within a species are probably due to the acquisition methods and experimental error. We can consider the magnitude of this error in an examination of cladoceran estimates. From 1985 to 2009, many species of *Daphnia*, and an array of other cladocerans, were the subject of DNA investigations (Rasch, 1985; Beaton, 1988; Beaton and Hebert, 1989; Beaton, 1995; Dufresne and Hebert, 1995; Korpelainen et al., 1997; Vergilino et al., 2009). Of the surveyed species, *D. pulex* was by far the most intensively studied, and determinations

based on multiple methods have been published (Table 16.1). Differences across estimates appear attributable to the method used for determining the C-value. Scanning Feulgen densitometry (by far the most commonly used method for this group) seems to have consistently resulted in an overestimation. Flying spot densitometry and flow cytometry both produced estimates slightly larger (0.23−0.33 pg) than predictions based on the genome sequencing initiative (0.204 pg). However, Colbourne et al. (2011) remarked that the 200-Mb draft genome assembly probably represented 80% of the entire genome, with the remainder comprising some duplicated genes and heterochromatic regions. This would shift the estimate originating from genomic sequencing from 0.204 to 0.256 pg. Furthermore, a clone of *D. arenata* (not *D. pulex*) was used. Based on this predicted genome size, measurements produced using flow cytometry and flying-spot densitometry are both slight underestimates.

Flow cytometry for *D. arenata*, *D. tenebrosa*, and *D. pulex* consistently produces values that are 54−62% of those obtained using scanning densitometry. Because the genome size predictions for other cladoceran members were determined by scanning microdensitometry, their DNA contents probably also represent overestimates and are even smaller than those reported by Beaton (1988, 1995). Consistent overestimation means that the reported C-values, whereas suspect for determining precise DNA contents, should still allow comparisons within a technique (but not between techniques). It is possible that the tissues and species used as the standards [often blood smears from a variety of fish species (Beaton, 1988)] were too dissimilar and biased the conversion from pixels to DNA content. Regardless, the general finding that cladoceran genomes are minute, based on the scanning densitometry estimates, is likely not affected by this bias. As such, current estimates for cladocerans, such as *Bosmina longirostris*, *Daphnia*

TABLE 16.1 Selected *Daphnia* Genome Estimates for Comparison of Methods

Species	Method of Estimation	C-Value (pg)	References
D. pulex	Flow cytometry	0.28−0.33	Korpelainen et al. (1997)
D. pulex	Flow cytometry	0.23	Vergilino et al. (2009)
D. pulex	Flying spot Feulgen densitometry	0.23	Rasch (1985)
D. pulex	Scanning Feulgen densitometry	0.37−0.38	Beaton and Hebert (1989) and Beaton (1995)
D. arenata	Flow cytometry	0.24	Vergilino et al. (2009)
D. arenata	Scanning Feulgen densitometry	0.42	Beaton (1995)
D. pulex/*D. arenata*	Genome sequencing	0.204	Colbourne et al. (2011)
D. tenebrosa	Flow cytometry	0.29	Vergilino et al. (2009)
D. tenebrosa	Scanning Feulgen densitometry	0.53	Dufresne and Hebert (1995)
D. tenebrosa	Scanning Feulgen densitometry	0.58	Beaton (1995)

thomsoni, and *Scapholebris kingii* (0.19, 0.21, and 0.16 pg, respectively), are among the smallest for a crustacean, with only two cyclopoid copepods possessing C-values less than 0.2 pg (Gregory, 2016).

16.1.4 Patterns in Interspecific Variation and Ecological Correlates With Genome Size

The 62 cladoceran C-value estimates form a bimodal frequency distribution (Fig. 16.2), with most C-values being about 0.25 or 0.4–0.45 pg. More than 90% of the available estimates are from representatives of the order Anomopoda (most of which are for the two *Daphnia* subgenera) and only one or two estimates are drawn from each of the other three orders. With such poor representation across the group, few conclusions can be drawn and there are no obvious patterns that might indicate a genome size increase or decrease from basal to derived members (Table 16.2). In addition, C-values show similar ranges across the two *Daphnia* subgenera.

In an examination of DNA content variation among species of *Daphnia*, Beaton (1995) could find no association between genome size and habitat preference; the ranges for species preferring pond environments were approximately the same as those that prefer lake habitats. Because pond-dwelling cladocerans tend to be larger than those found in lakes, an association between body size and DNA content might not be expected. Latitudinal association with polyploid clones that have been found for some members of *Daphnia* has been suggested. Ploidy assignments have been made using a combination of isozyme patterns and DNA content measurements. The use of in situ hybridization, as performed by Colbourne et al. (2011), may offer an alternative means of confirming ploidy assignments when inferences made by allozyme

TABLE 16.2 Ranges of C-Value Estimates

Order	Genus/ Subgenus	No. Species	C-Value Range (pg)
Anomopoda		45	0.16–0.53
	Ctenodaphnia	13	0.21–0.53
	Daphnia	23	0.24–0.58
Ctenopoda		2	0.22–0.47
Haplopoda		1	0.28
Onychopoda		1	0.22

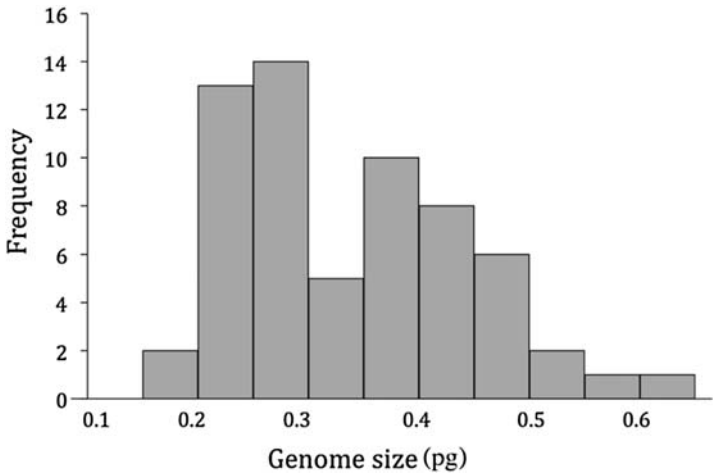

FIGURE 16.2 Distribution of genome size estimates of cladoceran members.

and DNA content analysis are inconclusive. Adamowicz et al. (2002) summarized the geographical range of polyploid representatives of the *D. pulex* complex as being the dominant form at latitudes above 70°N and 46−54°S (in Argentina). Triploids are believed the dominant polyploid level in the Canadian arctic (with tetraploids observed less frequently), whereas only tetraploids were recovered in Argentina (Adamowicz et al., 2002; Vergilino et al., 2009).

Generally, eukaryotic genome size is positively correlated with cell size, nuclear volume, cell-doubling rate, and so on (Cavalier-Smith, 1985), so similar relationships might be expected among cladocerans. When a limited number of representatives are chosen, a relationship between genome size and body size appears strong in this group; the large-bodied *Eurycercus glacialis* (0.63 pg) and *D. magna* (0.4−0.53 pg) have among the highest C-values for the group, whereas the tiny *B. longirostris*, *S. kingii*, and *Ceriodaphnia reticulata* (0.19, 0.16, and 0.23 pg, respectively) possess the group's smallest estimates (Beaton, 1988). However, using the larger *Daphnia* data set, a much weaker relationship was found. Some of the largest members of the genus (e.g., *Daphnia cephalata*, *Daphnia longicephala*, and *Daphnia magniceps*, with respective estimates of 0.27, 0.27, and 0.30 pg) each possess a genome size comparable to the small-bodied members (e.g., *Daphnia ambigua* and *Daphnia parvula*, with estimates of 0.24 and 0.28 pg, respectively) (Beaton, 1995). There can be two ways to achieve a large body: increase cell size (and by extension genome size) or increase cell number. The weak relationship observed points to the employment of the latter option by at least some species.

Unlike body size, the ability to alter head shape as defense and egg volume both correlate with genome size (Beaton, 1995). Members of *Daphnia* that are capable of forming an inducible head defense possess small genomes, whereas those the head shape for which is canalized tend to have larger genomes. This correlation is not perfect, but Beaton (1995) found it statistically significant. Head defenses (neck teeth, spines, helmets, and crests) are all formed from the epidermal sheet situated just under the carapace via an increase in cell numbers (Beaton and Hebert, 1997). In *D. pulex*, for example, neck teeth are present only during an animal's first two or three instars, but exposure to the pertinent predator kairomone is required from early embryogenesis onward to attain the most pronounced structure (Miyakawa et al., 2010). Therefore, there must be a critical period in development during which induction is initiated, but because the embryonic period is quite brief, neck-teeth formation must be rapid. The other induced head shapes are similarly created in just a few instars, and the fast response time is probably necessary to minimize the time that animals are vulnerable to predation. Because cell-cycle length is positively correlated to DNA content across many taxa (e.g., Bennett, 1972; Shuter et al., 1983; Cavalier-Smith, 1985), the logical extension seems to be that, for species under variable levels of predation pressure, a smaller genome would be advantageous. Higher cell-proliferation rates during key developmental periods would allow for the prompt formation of defensive structures.

The second strong ecological correlate observed with genome size was a positive one with egg size (Beaton, 1995). This relationship has also been observed for selected taxa, for example, among actinopterygian fish (Hardie and Hebert, 2004), as well as members of the rotifer, *Brachionus* (Stelzer et al., 2011). This may be a surprising result, as egg size will be affected by the amount of yolk laid down, as a reflection of environmental conditions (see Section 11.3.3). However, Hardie and Hebert (2004) noted that in fish, egg size limits fecundity, which is also likely to occur in cladocerans. A trade-off between brood size and neonate size may occur. Among small-bodied organisms, the size of the brood pouch may severely limit the number of eggs, but in larger species, many

small eggs or fewer larger ones are both viable options. Some of the largest species of *Daphnia* (such as *D. cephalata* and *D. longicephala*) have opted for large clutches of small eggs, leading to small neonates.

16.2 CYTOGENETICS

16.2.1 Background

Chromosome counts exist for only 5–6% of the cladoceran species (and most belong to a single family). There are at least two reasons why cytogenetic work on this group has been hampered. First, as a whole, this group possesses miniscule chromosomes owing to their condensed genomes packaged into reasonable chromosome numbers. Second, the choice of tissue and methods employed to obtain karyotypes have been linked to variable counts (Zaffagnini and Trentini, 1975). The production of a suspension of cells for examination is not an easy proposition with cladocerans. Employing adults for this endeavor is fraught with difficulties, including the separation of cells, as well as the confounding issue of rampant somatic polyploidy in members of this group (Beaton and Hebert, 1989). Using newly deposited subitaneous eggs (sometimes referred to as *summer eggs*), a technique that has been frequently employed (Zaffagnini and Sabelli, 1972; Zaffagnini and Trentini, 1975; Trentini, 1980), will generate cells that can be squashed, but pinpointing the ideal interval for egg collection is critical and the number of viable smears will be limited. Alternatively, whole animals can be fixed, blocked, and sectioned with a microtome, but the resulting counts can be variable when chromosomes in a cell do not lie within the same plane (Zaffagnini and Trentini, 1975). As a third viable method, preparations of chromosome spreads can be obtained from early embryos or from the epidermis underlying the carapace of juveniles or adults. In *Daphnia* (and presumably in other cladocerans), the epidermis is composed of a single sheet of diploid cells, with polyploid cells restricted to the tissue margins (Beaton and Hebert, 1989, 1994a). A synchronized cycle is often observed in the epidermis, with a brief window of opportunity to examine metaphase chromosomes occurring a few hours postecdysis (the first 12 h typically). Although this method allows examination of large numbers of mitotic figures, tissue collection is time sensitive. Regardless of the tissue or stage employed, to ensure that the brief metaphase period in the cell cycle is observed, the population of cells may be treated with a compound (such as colchicine) to inhibit spindle formation. Once the material is fixed onto a slide, it can be treated with one of several DNA stains (acetoorcein is a simple and commonly used dye) prior to visualization.

16.2.2 Karyotypes

A minute genome, combined with diminutive body size and a constrained growth pattern, has probably contributed to the paucity of published cytogenetic work for this group. In addition, information regarding centromere location and chromosome arm length has been difficult to obtain (because the chromosomes are so small!). With the exception of a handful of studies published since 1995, the bulk of information has been limited to chromosome numbers. Overall, diploid counts in the group have been obtained for members of only two of the orders: Anomopoda and Onychopoda (Table 16.3). Chromosome complements of many other members of the group are certainly required.

At least some of the published images have been obtained from colchicine-treated tissues. Trentini (1980) and Zaffagnini and Trentini (1975) obtained chromosome spreads of parthenogenetic eggs using a squash method. A similar technique, but employing colchicine-treated embryos to create a suspension of metaphase-

TABLE 16.3 Diploid Chromosome Counts for Cladocerans

Order/Family	Genus/Species[a]	2*n*
ANOMOPODA		
Daphniidae	*Ceriodaphnia*	
	Ceriodaphnia laticaudata[s,t]	14
	C. reticulata[t]	14
	C. pulchella[t]	20
	Ctenodaphnia	
	Daphnia angulata[u]	20
	D. cephalata[u]	20
	Daphnia exilis[u]	20
	D. longicephala[u]	20
	D. lumholtzi[u]	20
	D. magna[l,s,t,u]	20
	Daphnia salina[u]	20
	Daphnia similis[u]	20
	Daphnia tibetana[x]	24
	Daphnia	
	Daphnia catawba[u]	24
	Melanized sp. nov.[u]	24
	Daphnia middendorffiana[r,u]	24
	Daphnia minnehaha[u]	24
	D. obtusa[s-u]	24
	D. pulex[h,i,l,o,r,u]	24
	D. pulicaria[u]	24
	Daphnia schodleri[u]	24
	D. curvirostris[t,u]	20
	Daphnia galeata mendotae[u]	20
	Daphnia hyalina[s]	20
	D. longispina[t]	20
	Daphnia rosea[u]	20
	Simocephalus	
	S. vetulus[s,t]	20
	Simocephalus exspinosus[t]	20
Moinidae	*Moina*	
	M. affinis[s]	20
	M. affinis[y]	26
	M. macrocopa[j,v,y]	22
	M. micrura[y]	24
	M. mongolica[w,y]	24
	M. rectirostris[y]	24
	M. rectirostris[n]	30
Onychopoda	*B. longimanus*[c]	4?
Polyphemidae	*P. pediculus*[d]	8
ABERRANT COUNTS		
Anomopoda	*Ctenodaphnia magna*[o]	28–32
	D. magna[m]	8 F
	D. pulex[e]	8 (7–10) F
	D. pulex[k]	8 F, M; 9 S
	D. pulex[p]	20
	D. pulex[q]	16
	D. pulex[g]	8–10 M, S
	S. vetulus[f]	8–10 M
	M. paradoxa[b]	8
	M. rectirostris[b]	8

?, questionable count; *F*, during oogenesis; *M*, during spermatogenesis; *S*, during mitosis of somatic cells.

[a] Numbers represent count source.
[b] *Weismann and Ishikawa (1889) in Makino (1951).*
[c] *Weismann and Ishikawa (1891) in Makino (1951).*
[d] *Kuhn (1908) in Makino (1951).*
[e] *Kuhn (1908) in Trentini (1980).*
[f] *Chambers (1913).*
[g] *Taylor (1914) in Trentini (1980).*
[h] *Fanghaut (1921) in Trentini (1980).*
[i] *Schrader (1925) in Trentini (1980).*
[j] *Allen and Banta (1928) and (1929) in Makino (1951).*
[k] *Rey (1934) in Trentini (1980).*
[l] *Mortimer (1936) in Trentini (1980).*
[m] *Lumer (1937) in Trentini (1980).*
[n] *von Dehn (1948).*
[o] *Rossetti (1952) in Trentini (1980).*
[p] *Ojima (1954).*
[q] *Bacci et al. (1961).*
[r] *Zaffagnini and Sabelli (1972).*
[s] *Zaffagnini and Trentini (1975).*
[t] *Trentini (1980).*
[u] *Beaton and Hebert (1994b).*
[v] *Wenqing et al. (1995).*
[w] *Wenqing et al. (1999).*
[x] *Zhao et al. (2004).*
[y] *Zhang et al. (2009).*

arrested cells, was used for several *Moina* spreads (Wenqing et al., 1999; Zhang et al., 2009). Beaton and Hebert (1994b) obtained spreads using thoracic epithelium of mature females treated with a weak colchicine solution.

16.2.3 Order Anomopoda

Daphnia has been far and away the most intensely surveyed genus of the group, with counts for 22 species. Within this genus, chromosome complements show conservation with $2n$ numbers of only 20 or 24. Within the subgenus *Daphnia*, species composing the *D. pulex* group, all share the same chromosome count ($2n = 24$), whereas those making up the *Daphnia longispina* complex all possess $2n = 20$ chromosomes (Table 16.3). It should be noted that *Daphnia curvirostris* was historically classified within the *D. pulex* complex based on morphological characters, so its diploid complement of 20 was considered a puzzle. This led Beaton and Hebert (1994b) to question its placement. Subsequently, molecular analyses have confirmed that this species is most closely aligned with members of the *D. longispina* complex, concordant with chromosome numbers (Lehman et al., 1995; Colbourne and Hebert, 1996; Kotov et al., 2006). All members of the subgenus *Ctenodaphnia*, possess 20 chromosomes, with the lone exception of *Daphnia* (previously *Daphniopsis*) *tibetana* ($2n = 24$). Zhao et al. (2004) established that the diploid karyotype for *D. tibetana* has three pairs of metacentric (M) and nine pairs of telocentric (T) chromosomes ($2n = 24 = 6\text{M} + 18\text{T}$), an arrangement is reminiscent of the findings of Colbourne et al. (2011) for *D. pulex* (*arenata*). It has yet to be explored whether any other species of *Ctenodaphnia* shares the $2n = 24$ count with *D. tibetana* or confirms its eccentricity in the subgenus.

There have been both lower and higher reported chromosome counts for *D. magna* and *D. pulex*, but these are generally considered unreliable. For example, those obtained from tissue sections have generally been refuted and dismissed, sometimes by the authors themselves (Chambers, 1913 and Kühn, 1908, based on my translation of Zaffagnini and Trentini, 1975). A diploid count for *D. magna* by Rossetti (1952, in Trentini, 1980) represents the only aberrant set of counts for which we have no evidence favoring their rejection, aside from the overwhelming number of studies contradicting his counts (Mortimer 1936 in Trentini, 1980; Zaffagnini and Trentini, 1975; Trentini, 1980; Beaton and Hebert, 1994b). All aberrant records have been placed at the end of Table 16.3 simply for reference.

The sequencing initiative by the *Daphnia* Genomics Consortium succeeded in characterizing the nuclear genome of the water flea, *D. pulex* (*arenata*), including a detailed description of chromosome structure in this species. Colbourne et al. (2011) categorized the chromosomes (ranging in length from about 1 to 6 μm) into three broad classes based on size: the first group included only one chromosome pair, the second group comprising the next three largest pairs, and the third included all remaining chromosomes. Only about 25% of the total chromosome area was found to be heterochromatic and G-bands were only found on the largest four chromosome pairs. Although no attempt was made to identify the position of the centromere, the three largest pairs appear meta- or subtelocentric based on their image (Colbourne et al., 2011). The chromosome ends were characterized as telomeres made of long stretches of repeated TTAGG, the same sequence used by *Bombyx mori* and similar to that found at the ends of vertebrate chromosomes. Finally, fluorescence in situ hybridization (FISH) was used to locate the ribosomal RNA (rRNA) arrays (Colbourne et al., 2011). Using two probes, Pokey, a transposon that inserts into a specific site of the large subunit rDNA (Eagle and Crease, 2012) and a region of the intergenic spacer (IGS), Colbourne et al. (2011) confirmed that the rRNA genes were present as a single tandem array on one chromosome pair, with the transposon inserted along the entire length of the array.

Chromosome numbers for *Ceriodaphnia* have been published for only three members, with two diploid complements observed: 14 and 20 (Table 16.3). It is interesting to note that *C. reticulata* ($2n = 14$) and *Ceriodaphnia pulchella* ($2n = 20$), which can co-occur, exhibit differences in both body and egg size (Burgis, 1967). What might not be expected is that *C. pulchella* has the larger chromosome number, but a smaller egg and body size. With no phylogenetic assessment of the genus found in the literature, we cannot speculate on the basis for the chromosome variation.

With only two species of *Simocephalus* studied, diploid chromosome numbers appear to be conserved at 20 (Table 16.3). Chambers (1913) reported an aberrant diploid count of 8−10, but as it was based on tissue slices, the count is considered unreliable. *Simocephalus* and *Ceriodaphnia* have been linked as sister taxa based on a phylogenetic reconstruction using three genes (deWaard et al., 2006a,b). Chromosome counts for members of these two genera ($2n = 20$ and 14, respectively) show the largest separation observed across the Anomopoda to date (Trentini, 1980). Overall, among the members of the family Daphniidae, only three reliable counts have been reported, $2n = 14$, 20, and 24.

With just six species of *Moina* surveyed, diploid chromosome numbers in the genus appear restricted to a range between 22 and 26, although counts for *Moina rectirostris* (syn. *Moina brachiata*) have been reported as 8 and 30, in addition to 24, and a count of 8 was reported for *Moina paradoxa* (Table 16.3). The highest and lowest counts must be regarded as suspect; von Dehn's (1948) estimate ($2n = 30$) was based on tissue sections, and a description of how Weismann and Ishikawa (1889, in Makino, 1951) collected their data ($2n = 8$) could not be obtained. Similarly, the diploid count of $2n = 8$ for *M. paradoxa* should be considered suspect unless confirmation for the species is possible. Zhang et al. (2009) obtained a diploid count of 24 for *Moina micrura*, *Moina mongolica*, and *M. rectirostris* with karyotypes of 10M + 14T, 6M + 18T, and 10M + 14T, respectively. Their spreads of *Moina affinis* revealed $2n = 26$ chromosomes (in contrast to Zaffagnini and Trentini, 1975, who obtained a count of 20), with a karyotype of 12M + 14T. Finally, for *Moina macrocopa*, a diploid karyotype of $2n = 22 = 10M + 12T$ has been described (Zhang et al., 2009).

The basis of the discrepancies observed among species of *Moina* (especially for *M. affinis*) must be investigated further. Errors in the counts, misidentification of individuals, or the presence of cryptic species complexes are possible explanations. Obtaining karyotypes from additional individuals, populations, and species should be undertaken to definitively establish the correct counts.

16.2.4 Order Onychopoda

Only two early counts have been reported for this order and these should be viewed with some skepticism. The diploid counts of 4 and 8 need to be confirmed and several more representatives of this order surveyed before any generalizations are possible.

16.3 ENDOPOLYPLOIDY

Endoreplication, or somatic polyploidy, is observed when DNA replication occurs in a cell without cytokinesis. Separating the process of DNA replication from one or more steps of cell division can lead to a single reconstituted cell (rather than two daughter cells) that possesses twice as much chromatin compared to its original condition. Repetition of this abbreviated cycle produces an enlarged cell and nucleus containing $2n$ copies of the nuclear genome (in which n is the number of completed endocycles or endomitotic cycles). Endocycles bypass mitosis entirely, so chromosomes do not condense, creating multistranded chromatin as seen in larval *Drosophila* salivary gland cells.

Endomitotic cycles, on the other hand, typically include prophase, metaphase, and anaphase, but during telophase a single nuclear membrane is formed around all of the chromatin (see Lee et al., 2009 for a brief comparison). As a consequence, the latter process will result in multiple sets of the haploid chromosome complement (endopolyploidy).

The sweeping description of endoreplication hints at the occurrence of some accident or abnormality, as is the case with some cancerous tumors. However, endopolyploidy is a ubiquitous phenomenon, and routinely is a necessary and normal act in the developmental process of most multicellular organisms. Trophoblast cells of rodents, many plant embryo suspensor cells, and the giant neurons of the sea hare, *Aplysia californica*, are but a few examples of this widespread phenomenon (Nagl, 1978). In general, polyploid cells range from 4 to 10^6 C (in which C represents the DNA content of a single chromosome set) and those cells with the highest levels are often associated with secretory roles, although the neurons of *Aplysia* (approximately 10^6 C) may contradict this pattern. The widespread nature of somatic polyploidy could be an evolutionary mechanism for allowing abrupt shifts in traits or physiological responses (Beaton and Hebert, 1997).

Some of the first reports of endopolyploidy in cladocerans came from examinations of labrum and fat cells (Cannon, 1922; Jaeger, 1935; Sterba, 1956a, 1957a). More recently, Beaton and Hebert (1989, 1997, 1999) established the extent of polyploidy for multiple *D. magna* and *D. pulex* tissues, with several substantive patterns revealed. First, most examined tissues in mature females were found to contain multiple polyploid cells, each with a DNA content ranging from 4 to 2048 C (Tables 16.4 and 16.5). The Feulgen staining pattern of many of the moderately polyploid cells (32–64 C) appears to be far more heterogeneous than those at 2–16 C (Beaton, unpublished; Sterba, 1956a, 1957a). Two tissues, the gut and the epipodites (the leaf-like lobes located at the base of each of the five pairs of thoracic appendages), contain only polyploid cells and the labrum contains cells reaching the highest levels (1024–2048 C). Overall, Beaton and Hebert (1997) estimated that nearly half of each individual's DNA was packaged in a polyploid state; with no mitotic figures recorded from polyploid cells other than those in the gut, it is assumed that polyteny does not occur in *Daphnia*.

TABLE 16.4 Typical Number of Fat and Epipodite Cells per Abdominal Appendage Pair of *Daphnia pulex* and *Daphnia magna*

Appendage Number	Fat Cells		Epipodites	
	D. magna	*D. pulex*	*D. magna*	*D. pulex*
1	41–42	16–17	107–108	45–46
2	46–47	26–27	122–123	58–59
3	125–126	49–50	123–124	82–83
4	109–110	40–41	137–138	85
5	45–46	26–27	130–131	86

Summarized from Beaton and Hebert, 1989.

Second, both tissue and species specificity was found with regard to levels and number of cells involved (Tables 16.4 and 16.5). For example, in newly mature females of both *D. magna* and *D. pulex*, the labrum possessed eight polyploid cells, but levels differed between species (Beaton and Hebert, 1997). In addition, for a given species, epipodites of mature females contained cells that were between 8 and 32 C, but the number of cells in each epipodite differed both among thoracic limbs and between species (Table 16.4).

Third, the number of polyploid nuclei in a tissue, once established (typically before instar three or four), remained constant with age (Beaton and Hebert, 1999). When individual polyploid cells in the epidermis are destroyed via laser ablation they are not replaced (Beaton, personal communication), suggesting that a genetic control to establish these cells is in place. The

TABLE 16.5 Summary of Occurrence of Endopolyploidy in Adult Female *Daphnia*

Tissue	Number of Polyploid Cells		Levels Observed (C)	Specific Comments
	Daphnia magna	*Daphnia pulex*		
Epidermis	ND	35	8–64	16–32 C is most common
Epipodites	621	357	8–32	16 C is most common
Fat cells, limbs	371	161	64–256	128–256 C are more common
Gut	All	All	4–16	4 C for almost all nuclei
Labrum	8	8	64–2048	Distinct patterns for species
Rostrum	156	83	8–128	32–64 C is most common
Shell gland	300	102	8–256	16 C is most common

Number of polyploid cells represents the counts for the entire tissue (e.g., for all five pairs of limbs). *ND*, cell counts (and corresponding ploidy levels) were not determined.
Summarized from Beaton and Hebert, 1989.

inference can also be made that polyploid cells in most tissues cannot exit the endomitotic cycle and return to the typical cell cycle to restore cell numbers. The exception to this rule, however, was observed in gut cells, which appear to reach the tetraploid level by instar 2, and then return to a normal cell cycle, which allows tissue growth without further ploidy increase (Beaton and Hebert, 1999). In general, polyploid cells do not return to the cell cycle once they have opted for the endomitotic cycle, so this finding is particularly intriguing. The significance of digestive cells maintaining a low-ploidy level has not been investigated.

Finally, Beaton and Hebert (1999) found tissue specificity in *D. pulex* with regard to increasing ploidy levels (Table 16.5). During the first instar, 4 C was the common level found in most tissues, but specific labral cells had already reached 64 C. Over subsequent instars, the levels in labral cells continued to increase (although a doubling of the DNA content with each instar was not observed), and two of the eight polyploid nuclei reached 2048 C by instar 14 when the study ended. The tissues most involved in feeding and growth, that is, the epidermis (which lays down the new carapace each molt), the labrum, and the fat cells of the limb central core all showed substantial ploidy level increases across the instars. By comparison, the cells located in the epipodites typically remained at 16 or 32 C from the sixth to the thirteenth instar.

The functional significance of possessing polyploid cells in some tissues can be inferred. For example, a band of polyploid cells along the underside of the rostrum is likely important for chemoreception. Glandular polyploid cells in the labrum may aid in the formation of the food bolus and the start of digestion. If true, one wonders if polyploid patterns in this tissue

(and perhaps thoracic limb fat cells) are related to the diverse feeding strategies (detritivory, herbivory, carnivory, etc.) exhibited among the group.

The presence of 20–25 polyploid cells distributed in *Daphnia* along the ventral margins and dorsal fold in the epidermal sheet that lies adjacent to the carapace may act as the primary developmental centers to control the growth rate of the animal. Similar types of cells in the cephalic epidermis have also been hypothesized to act in this manner in controlling formation of inducible defenses, based on multiple lines of supporting evidence. First, mitotic rates in diploid cells near polyploid cells were higher than in cells in more distant positions (Beaton and Hebert, 1997). Second, pilot loss of function studies indicate that ablation of selected polyploid epidermal cells in the head of helmeted *Daphnia lumholtzi* lead to a 10–40% decrease in the size of the helmet in the next instar (Beaton, personal communication), suggesting that the polyploid cells are necessary for helmet formation. Finally, these inducible structures have been linked to cholinergic and γ-aminobutyric acid (GABA)-ergic signaling (Barry, 2002; Weiss et al., 2012) and Weiss et al. (2015), employed whole-mount in situ staining to confirm the presence of the neurotransmitter, dopamine, in cephalic polyploid cells of two species of *Daphnia*. Further investigations will be required to pinpoint the entire pathway creating and maintaining defensive structures.

It would be interesting to know how ploidy levels (and polyploid cell number) compare for members of the other three orders, as well as for those with distinct physical characteristics, such as *Sida*, *Holopedium*, and *Leptodora*. Each of these species presumably uses the epidermal tissue for different functions. In *Holopedium*, it likely lays down its distinctive jelly coat, whereas a portion of the epidermis, at least, forms the "sucker" found on the dorsal surface of *Sida*; in addition, the carapace seems to have "fused" with the thorax in *Leptodora* (Olesen et al., 2003), but ploidy levels have yet to be established. The function of polyploidy in some tissues will be considered further in the next section.

16.4 CYTOLOGICAL OBSERVATIONS FOR SPECIFIC TISSUES

16.4.1 Brain and Central Nervous System

Chapter 13 provides an excellent overview of the Cladoceran nervous system and sense organs, including a functional description of neurosecretion. As a complement to this chapter, we provide here a more detailed description of the structural makeup of the brain and the neurosecretory cells. Branchipod brains are commonly divided into three major masses: the "protocerebrum," "deutocerebrum," and "tritocerebrum" (Bullock and Horridge, 1965; Martin, 1992). These three masses were recently characterized in a comparative study on *D. pulex*, *D. lumholtzi*, and *D. longicephala* (Weiss et al., 2012). Situated inferior to the compound eye, in the medial and anterior region, the brain of all three species innervates the organs and appendages through the ventral nerve cord. The protocerebrum is the largest part of the *Daphnia* brain, with a dorsoventral diameter of 100 μm in adult *D. lumholtzi* and *D. longicephala*, and 50 μm in the second juvenile instar of *D. pulex* (Weiss et al., 2012). After Sterba (1957b) identified several specialized cell groups in the cephalic region of *Daphnia* that released secretions, a detailed description of sites with presumed neurosecretory function in *D. magna* was undertaken by Halcrow (1969). Several frontal sections were stained with paraldehyde-fuchsin (PAF) and the presence and location of fuchsinophilic granules were noted in each section. Three to four cells on the anterior and several cells on the ventral surface of the brain

stained densely with PAF. On the ventral surface, the secretory cells are 10 μm long with nuclei 4 μm diameter, roughly cuboidal, and lie near the junction of the naupliar eye. Circumenteric connectives found posterior to the brain have one cell of neurosecretory function. These cells are 10 μm × 14 μm with nuclei 5.5 μm diameter. Each of these connectives also has a bulge, approximately 4 μm × 12 μm, on the inner surface that stains a deep violet color with PAF. Moving posteriorly, the ventral nerve cords form junctions with the second ventral commissure. One cell, sized 9 μm × 30 μm with distinct nuclei 6-μm diameter, at each of these junctions contains distinct granules. Halcrow (1969) suggested that there was considerable variation when all of the previously mentioned regions were stained in different stages of the molt cycle.

16.4.2 Nuchal Organ and Epipodites

As a rule, cladocerans can actively regulate the osmotic pressure of their fluids to maintain homeostasis of their water content (Aladin and Potts, 1995; and see Chapter 8). Within the order, 95% of the species are confined to freshwater, 3% are found in brackish water, and only 2% are restricted to marine habitats (Bowman and Abele, 1982, in Weider and Hebert, 1987). Osmoregulatory strategies of the group are as diverse as the environments that they inhabit. Hyperosmotic regulation of hemolymph is found in freshwater animals and those living in slightly brackish, whereas hypoosmotic regulation occurs in marine individuals (Aladin and Potts, 1995). A combination of hyper- and hypoosmotic regulation is found in cladocerans from both brackish and highly mineralized waters (Aladin and Potts, 1995).

The two primary structures utilized by the group for osmoregulation are the epipodites and the nuchal, or neck organ (Aladin and Potts, 1995). Although commonly thought of as a gill, Koch (1934) suggested that the selective permeability of the epipodites to silver stain suggested that they have an ion-regulatory role. The nuchal or neck organ has been described in a variety of larval and adult crustaceans, including branchiopods, copepods, and malacostracans (Aladin and Potts, 1995). Among malacostracans, the nuchal or neck organ has been suggested to serve in chemoreception, mechanoreception, and baroreception. Branchiopod neck organs may have evolved from these sensory functions; as with these other functions, salt uptake is also only effective where a thin cuticle is present (Aladin and Potts, 1995). It seems probable that the neck organ first evolved in a branchiopod nauplius to allow salt uptake in freshwater environments. This organ is now retained in adult cladocerans that have raptorial limbs and no longer possess epipodites (Aladin and Potts, 1995). Members of the four orders of Cladocera (Ctenopoda, Anomopoda, Haplopoda, and Onychopoda) may use one or both of these ion-regulating systems (Table 16.6). Among the Ctenopoda, because epipodites are well developed in some members (*Sida*), but are small for others (*Penila*), reliance on the nuchal organ for ion regulation will be varied. In *Sida*, for example, the nuchal organ has been modified, hijacking the ion regulatory function in favor of an attachment device (Olesen, 1996; Peñalva-Arana and Manca, 2007). In Anomopoda, such as *Daphnia*,

TABLE 16.6 Variation in Osmorgulatory Structures Present in Orders

Order	Epipodites	Nuchal Organ
Anomopoda	Present	Limited to embryo and first instar
Ctenopoda	Present in varying sizes across species	Modified to function for attachment
Haplopoda	Absent	Present in all stages, surrounding the head within the shield
Onychopoda	Absent	Well developed and incorporated in head shield

the nuchal gland persists from embryo to the first instar, although it may become inactive just a few hours after release from the brood pouch (Benzie, 2005). This group possesses epipodites upon release from the brood pouch. In contrast, haplopods possess only the nuchal gland, which develops into a broad shield containing numerous mitochondria-rich cells. Similarly, members of Onychopoda possess only a well-developed nuchal gland (Aladin and Potts, 1995).

Sandwiched between the outside medium and the hemolymph, both neck organ and epipodite are covered by a thin cuticle (Aladin and Potts, 1995). The ultrastructure of these structures is very similar: cells of both contain dense cytoplasm with numerous mitochondria (Aladin, 1991). Furthermore, their cell structure is unaffected by the type (hyper- or hypoosmotic) of ion regulation employed. Structurally, ion-transporting cells in these tissues share similarities with cells of similar function in the gills of other crustaceans, those in insects and fishes, and the salt-secreting glands of reptiles and birds (Aladin and Potts, 1995).

The structure of the nuchal organ in *Daphnia* has been well studied. First instar individuals have a nuchal organ that, upon external observation, appears as an expanded portion of a dorsal ridge of the head. In *Daphnia himalaya*, a groove appeared to mark the margin of the structure, which has a mediodorsal hole (Peñalva-Arana and Manca, 2007). Schwartz and Hebert (1984), using a simple silver-staining technique, reported that the position of the organ could be used as a diagnostic trait between the subgenera, *Ctenodaphnia* and *Daphnia*. Benzie (2005), on the other hand, asserted that its location is not specific to a subgenus, but it is situated at the border between cephalic and dorsal shields. Internally, lying just below the cuticle, the nuchal organ consists of two cell types (referred to as *dark* and *light*) that fill a large portion of the hemocoelic space (Halcrow, 1982). The higher proportion of microvilli on the apical surface of both cell types makes them noticeably different than the surrounding squamous cells. The darker of the two cell types has microvilli that sometimes branch and may be smaller in diameter compared to those of the light cells. Deep within both cells and at the bases of microvilli are numerous mitochondria with prominent cristae. It is possible to define the border of the nuchal organ with permanganate staining: the cuticle overlying it is densely stained, whereas the thicker surrounding cuticle is not (Halcrow, 1982). In *D. magna*, increased membrane surface area (microvilli) and numerous mitochondria are common features of nuchal organ cells that have osmoregulatory or ion-transporting roles in other crustaceans. This structure remains functional as an ion transporter for a brief period; only approximately 12 h in the free-swimming first instar animals (Halcrow, 1982). Due to its rapid loss of function, it has been suggested that the nuchal organ is most beneficial in these animals during their development in the brood pouch, when limb movement is severely limited or nonexistent. These cells remain large in older animals, but have reduced numbers of microvilli and mitochondria (Halcrow, 1982).

The nuchal organ of *Leptodora* is a saddle-shaped area of integument almost completely encircling the head (Halcrow, 1985). The structure is similar to that of other branchiopods in mitochondrial abundance, varied cell types, surface area, and distinct integumental boundary. However, the microvilli that cover almost the entire apical surface of the epidermal cells in the nuchal organs of *Artemia* and *Daphnia* are absent in *Leptodora*. This structural difference may be due to the organ's larger surface area in *Leptodora* or to a difference in metabolic activity, but is not believed to result from functional differences. Unlike the nuchal organ of *Artemia* and *Daphnia*, it persists in *Leptodora* adults, probably because epipodites are lacking on their stenopodial limbs (Halcrow, 1985).

Marine members of the Onchopoda retain an undiminished neck organ throughout their lives

(Meurice and Goffinet, 1983). Light, scanning, and electron microscopy of four mature and immature representatives (*Podon intermedius*, *Evadne nordmanni*, *Evadne spinifera*, and *Evadne tergestina*) revealed that the neck organ hangs as a mass from the dorsal carapace in the middle of the interantennary cephalic shield (Meurice and Goffinet, 1983). The cells composing the organ have an apical zone with a dense cytoplasm that surrounds the nucleus and a basal zone containing many mitochondria distributed throughout a lacunar system (Aladin, 1991). Dense bundles of microtubules are found in the apical cytoplasm and many free polysomes fill the cytoplasmic interstices. A small number of cisternae near the nucleus make up the rough endoplasmic reticulum (Aladin, 1991). Juxtaposed plasma membranes are held together by septate desmosomes, whereas a thin basement membrane defines the ventral cell mass of the organ (Aladin, 1991).

The most interesting features of the *Podonidae* neck organ are the small number and the structure of the cells that compose the tissue. The organ comprises at most 12 cells, compared to 50 to 60 cells in *Artemia*, and the ultrastructure of all cells are similar, in contrast to the two cell types found in *Daphnia* (Meurice and Goffinet, 1983). Comparisons between environmental salinities and hemolymph concentrations indicate that a hypoosmotic regulating mechanism is utilized by marine gymnomeran Cladocera.

Early research suggested a respiratory function for epithelial epipodite cells. Because this tissue's cuticle is approximately 4–5 times thicker compared to surrounding limb epithelia, epipodites are unlikely sites for gas exchange (Goldmann et al., 1999). But a role in oxygen transport has been linked to these tissues. In *D. magna*, for example, hemoglobin synthesis has been restricted to epipodite cells (as well as limb fat cells; see later section) (Goldmann et al., 1999). Furthermore, messenger RNA (mRNA) of an aryl hydrocarbon nuclear translocator homolog (ARNT, also called hypoxia-inducing factor 1β) is expressed in adult epipodites (Tokishita et al., 2006).

Beyond the first instar, epipodite epithelial cells presumably serve the primary ion-regulatory role in *D. magna* (Halcrow, 1982). Two cell types, dark and light, are found arranged in a jigsaw-type pattern in the epipodite epithelia (Kikuchi, 1983). Dark cells have many infoldings, large mitochondria, and a complex tubular system, and show an accumulation of chloride ions, suggesting an ion-transporting function. The light cells have strong infoldings of both basal and lateral cell membranes and tubule-like protrusions toward the blood space, but an osmoregulatory function has not been suggested (Kikuchi, 1983; Goldmann et al., 1999). It is possible that the two populations of epipodite cells have separate functions, with the light cells playing a role in oxygen transport, but this has yet to be considered. Certainly, further investigations into the full role of this tissue in oxygen transport is needed.

The presence of two cell types in the epipodites raises some interesting questions. Beaton and Hebert (1999) found that *D. pulex* epipodites routinely maintained nuclei with two ploidy levels, 16 and 32 C (with occasional 8 and 64 C cells) from the sixth through to the thirteenth instar. Could the cells with differing ploidy levels correspond to the light and dark cell types? In addition, how is the polyploid nature of these cells important for their function? Furthermore, we should ask if osmoregulatory capability is sustained beyond the sixth or seventh instar, because ploidy levels (and number of cells, for that matter) do not appear to increase, despite the continued increase in body size. It seems logical that, without ploidy increases, as the animal grows each epipodite cell must experience a greater osmoregulatory load, and at some point the control of ion concentration could be compromised.

A third osmoregulatory structure utilized by many cladocerans is the closed brood pouch. In marine members, when the brood pouch contains developing eggs and embryos, the osmolarity of

the marsupial fluid equals that of the hemolymph, but once the embryos develop a neck organ, the fluid ion concentration rises to that of the surrounding sea (Aladin, 1991). The ability to regulate the embryonic environment and protect developing embryos allows Cladocera to inhabit high-osmolarity water (Aladin, 1991).

16.4.3 Labrum and Fat Cells

Three structures that are essential in digestion and energy storage, the labrum, gut, and fat cells, have been the subject of some study. In *Daphnia*, the digestive tract is basically an extended tube, consisting of a foregut, midgut, hindgut, and a pair of intestinal ceca. The walls of both the foregut and hindgut are composed of cuboidal epithelial cells (about 5 μm tall) lined with chitinous intima (Schultz and Kennedy, 1976a). The midgut can be distinguished from foregut and hindgut based on cell structure: midgut cells are taller (about 30.5 μm), the nucleus is basally located, mitochondria are found anteriorly, and the apical membrane possesses long microvilli. Cell density along the gut remains relatively constant across age classes despite increases in the length of the tract as the animals grow. Because the ploidy level of all gut cells stabilizes at 4 C, it is likely that these cells exit their endomitotic cycle and return to a mitotic cell cycle (Beaton and Hebert, 1999). This is quite unusual, but has not been studied and further investigation is required.

Early descriptions of the labrum depicted the structure as glandular and organized into two groups of cells in *Simocephalus sima* and *Acanthocercus* (Cunnington, 1903 and Schödler, 1846; both in Cannon, 1922). In *S. sima*, the four large distal cells (the shape for which was likened to a hollow bowl) were thought to be replaced when they "lose their secretory power" by the proximal group of cells, which were smaller and thought to be epidermal in origin (Cunnington, 1903; in Cannon, 1922). The distal cells were linked to the outside by a duct leading to the inner surface of the labrum. Cannon (1922) similarly found two groups of cells in the labrum of *Simocephalus vetulus*: the proximal group was separated into two lateral parts of 20 epidermal cells each, both situated near the nerve of the first antenna. It is likely that these cells correspond to the polyploid cells of *Daphnia* that are located along the underside of the rostrum (Table 16.5). The distal group of cells consisted of eight large cells (four per side) arranged in pairs, with anterior cells connected to the posterior ones. The two posterior cells "embraced" two "duct cells" that were slightly larger than muscle cells (Cannon, 1922), so presumably were 2−4 C.

Sterba (1957a) recognized three gland cell types in an examination of *Daphnia* (presumably *D. magna*), which he referred to as head bottom, replacement, and main cells (the names of which are based on a partial translation of his work). He found that the multiple head bottom cells, which are proximally located, reached 128 C and the medial paired replacement and distal main cells reached 2048 C. Sterba (1957a) also noted that the cells within each cell type were linked via plasma bridges. Beaton and Hebert (1989, 1999) similarly observed polyploid nuclei in *D. magna* and *D. pulex* arranged in three groups (Table 16.5). From proximal to distal, the first group comprised four cells (maximum of 512 C), the second group had two extremely large cells (maximum of 2048 C), and the most distal group of two cells reached 256 C in *D. pulex* and 1024 C in *D. magna*. In *D. pulex*, the two largest cells had Y-shaped nuclei, suggesting a similar structure to that found in the labrum of the conchostracan, *Caenesteriella* (Larink, 1992). None of the four most distal cells in *D. magna* exhibited the Y-shaped or folded nuclear conformation found in *D. pulex*, despite possessing commensurate ploidy levels. Overall, there is congruence in the general pattern of polyploidy observed in the labrum (Sterba, 1957a; Beaton and Hebert, 1989, 1999), and this tissue appears to possess cells with the highest ploidy level in the animal.

Lipid and glycogen can constitute a massive portion of the volume of a daphnid's fat cells, which are situated in the body core along the length of the gut and in the endopodites of thoracic appendages near the base of the epipodites. The level of lipid reserves in these cells appears intimately related to an organism's reproductive status. Prior to peak ovary growth, the fat cells hold maximal lipid stores; as developing parthenogenetic oocytes fill with yolk, lipid and glycogen levels in fat cells diminish (Jaeger, 1935). These cells are the most likely sites of vitellogenin synthesis, a principal component of yolk (Zaffagnini and Zeni, 1986). In addition to lipid droplets, the cytoplasm of fat cells contain numerous free ribosomes, a well-developed rough endoplasmic reticulum (indicating active protein synthesis), mitochondria, and small Golgi complexes with enlarged cisternae (Zaffagnini and Zeni, 1986). Sterba (1956a) noted that the mitochondria are uniformly distributed throughout the cytoplasm until lipid stores fill the cells, at which point they arrange in chains (my translation) and are moved to the cell margins. The structural organization of fat cells in parthenogenetic *Daphnia obtusa* females appeared similar to those of adult female insects in the vitellogenic phase and to subepidermal fat cells of reproducing females of the amphipod, *Orchestia gammarellus*. These similarities support the hypothesis that the fat cells of *Daphnia* synthesize vitellogenin (Zaffagnini and Zeni, 1986).

The nucleus in each fat cell contains an irregularly shaped, large nucleolus, which occasionally appears as two or more pieces. In *Daphnia*, these cells are known to be highly polyploid (Jaeger, 1935; Sterba, 1956a). Beaton and Hebert (1989) found fat cells in the limb central core of *D. magna* and *D. pulex* females (typically corresponding to instar 4 or 5 for well-fed animals) that contained up to 256 times more DNA than is found in haploid cells. This level agreed with a study of fat cells completed by Sterba (1956a). An examination of ploidy shifts in *D. pulex* associated with growth and development revealed that the number of core limb fat cells was unchanged with instar, but that ploidy levels changed with food availability (Beaton and Hebert, 1999). Body size and nutritional status apparently influence the ploidy level present in this tissue; this is an expected finding, because fat cells store lipid reserves for potential oocytes. When food supplies were low, inadequate glycogen and lipid supplies were amassed, resulting in decreased egg production or the requirement of additional lipid synthesis by the ovaries during the period of sexual reproduction.

Fat cells have also been implicated with epipodite cells in the synthesis of hemoglobin (Hb), the primary oxygen-carrying molecule in cladocerans. In contrast to malacostracan crustaceans that use hemocyanin as their oxygen carrier, many branchiopods utilize Hb as their respiratory protein. The site of Hb synthesis had been pinpointed for members of several invertebrate phyla, such as Annelida and Nematoda and one insect (Bergtrom et al., 1976), but not for a crustacean until the fat cells of *D. magna* were identified as a primary manufacturing site. Goldmann et al. (1999) hybridized a probe based on a previously determined Hb cDNA sequence (Tokishita et al., 1997) to sections of adult specimens. Both fat and epipodite cells produced mRNA signals for Hb; stronger staining in epipodites compared to fat cells (Goldmann et al., 1999) may reflect relative levels of production.

16.5 REPRODUCTION

Cladocerans have evolved two forms of reproduction that may alternate depending on environmental conditions. For much of the growing season, a population will be composed of nearly all females that produce parthenogenetic eggs, which develop immediately. Early accounts asserted that eggs were created ameiotically, so simple mitotic divisions (apomixis) were invoked to retain their diploid condition. Under adverse conditions, a switch to the second

mode of reproduction occurs, and in most cladocerans, germ cells undergo meiosis to produce haploid gametes that must be fertilized to reconstitute the diploid state. The eggs resulting from this process do not develop immediately, but undergo a period of diapause prior to completing embryogenesis. We must remember that sex determination in this order is under environmental control. In cyclically sexual cladocerans, males are produced from clutches of parthenogenetic eggs before the sexual eggs can be formed.

Contrary to early research, a recent examination of the "parthenogenetic" process by Hiruta et al. (2010) revealed that, in *D. pulex*, these eggs are not produced mitotically but instead undergo an abortive meiosis. Using histological and immunochemical analyses, they observed two divisions, with paired homologous chromosomes aligning at the equatorial plate during the first division (as happens during meiosis). Bivalents were split to form two half bivalents that began separation, but reassembled as a diploid equatorial plate after anaphase I. During the second division, one of the separated sister chromatid sets migrated and was elevated above the egg surface to form a tiny polar body-like daughter cell, reminiscent of meiosis II (Hiruta et al., 2010). Hiruta and Tochinai (2012), on examining spindle assembly in *D. pulex*, revealed a distorted-spindle shape during abortive meiosis. Contrary to the normal mitotic silhouette, spindles appeared barrel-shaped and lacked centromeres. Furthermore, γ-tubulin appeared to be distributed along the spindle microtubules; in contrast, during mitosis this protein is concentrated around the poles (Hiruta and Tochinai, 2012).

16.5.1 Formation of Parthenogenetic Eggs

Paired ovaries lie along the length of the intestine in the thorax of cladocerans. The location of the germarium in the tubular ovary appears to vary across the orders; it is located in the posterior region in the Anomopoda and Haplopoda (Rossi, 1980; Kato et al., 2011a,b) and anteriorly in the Ctenopoda (Rossi, 1980). Among the Onychopoda, Jorgensen (1933) found the germarium of *E. nordmanni* to be near the anterior of the ovary, but Rossi (1980) positioned it at the posterior end in *Bythotrephes longimanus*. Examination of gonads from stained sections indicate that the diameter of each ovary is more than twice that of each testis in males (suggesting 4−5 times greater volume). As expected, testis tissue has a homogeneous consistency and surrounds a single lumen in *D. magna* (Kato et al., 2011a,b). Ovarian tissue, on the other hand, is far more irregular, with yolk granules, lipid droplets, and large oocytes filling the space (Kato et al., 2011a,b; Sumiya et al., 2014).

Within the ovaries are oocyte clusters, comprising one oocyte and three nurse cells. Zaffagnini and Lucchi (1965, in Zaffagnini, 1987) reported that, in *D. magna*, intercellular bridges arising from incomplete cytoplasmic divisions connect the cells within each cluster, indicating one oogonium to be the origin of the four cells. At the time of molting, the oocyte nuclear envelope degenerates and meiosis begins (Sumiya et al., 2014). Egg deposition into the brood pouch occurs soon after. The oocyte is the second or third cell to exit the germarium, and the four-cell cluster forms a single row, although in *Daphnia* the nurse cells are shifted dorsolaterally or laterally (Rossi, 1980; Zaffagnini, 1987). At this time, the oocyte's cytoplasm appears homogeneous and the nucleus contains a large nucleolus. Somatic cells in the area form an incomplete follicle surrounding just the oocyte (Zaffagnini, 1987). The presence of follicular cells may be unique to *Daphnia*; their absence has been noted in members of all orders (e.g., *Bythotrephes*, *Leptodora*, *Moina*, and *Sida*) (Rossi, 1980).

In *Leptodora*, *Moina*, and the onychopods, embryos develop in a brood pouch and are nourished by a maternal glandular structure, the Nährboden, which was first described by Weismann (1877, in Patt, 1947). Using *Polyphemus pediculus* as a model, Patt (1947) confirmed

Weismann's findings. The structure can be seen for the first time during embryogenesis as a line of cells situated between the gut and future brood pouch (see Fig. 11.1). Recent work confirms what early figures suggest; the cells composing the Nährboden of *P. pediculus* are polyploid, reaching as high as 256 C (Shea pers. comm.). Patt (1947) reported that as the animal grows the structure also grows via cell expansion (and ploidy levels increase, according to Shea, pers. comm.). In these species, the Nährboden appears not to be functional when females produce amphigonic eggs, but whether the tissue is resorbed or simply quiescent is unclear. When parthenogenetic eggs are deposited into the brood pouch, Nährboden cells increase in size to a maximum just before embryos possess limb buds. These observations almost certainly indicate that the structure is composed of polyploid cells, and DNA contents should be examined throughout embryogenesis.

Initially, the oocyte of *Daphnia* is indistinguishable from nurse cells. The cytoplasm appears homogeneous and the nucleus contains a large, central nucleolus (Zaffagnini, 1987). As the oocyte initiates growth, cytoplasmic RNA concentration increases; as the oocyte begins to differentiate, the RNA concentration decreases slightly. At the onset of vitellogenesis, the oocyte appearance is clearly different from the nurse cells; the cell is much larger, the cytoplasm appears more granular, its RNA concentration drops further, and lipid droplets become visible (Zaffagnini, 1987). The presence of oil droplets is one of the first outward signs that distinguish the oocyte from nurse cells.

During vitellogenesis, the ooplasm accumulates yolk globules via pinocytosis, and as individual globules enlarge, they concentrate in the cell periphery. Lipid droplets also increase in size and number, and mitochondria are sometimes observed to cluster around them (Zaffagnini and Lucchi, 1965, in Zaffagnini, 1987). Microvilli develop on the surface of the oocyte near the follicular cells and pinocytotic vesicles and endoplasmic reticulum form in the immediate vicinity (Zaffagnini, 1987). Kessel (1968) observed periodic accumulations of yolk bodies in the cisternae of the rough endoplasmic reticulum. The nucleus also experiences change; as the nucleolus grows, its interior becomes less dense. Furthermore, the nucleolar RNA concentration increases throughout differentiation until vitellogenesis, indicating active RNA synthesis.

The function of the nurse cells, therefore, seems less clear. As vitellogenesis proceeds, the nurse cells progressively shrink and degenerate. Simultaneously, RNA-rich bodies appear around each nucleus before being transferred to the oocyte. However, because the oocyte actively manufactures RNA and the nurse cells do not synthesize lipid or yolk, the nutritive function of the nurse cells does not appear essential (Zaffagnini, 1987).

16.5.2 Formation of Amphigonic Eggs

Generally one or two sexually produced eggs are formed at a time. The ovaries of examined members of Onychopoda and Anomopoda (*Evadne*, *Podon*, and *Daphnia*) appear similar in parthenogenetic and sexual females (Zaffagnini, 1987; Egloff et al., 1997). An early examination of a chydorid and macrothricid, (*Acroperus elongatus* and *Lathonura rectirostris* respectively), revealed a gland situated adjacent and dorsal to the ovaries in sexual but not parthenogenetic females (Makrushin, 1970). As it appeared to secrete material between molts during the formation of amphigonic eggs, Makrushin (1970) supposed the gland, which he showed as running the length of each ovary, is involved in egg case formation.

The amphigonic oocyte is formed with three nurse cells in the same manner as a parthenogenetic oocyte. The nucleus and nucleolus of both oocyte types also appear similar. However, in marine onychopods, the brood chambers of gamogenic (sexual) and parthenogenetic females differ. Cells of the former are cuboidal or

hexagonal and may contribute to the egg's chitinous layer, whereas cells of the latter are transparent and amorphous (Kuttner, 1911 and Rivier, 1968; both in Egloff et al., 1997). Several characteristics have been reported for *Daphnia* that distinguish sexual from parthenogenetic eggs: the amphigonic oocyte has a more granular cytoplasm and contains no lipid droplets; and the yolk globules do not accumulate in the periphery, but fill the ooplasm uniformly with many tiny yolk balls that have phospholipid composition and concentration differences compared to a parthenogenetic oocyte (Zaffagnini and Minelli, 1968, in Zaffagnini, 1987). Furthermore, although the nurse cells of both types of oocytes seem similar in both appearance and RNA content, those of the sexually produced oocytes cease growth by the time that vitellogenesis is initiated (Zaffagnini, 1987). Finally, upon completion of vitellogenesis, the amphigonic egg occupies nearly the entire ovary volume (one egg per ovary).

16.6 CONCLUDING REMARKS

Although a great deal of cytological information has been amassed for selected cladocerans, there is still a great deal of work left to be done. For much of this chapter, we have attempted to highlight some cytological patterns that have emerged from the last 100 or so years. However, some prominent gaps in our knowledge are evident, even from a cursory examination. Much of our understanding of the subject is based on studies of one or two species of *Daphnia* and occasionally of one additional cladoceran member. Only four genome size estimates are known for representatives from outside the order Anomopoda. Our understanding of chromosome numbers is also limited to Anomopoda, except for two ancient and unsubstantiated estimates. Many more surveys of DNA content and chromosome number, which represent some basic science, are required. The ultrastructure of some tissues has been carefully completed for several tissues (but again with heavy emphasis on *Daphnia*). However, questions of the function and development of some structures will benefit substantially from the burgeoning molecular tools that are available to researchers today. A much clearer understanding of the mechanism used for creating parthenogenetic eggs has only recently been described, due in no small part to the developments in immunohistochemistry. The function of nurse cells needs to be reevaluated via gene expression and in situ hybridization studies. Similarly, molecular studies focused on structures such as the Nährboden of *Polyphemus* will clarify the types of material that cells of this tissue provide to parthenogenetic embryos and how they function when amphigonic eggs form. There is clearly much left to be investigated.

CHAPTER

17

The Genomics of Cladoceran Physiology: *Daphnia* as a Model*

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17.1 INTRODUCTION

Omics studies aim at exploring the molecular and genetic profiles of organisms to unravel the evolution, the structure, the complex interactions, and functions of its components using a holistic approach. Rather than focusing on a single gene, the field of genomics allows researchers to plunge into a vast matrix of genetic interactions and gene functions. This exciting research field is incorporated into crustacean physiology research (Stillman and Hurt, 2015). Studies in cladoceran genomics have transformed the field of cladoceran physiology, and likewise, physiological research allows for a better understanding of cladoceran genomics by helping to identify orphan genes. Functional genomics emerge at the crossroads of both research fields.

Understanding the tolerance of organisms to environmental change and the underlying mechanisms that define the thresholds of physiological capacity are major objectives for modern biological investigation (e.g., Hofmann and Todgham, 2010). Against the background of a rapidly changing world, there is an increasing emphasis on linking organism–environment

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interactions to physiological responses and phenotypic plasticity. To obtain a thorough understanding of the origin and maintenance of phenotypic variation of populations in the natural world, i.e., how environmental stimuli affect individual fitness and the evolutionary potential of populations, we need to decipher the molecular machinery that regulates phenotypic responses to environmental conditions. These responses span the short-term physiological changes of acclimation to long-term genetic alterations of adaptation. Linking organismal change across these disparate time frames requires delving deeply into the genetic mechanisms that underlie phenotypic plasticity. A plastic (flexible) phenotype is the outcome of modification to the functional genome, for example by altering transcription or translation. "Environmental change that emerges across generations can also be accommodated by plasticity, or alternatively may drive structural change of genomes by adaptive and demographic evolutionary processes that sort allele frequencies within populations across generations" (Whitehead, 2012).

Making connections between the functional genome and the interaction with the environment that gives rise to the phenotype is a challenging task. One central difficulty is that environmentally sensitive physiological traits are rarely controlled by single, or a few, genes of major effect. They are often highly polygenic traits (West-Eberhard, 2005; Aubin-Horth and Renn, 2009; Ayroles et al., 2009; Flint and Mackay, 2009), which dictates that entire regulatory networks need to be considered.

Studying the genetic basis of cladoceran physiology provides an ideal context for understanding the generality of genomic mechanisms underlying physiological responses. Cladocerans, with their pronounced phenotypic plasticity and particular mode of reproduction, are useful organisms to study genome evolution (Colbourne et al., 2011). Only through a better understanding of the phenotype (e.g., morphology and physiology) and of the genomic basis of phenotypic variation can linkages be explored between genes and physiological functions.

Functional genomics allow a new approach to explore the mechanisms that drive physiological change and help to unravel key processes that enable organisms to cope in their natural environment (Aubin-Horth and Renn, 2009; Bell and Aubin-Horth, 2010). This area of investigation is embodied by the emerging field of *ecological and evolutionary genomics* (EEG), which seeks to link gene functions to phenotypes and ecological factors (e.g., Renn and Siemens, 2010; Pavey et al., 2012). *Gene–environment* ($G \times E$) *interactions* can be best studied in animals with well-known ecologies on a physiological timescale as well as in an evolutionary context (Carroll et al., 2007; Hoffmann and Willi, 2008; Pfrender, 2012; Yampolsky et al., 2014; for a review, see Hodgins-Davis and Townsend, 2009). Cladocerans lend themselves to such research, as their ecology is well studied, the genomic resources required are available for the model organism *Daphnia*, and ancient phenotypes can be resurrected (Stollewerk, 2010; Colbourne et al., 2011; Ebert, 2011; Tautz, 2011; Orsini et al., 2013, 2016a). The genomic repertoire and phenotypic plasticity in the Cladocera are linked to millions of years of evolution and to the evolution of the aquatic environment itself (Van Damme and Kotov, 2016). The importance of cladocerans as model organisms in environmental and functional genomics is considerable, because "by examining genome structure and the functional responses of genes to environmental conditions within species with traceable ecologies, we further our understanding of gene–environment interactions in an evolutionary context" (Colbourne et al., 2011). Current genomic research seeks to address the following key **questions** in cladoceran physiology:

1. Are physiological responses reflected by responses in the functional genome?
2. What are the genomic elements or regulatory programs that allow plastic responses, i.e., by

which mechanisms is a change in the environment sensed, integrated, and transformed into distinct physiological responses?
3. How are physiological systems integrated, and how do genes interact to describe the regulatory networks underlying the biological processes and pathways involved?
4. How genetically variable are Cladocera genotypes (including past and present genotypes) and how do they reflect on their plasticity?

In this chapter, we explore the current knowledge on the genetic basis of cladoceran physiology drawing largely from our growing understanding of *Daphnia*, which has been the most extensively studied taxon in the group, and we discuss *Daphnia* as a model in physiological genomics.

17.2 HISTORY OF RESEARCH: THE PREGENOMICS ERA

The genus *Daphnia* has been the focus of biological and ecological research for over a century, making it one of the oldest study systems in ecology, physiology, and evolutionary biology among freshwater invertebrates (e.g., Ebert, 2011; Lampert, 2011). When it comes to genetics, it is currently the best-studied cladoceran. Today's in-depth knowledge of these organisms results from their prominent ecological role as keystone species in freshwater ecosystems. The role of cladocerans is historically important. Their evolution is thought to have had an influence on the evolution of aquatic ecosystems through time and even the genus *Daphnia* has been present at least since the end of the Jurassic (Van Damme and Kotov, 2016 and references within).

Extensive genetic investigation at the population level and a phylogenetic framework for several groups of cladocerans form the backdrop for understanding the evolution of physiological traits. In the pregenomics era of the past century, the genetic architecture of natural populations of *Daphnia* was investigated through population genetic, quantitative genetic, and phylogenetic approaches. Among the early studies were examinations of the characteristic alterations in the phenotype in response to predator chemical cues (Woltereck, 1909) and the regulation of sexuality and diapause (Banta and Brown, 1939). Another adjustment of the physiological properties is the animals' response to low-oxygen conditions leading to hemoglobin induction. As this is literally a very obvious plastic trait turning the animals from transparent pale to red, it already gained attention early (Fox, 1945, 1948; Fox et al., 1949).

With the advent of readily available molecular genetic techniques (i.e., protein gel electrophoresis) in the 1970s, genetic studies revealed populations and species with high levels of genetic variation indicative of local adaptation. In the following decades, research on cladoceran genetics shifted with the techniques available from allozyme studies (1970–90) to the amplification and the sequencing of nuclear and mitochondrial genes (Hebert and Taylor, 1997). Allozyme analysis provided a useful overview of population structure and is still often used as a diagnostic tool to identify naturally occurring hybrids (Hebert et al., 1989a,b; Cerny and Hebert, 1999). Later, DNA sequencing became (and remains) the most widespread technique to understand speciation and phylogeny in the group.

Cladoceran phylogenies have been variously constructed with mitochondrial genes [12S, 16S ribosomal genes, and cytochrome oxidase I (COI)] (e.g., Crease and Little, 1997; Colbourne et al., 1998; Crease, 1999), nuclear genes [18S, 28S ribosomal genes, and enhancer factor 1 (*EF-1*)] (e.g., Crease and Taylor, 1998), or combinations of both (e.g., Adamowicz et al., 2009). Somewhat surprisingly, research that examines phylogenetic relationships at the higher taxonomic levels in the Anomopoda is currently

limited. Molecular phylogenies often fail to resolve relationships among the deeper cladoceran branches (de Waard et al., 2006a,b; Stenderup et al., 2006; Van Damme et al., 2007a,b; Van Damme and Kotov, 2016). Within cladoceran genera, DNA markers have been proven useful, as repeatedly shown for *Daphnia* in a genus-wide phylogenetic context (e.g., Lehman et al., 1995; Adamowicz et al., 2009). In addition, DNA markers have been shown useful in the exploration of the genetic diversity and biogeography of selected species groups (Taylor et al., 1996; Hebert et al., 2003; Thielsch et al., 2009; Kotov and Taylor, 2010; Crease et al., 2012; Kotov et al., 2016). The phylogenetic relationships within many cladoceran families, genera, and species have not been well investigated beyond the Daphniidae, and for some groups the first molecular phylogenetic studies have emerged only recently (e.g., Bosminidae; Kotov et al., 2009; Chydoridae; Sacherová and Hebert, 2003; Belyaeva and Taylor, 2009; Kotov et al., 2016; Leptodoridae; Xu L. et al., 2011; Polyphemidae; Xu S. et al., 2009). As a result, no other large cladoceran genus has been genetically characterized as well as *Daphnia* (so far). The rapid development of a genetic characterization of *Daphnia* prompted Hebert (1987) to predict that "the future may well also see the broader use of cladocerans as model systems to examine problems of more general theoretical importance."

Parallel to the development of population genetic and phylogenetic studies using molecular markers, quantitative genetic studies leveraging the clonal reproductive mode of *Daphnia* investigated the extent to which the phenotypic variation in life-history traits and fitness has a genetic basis. These studies revealed links between phenotypic variation, mutation, and heritable genetic variation (e.g., Lynch, 1985; Spitze, 1993; Pfrender and Lynch, 2000). Building on this fundament of population- and quantitative-genetic studies, novel approaches linking quantitative genetic variation with environmental context were advanced in *Daphnia*. These studies make a statistical association between the patterns of population subdivision in quantitative traits and molecular genetic markers. Excess levels of population subdivision in these traits and markers provide evidence of natural selection and local adaptation (Spitze, 1993; Lynch et al., 1999; Morgan et al., 2001). Following the seasonal changes of the contribution of specific genotypes representing quantitative traits within the population allows correlation of physiological parameters to changing environmental conditions.

For example, the temperature reaction norms of respiration, ventilation of the external medium, and perfusion within the body indicated by the heart rate were correlated to specific genotypes of *Daphnia longispina, Daphnia galeata*, and their hybrids in a German reservoir (Pinkhaus et al., 2007; Paul et al., 2012). Genotypes with low tolerance toward elevated temperatures even after acclimation to warmer water were abundant in the winter months, whereas genotypes with high physiological performance in subsequent warmer conditions were dominant in the summer, and an important role of the phosphoglucomutase locus was identified (Paul et al., 2012). Further studies in intra- or interspecific differences of the distribution in the habitat revealed a temporal and spatial separation concerning genotypes differing in their hemoglobin expression (see Section 17.5.1). Hemoglobin-rich individuals were observed in deeper layers (Sell, 1998; Wiggins and Frappell, 2002) representing species (Sell, 1998) or genotypes of the same species (Pinkhaus et al., 2007) with elevated induction potential for hemoglobin. In contrast, the observed seasonal patterns of hemoglobin content in *Daphnia* may rather be affected by food conditions and energy allocation toward reproduction and the offspring in the summer than being the result of changes in genotype structure (Schwerin et al., 2010). For the contribution of genotypes to temporal and spatial subsets of the population, the spring phase of population increase seems of

outstanding importance. In lakes with overwintering *Daphnia*, the population growth can start from parthenogenetic individuals. Spring population increase after a long phase of ice coverage mainly relies on individuals hatched from ephippia (Rother et al., 2010; Zeis et al., 2010). Thus, in lakes of the temperate climate zone with long cold-winter periods with ice formation on the lake, the population experiences a genetic bottleneck every year. During the subsequent exponential population growth phase the genotype structure is determined by the recruitment strategy. Dominant parthenogenetic reproduction in spring leads to a clonal population structure until sexual reproduction occurs, mostly in autumn (Hülsmann et al., 2012). Of course, this crucial phase of winter and spring conditions affecting the population structure is sensitive to the expected climate changes. The genotypes present in the dormant eggs (ephippia) are not only the important genetic reservoir for the inoculation of the spring population, but they can also illuminate the contribution of genotypes to past populations.

The previous example also illustrates the amazing benefit of Cladocera to compare physiologies of genotypes between past and present, hatched from ephippia from lake sediments, to analyze their physiological properties. This approach has been extended to examine patterns of adaptation through time by leveraging the historical legacy genotypes in diapausing embryos trapped in lake-bottom sediments (Cousyn et al., 2001; Brendonck and De Meester, 2003; Brede et al., 2009; Decaestecker et al., 2007, 2013; Orsini et al., 2012, 2013), currently extended into paleogenomics (see Section 17.7).

17.3 *DAPHNIA* AS A MODEL SYSTEM FOR PHYSIOLOGICAL GENOMICS

Although studies before genomics have been essential in establishing the framework for a detailed investigation of physiology and physiological mechanisms, they are best suited to describing patterns of genetic variation within and among species and provide limited insight into mechanisms. Linking the extensive phenotypic variation in physiology and the wide range of habitats occupied by cladocerans requires new approaches. Such research can be leveraged by modern high-throughput DNA and RNA sequencing technologies and draw on the rapidly expanding resource of genome sequences, functional genomic assays of gene regulation, and proteomic and metabolomic data.

The genomic investigation into the tremendous phenotypic and physiological variation in cladocerans provides insights into physiological mechanisms that were unattainable in the past. In the postgenomic era, responses can be examined for a wide range of genes at once. An example is the STRESSFLEA consortium, that generated a comprehensive RNA sequencing (RNA-Seq) data set by exposing two inbred genotypes of *Daphnia magna* and a recombinant cross of these genotypes to a range of environmental perturbations. Gene models were constructed from the transcriptome data and mapped onto the draft of the *D. magna* genome. This transcriptome data set, together with the available draft genome sequence of *D. magna* and a high-density genetic map, is a key asset for future investigations in environmental genomics (Orsini et al., 2016a).

The field of cladoceran genomics is still in the early stages of development, yet advances rapidly. In 2002, the first genome project for a cladoceran species (i.e., *Daphnia pulex*) was launched (see Section 17.4). The initiative aimed at creating a leading model system for ecological, ecotoxicological, and evolutionary genomics (e.g., Colbourne et al., 2011; Stollewerk, 2010; Lampert, 2011; Miner et al., 2012). With the first draft of the *D. pulex* genome made available in 2006, the first annotated crustacean genome helps to bridge "the gap between genotype and

phenotype" in an ecologically relevant framework (Dow, 2007; Gregersen, 2009; Colbourne et al., 2011). Based on these resources, *Daphnia* can now be considered as a useful model system for *physiological genomics*.

One of the most intriguing features in *Daphnia* biology is its extraordinary ability to cope with changes in the environment. Cladocerans can produce extremely divergent phenotypes in response to environmental challenges, which include altered morphologies (Tollrian and Dodson, 1999; Laforsch et al., 2004; Petrusek et al., 2009; Herzog et al., 2016), changes in their physiology (Kobayashi and Yamagata, 2000; Lamkemeyer et al., 2003; Paul et al., 2004) and behavior (Wiggins and Frappell, 2002; Decaestecker et al., 2002; Lamkemeyer et al., 2003; Zeis et al., 2004a,b, 2005), and switches in the reproductive mode from parthenogenetic to sexual reproduction cycles (Zaffagnini, 1987; Olmstead and LeBlanc, 2003; Decaestecker et al., 2009).

Modern genomic approaches have transformed cladoceran research. Investigators working with this group of organisms are poised to tackle one of the main objectives in modern biology, to identify the mechanistic basis of plastic responses to environmental change. At present, researchers explore how the developmental and physiological "program" of an organism can be modified in response to distinct environmental stimuli. However, the latter requires "first and foremost the understanding of its genomic make-up" (Aubin-Horth and Renn, 2009). Even though numerous investigations in diverse taxa have reported the existence of environmentally induced phenotypic plasticity, there has been relatively little success in identifying the empirical patterns of cellular control mechanisms that govern the plastic traits in question. This lack is mainly due to a paucity of study organisms with well-developed genomic resources that are also empirical models for investigating phenotypic plasticity. Currently, knowledge on genes, genomes, and their regulation is predominantly based on traditional model systems such as *Caenorhabditis elegans*, *Drosophila melanogaster*, zebrafish, and mice. As all these species are of limited relevance in their natural habitats and ecosystems, and consequently lack a significant context outside the laboratory, available data are limited on how environmental factors may contribute to genetic and phenotypic evolution. Moreover, many genes that respond to ecological conditions have unknown functions, and information from laboratory model species may be insufficient. Consequently, ecological genomic approaches require empirical annotations of new genome sequences from a broader range of species tested under a variety of natural and ecologically relevant conditions (see Section 17.6). As a model for ecological and evolutionary research (e.g., Lynch et al., 1994; Lampert, 2011; Miner et al., 2012), *Daphnia* bridges this gap, as it allows us to increase our understanding of the linkages between phenotypic responses and underlying genotypic and environmental effects. There are several compelling reasons why *Daphnia* is particularly well suited to this research agenda:

1. A complete genome sequence for multiple species is available (*D. pulex, D. magna*), a transcriptome for *D. galeata* and genome-wide SNPs for *Daphnia pulicaria* (Colbourne et al., 2011; Huylmans et al., 2016; Orsini et al., 2016a; Muñoz et al., 2016).
2. The data resources are publicly accessible [including genomic and complementary DNA (cDNA) libraries, microarrays, tiling arrays, genetic linkage maps, web-based bioinformatics portals, and annotation and gene expression databases].
3. The phylogenetic position of *Daphnia* in the arthropods is excellent for comparative genomics (i.e., branchiopods, which are basal crustaceans) (e.g., Stollewerk, 2010).
4. Their maintenance in the laboratory is relatively cheap and easy.

5. Their large brood sizes and fast reproductive cycle are ideal for experimental genetics (with the generation time in the laboratory rivaling that of nearly all model eukaryotic systems).
6. The clonal nature of these organisms provides an outstanding opportunity to study the genetic responses to environmental stimuli in a defined and constant genetic background with unlimited replication (which is relevant for e.g., epigenetics research; see Asselman et al., 2017).
7. *Daphnia* are transparent throughout life, allowing studies of tissue-specific gene expression at any life stage (e.g., to changes in oxygen).
8. The extensive phenotypic diversity of this species provides ample raw material to study gene function and genome by environment (G×E) interactions. Recently, this has been associated with *Daphnia*—microbiome research indicating a strong role of the microbiome in modifying these G×E interactions (Sison-Mangus et al., 2015; Callens et al., 2016; Macke et al., 2017), more in particular with respect to cyanobacterial stress (Macke et al., *Nature Communications* in revision).
9. *Daphnia*-specific genes have been shown to respond rapidly to environmental disturbances.
10. *Daphnia* can be resurrected from egg banks, allowing ancient phenotypes and genotypes to be studied and compared to contemporary populations (see Section 17.7).
11. Research is supported by a large, and expanding, community of scientists, i.e., the *Daphnia* Genomics Consortium (DGC).

Our understanding of the cellular processes by which plastic responses to environmental stimuli are triggered is still very limited (Aubin-Horth and Renn, 2009). Genome-wide association studies that aim at linking genes to phenotypes strongly suggest that complex traits are governed by many loci (Flint and Mackay, 2009). In *Daphnia*, most of the alternative phenotypes can be generated from the same genetic background through the perception of cues from the environment. These cues lead to modifications of gene regulation that alter the physiological state and developmental trajectory of individuals (e.g., the formation of males). The clonal character of *Daphnia* facilitates dissection of the genetic and environmental components of plastic responses (Shaw et al., 2008; Simon et al., 2012). The remarkable ability of these organisms to cope with environmental change is associated in part with its unique gene inventory and genomic structure (see Section 17.4).

Concerning a mechanistic understanding of environmentally induced differential gene expression, studies on the role of signaling pathways and transcription factors have gained importance. Those important key players form the basis of regulatory networks and concerted responses of a multitude of gene loci. One important example, the hypoxia-inducible factor (HIF) regulated in response to oxygen conditions and binding to hypoxia-responsive elements (HREs) in the promoter regions of genes, has been studied in *Daphnia* (Gorr et al., 2004) and is described in detail later (see Section 17.5). Further examples of transcription factors involved in coordinating responses to environmental stimuli include the heat-shock factor (HSF) as a key regulator of heat-shock protein induction at elevated temperatures (Klumpen et al., 2017) as well as illuminating a role of HIF also in the context of temperature acclimation (Becker et al., 2011a; Klumpen et al., 2017). Analyses of the promoter regions of differentially expressed genes concerning binding sites for the large variety of transcription factors that govern gene expression patterns will help to shed light on the mechanisms of environmentally induced gene expression.

To decipher the relationship between phenotypic plasticity and adaptive evolution, it is important to understand the genetic basis of

interactions between genotype and environment (G×E interactions; Miner et al., 2005; Pigliucci, 2005; West-Eberhard, 2005). The regulation, expression, and function of many genes are highly context dependent, and are only manifested under particular environmental conditions. It is presently unclear to what extent such genetic mechanisms are species specific. Ecological genomics aim to understand how natural populations adapt to their local environments. *Daphnia* is known to have colonized radically different environments on multiple occasions, with a characteristic pattern of convergence of adaptive traits linked to specific habitats (e.g., Colbourne et al., 1997). Consequently, the genetic variation found within natural populations of *Daphnia* provides an exceptional opportunity to evaluate the nature of genomic and phenotypic adaptations (e.g., temperature; De Meester et al., 2011; Yampolsky et al., 2014; Geerts et al., 2015; Orsini et al., 2016b). Comparing different *Daphnia* lineages that show habitat-specific reaction norms allows us to decipher whether similar environments can and do induce similar genetic outcomes, and whether different *Daphnia* lineages evolve the same way to meet these environmental challenges (see e.g., Colbourne et al., 1997; Pfrender et al., 2000; Mitchell and Lampert, 2000; Scoville and Pfrender, 2010).

The availability of the *Daphnia* genome (see Section 17.4), coupled with a wide range of phenotypic diversity, make this organism an outstanding model system to explore the genetic basis of complex phenotypic traits, including physiology (e.g., Miner et al., 2012). It allows unprecedented insight into the responses to environmental perturbations by tackling physiological acclimation and evolutionary adaptation processes in response to the environment (e.g., Yampolsky et al., 2014; Fritsch et al., 2014; Geerts et al., 2015; Roy Chowdury et al., 2015; Rund et al., 2016). In this chapter, we highlight a few studies related to temperature, oxygen, toxicants, eutrophication, and diurnal vertical migration (DVM); however, this is only a selection. The number of studies including *Daphnia* omics increases yearly, and fascinating work is being carried out by several research groups on predator−kairomone-induced effects (e.g., Rozenberg et al., 2015 and ongoing studies by R. Tollrian, C. Laforsch, E. von Elert), pH, salinity, and other stressors (e.g., Orsini et al., 2016b).

Although earlier investigations were often constrained to hunting for single genes associated with particular phenotypes, employing functional genomic methodologies at genome-wide scales offers a more comprehensive strategy. These tools will facilitate a genuine understanding of how the environment interacts with (and shapes) the genome and how genome regulation and variation are linked to phenotypic plasticity and phenotypic evolution.

17.4 *DAPHNIA*'S ECORESPONSIVE GENOME

Modern physiological studies will increasingly draw on a genomic perspective to discover underlying mechanisms and to understand the patterns of variation in natural populations occupying diverse environments and confronted with varied physiological challenges. Developing a genomic perspective requires a high level of genomic infrastructure, including genome sequences and functional genomic tools. Of all Cladocera, the widespread *D. pulex* (e.g., Crease et al., 2012) and *D. magna* are the best-developed species in this area. *D. pulex* is the first crustacean taxon to have its complete genome sequenced and annotated (Colbourne et al., 2011). The genome of a second species, *D. magna*, a well-studied ecotoxicological model, is now available (Routtu et al., 2014; Orsini et al., 2016b) and more species are being sequenced (*D. galeata*; P. Spaak, Swiss Federal Institute for Environmental Science and Technology/ETH (EAWAG); Huylmans et al., 2016). High-quality genomes of other model species among

the crustaceans are now available, such as the genome of the amphipod *Parhyale hawaiiensis* (Kao et al., 2016; see also Section 17.8.1).

What makes the first crustacean genome special? Even though the *D. pulex* genome is small compared to that of other organisms, measuring only c. 200 Mb, it contains almost twice as many genes as any other arthropod yet sequenced, and has a greater number of genes than in most other eukaryotic genomes, including the human genome. So far, 30,907 protein-coding genes are predicted on the 12 chromosomes. Sequencing and assembling this water flea's genome revealed a significant number of nonprotein-coding genes including microRNAs, ribosomal RNAs, transfer RNAs, and several families of transposable elements. For a detailed discussion on the *D. pulex* genome and its potential as a genetic model, we refer to Colbourne et al. (2011), on which the current section is based (unless stated otherwise). In addition to the latter work, the authors selected 50 thematic publications (the "companion papers" by the DGC, published between 2005 and 2011), which constitute the initial survey of the genome (see http://www.biomedcentral.com/series/Daphnia). The genome itself, and draft versions of *D. magna*, can be explored through the publicly available, interactive genome database, wFleaBase (Colbourne et al., 2005), which also includes data on the expression patterns of a large number of protein-coding genes under different experimental conditions.

Comparisons of the *D. pulex* genome with that of several insects revealed that intron sizes appear rather reduced in the *Daphnia* genome, with an average intron length of only 170 base pairs (bps). Moreover, the intron density in the cladoceran seems similar to that observed in *Apis mellifera*, but there are at least twice as many introns per gene than in *Drosophila*, for example. As outlined by Colbourne et al. (2011), the majority (i.e., 78%) of the intron gains observed in the *D. pulex* genome appear unique to the *Daphnia* lineage (or perhaps to the branchiopods). That is to say, the *Daphnia* genome is more compact with respect to the size of its introns, but not in the number of introns acquired.

Exploring the *Daphnia* gene inventory revealed a higher propensity for gene duplication and retention when compared to other organisms. Gene-duplication events account for approximately half of the genes in the genome. In fact, the rate of gene duplication in *D. pulex* seems three times as high as that observed for *Drosophila* and *Caenorhabditis*, and about 30% greater than that of the human genome. As Colbourne et al. (2011) showed, the elevated number of genes in the *D. pulex* genome results not only from acquiring but also from retaining a large number of genes. The retention of duplicate genes and the neofunctionalization of these paralogs and/or changes of their temporal/ontogenetic or tissue-specific expression pattern may be directly linked with the plasticity of cladocerans and their ability to respond to a large range of environmental challenges (Colbourne et al., 2011; Simon et al., 2012).

As gene duplication events are considered to play a key role in shaping genomes, they represent one of the most significant sources of evolutionary novelty. If, after duplication, selection maintains both copies of a gene, then one gene version commonly retains its original function, whereas the other may functionally diverge; the latter is called a *paralog* (for reviews, see e.g., Innan and Kondrashov, 2010; Proulx, 2012). Most of *Daphnia*'s paralogs have evolved context-dependent functional specifications and the expression patterns diverge with the age of the gene. Although duplicates that are more recent, which have different gene sequences, showed rather indistinguishable gene expression patterns under several tested environmental conditions, duplicates that are more ancient revealed divergent expression patterns over time. These duplication and divergence events in various gene families represent an impressive evolutionary mechanism that enables these

animals to express a selective suite of functionally distinct molecules depending on the environmental condition. The genome can thus mediate diverse cellular processes to maintain or restore cellular integrity in response to the environmental challenges faced. However, the *D. pulex* genome was also shown to contain a number of recent duplicates that have nearly identical nucleotide sequences but show rather differential gene expression patterns in response to different environmental parameters. These expression patterns may arise by integrating novel genes into a new genomic location (chromosome) or dissociating them from their previous regulatory framework (e.g., pathways).

Due to their high degree of homology, duplicated genes (and especially tandemly clustered genes) are prone to undergo genetic exchanges via unequal crossing over and unidirectional gene conversion (GC) (Hoffmann et al., 2008). In the *Daphnia* genome, 47% of all genes were revealed to contain tracts of GC; in comparison, in five different *Drosophila* species, only 12−18% of genes were shown to be affected by such genetic homogenization events. However, GC events were shown less common among more recent duplicates and within gene families containing only a few paralogs. An exceptional example of widespread GC events in the *Daphnia* genome comprises the di-domain hemoglobin (Hb) gene family (see Section 17.5).

Even though the high number of genes in the *Daphnia* genome may indicate that these genes arose by whole-genome duplication, the number of synonymous substitutions among all pairs of duplicated genes in *D. pulex* rather suggests high and steady rates of tandem duplication events in the lineage's evolutionary history. Similar to other genomes in which new duplicates are found in clusters, the *D. pulex* genome has approximately 20% of its genes in tightly arranged groupings. Each cluster can contain up to 80 paralogs. Two gene families that have gained and retained an exceptional number of duplicated genes in *D. pulex* include the photoresponsive genes of the opsin family (Rivera et al., 2010) and di-domain Hbs (Colbourne et al., 2011), which play critical roles in coping with complex light regimes or allowing adequate responses to oxygen deprivation in aquatic environments, respectively.

The expansion of gene families in *D. pulex* was shown also associated with distinct metabolic processes. By analyzing the functional role of paralogs, Colbourne et al. (2011) could show the co-expansion of genes in distinct metabolic pathways (e.g., androgen−estrogen metabolism and sphingolipid biosynthesis metabolism), which indicates that the duplicated genes may be interdependent. In fact, half of the expanded metabolic genes were found to belong to a subset of seven distinct pathways, and the expression patterns of these genes co-diverged according to their pathway and not according to their evolutionary history, which suggests the functional divergence of expanding genes within pathways.

A highly expanded gene family involved in protein metabolism are the digestive proteases trypsin and chymotrypsin. Within the set of 256 genes annotated as serine proteases in the *D. pulex* genome, 16 chymotrypsins and 40 trypsins were considered functional as judged from the presence of all residues of the catalytic triad of the active site (Dölling et al., 2016). Their differential gene expression can be correlated to temperature conditions; moreover, the presence of microcystins, protease inhibitors from cyanobacteria that specifically inhibit one of the protease subtypes, may be important for the observed shifts in protease activity (Dölling et al., 2016; Schwarzenberger et al., 2010).

Finally, a large number of genes (36%) in the *D. pulex* genome lack any detectable homology with other noncladoceran species. Phylogenetic analyses that account for the expansion and contraction of all gene families within pancrustacean and several deuterostome genomes suggest a net increase in the number of paralogs within the lineage leading to *Daphnia*. That is to

say, the vast number of lineage-specific genes appears to result from disproportionate expansions of particular gene families distinctive to this crustacean lineage and the fast divergence of most of these genes. Orthology-based analysis showed that many of these *D. pulex* orphan genes (i.e., without homologous sequences) do have orthologs in *D. magna* and *D. galeata* (Huylmans et al., 2016). Using whole-genome tiling arrays or expressed sequence tag (EST) sequencing, it was shown that *Daphnia*-specific genes in particular (as far as it is known) appear to play important roles in the organism's ecology. These "orphan genes" may particularly account for the plastic responses of *Daphnia*, as they were shown to have distinct gene expression patterns that are context specific (and may thus only be expressed under particular ecological conditions). Similarly, another set of *D. pulex* transcripts showed condition-dependent gene expression patterns in response to diverse environmental challenges. These transcriptionally active regions (TARs), which have predictable exon−intron intervals, are not yet assigned to any known gene annotation models, and thus need to be functionally and structurally characterized.

17.5 THE GENETIC BASIS OF PHYSIOLOGICAL PLASTICITY: A CASE STUDY

17.5.1 *Daphnia*'s Hemoglobin Genes

Hb is found in virtually all kingdoms of living organisms and is arguably among the best-studied proteins tied to natural conditions (Weber and Vinogradov, 2001). For *Daphnia*, hemoglobin was first characterized by Hünefeld in 1840 and reported by Lankester (1871). Its central role in maintaining cellular oxygen homeostasis has garnered markedly detailed molecular biological investigations since the late 1990s, including the comparative study of DNA and protein sequence, and of the molecular assemblage and functional properties of this respiratory protein across diverse lineages of animals (for reviews, see Weber and Vinogradov, 2001; Hardison, 2012; Storz et al., 2013). Although Hbs are remarkably diverse in structure and function, their sequence similarity and conserved gene structures indicate that all globins described to date are descended from a shared ancestral gene (Goodman et al., 1987, 1988; Hardison, 1998; Vinogradov et al., 2005). Expansion and diversification of the Hb gene family, both within and across species lineages, is frequently shown to correlate with the physiological oxygen demands that organisms face in their natural habitats (Weber et al., 2002; Storz et al., 2007, 2010; Storz and Moriyama, 2008; Tufts et al., 2013). Because an animal's ability to cope with environmental change is predominantly determined by its capacity to maintain performance and oxidative metabolism (e.g., Pörtner and Knust, 2007), variations in the protein structure and differential expression of distinct *hb* genes consequently represent central mechanisms that enable proper system functioning and survival. Due to the extensive background knowledge on the physiological role of Hb and its structure−function relationships, critical insights into the genetic and mechanistic bases of physiological acclimation and evolutionary adaptation processes are gained by investigating the structural variation and regulation of this protein in Cladocera.

Numerous investigations on the functional processes involved in oxygen regulation by Hb have been carried out in *Daphnia*, with initial studies dating back to the late 1940s and early 1950s (e.g., Fox et al., 1949; Fox, 1950; Green, 1956b). These aquatic poikilotherms are exposed to a wide range of environmental conditions (Lampert, 2003; Lampert and Sommer, 2007; Brede et al., 2009; Weider et al., 2010; Paul et al., 2012); both diurnal and seasonal fluctuations in oxygen and temperature are among the major abiotic challenges that *Daphnia* face. Their

DVM between the epilimnion and hypolimnion in deeper lakes demands a high plasticity in their molecular responses to cope with different oxygen conditions in nature within short time intervals. Both environmental hypoxia and temperature-induced mismatches between oxygen supply and demand provoke cellular oxygen deficiency in aquatic organisms (e.g., Ekau et al., 2010; Pörtner, 2010). Daphniids in particular, but branchiopods in general, show an extraordinary plasticity to cope with such environmental conditions; they have evolved various regulatory mechanisms that perceive change and compensate for substantial variation in ambient conditions (Guadagnoli et al., 2005). So far, the mechanisms by which *Daphnia* sense changes in the amount of oxygen in the ambient environment are not entirely understood. However, some studies (e.g., Gorr et al., 2004, 2006b) have revealed that cellular responses to environmental challenges in *Daphnia* are mediated via distinct regulatory elements that bind to intergenic regions and control the gene expression level of physiologically important proteins (see later discussion).

In response to short-term changes in the ambient conditions, water fleas can counteract oxygen deprivation via alterations in their ventilation and perfusion rates, which restore adequate oxygen supply to peripheral tissues and cells (Lamkemeyer et al., 2003; Paul et al., 2004; Pirow and Buchen, 2004). If oxygen shortage persists for a prolonged period of time (i.e., a few hours) (see e.g., Zeis et al., 2004a,b; Becker et al., 2011a), then the microcrustacean's oxygen transport capacity is improved by the induction of Hb and even a modification of the Hb structure (Fox et al., 1951; Kobayashi and Hoshi, 1982; Tokishita et al., 1997; Pirow et al., 2001; Zeis et al., 2003a,b; Gorr et al., 2004; Pirow et al., 2004; Gerke et al., 2011; Zeis et al., 2013). In response to severe hypoxia (approximately 2−3 kPa) (e.g., Zeis et al., 2003a,b; Gerke et al., 2011), *Daphnia* Hb levels drastically rise by a factor of 15−20, thus notably coloring adult animals red within a single molting cycle (Kobayashi and Hoshi, 1982; Pirow et al., 2001; Zeis et al., 2003a,b; Gerke et al., 2011). Other branchiopods, such as *Triops*, are known to show a similar response to hypoxia by modifying their Hb structure or elevating *hb* gene expression (Guadagnoli et al., 2005). Earlier investigations on the de novo synthesis of the respiratory protein in *D. magna* provided evidence of highly controlled and localized gene expression, with Hb synthesis sites restricted to the fat cells and the epithelial cells in the epipodites (Goldmann et al., 1999).

Oxygen acquisition in the hemolymph of *Daphnia* is not only adjusted by changes in the Hb concentration but also arises from an altered oxygen-binding affinity of the respiratory protein. Expression of higher-affinity Hbs in animals facing environmental change is assumed to reduce Hb synthesis costs due to an enhanced oxygen transport efficiency of these molecules in comparison to low-affinity variants (Kobayashi et al., 1994; Pirow et al., 2001). In the long term, adjustments in the cellular Hb repertoire allow *Daphnia* to inhabit oxygen-poor water layers, thereby providing access to their respective food resources, which may ultimately increase the animals' overall fitness (Fox et al., 1951; Sell, 1998; Wiggins and Frappell, 2002; Duffy, 2010).

Several investigations on the Hb protein level have thus far revealed that changes in the oxygen-binding affinity of the multimeric Hb molecule are a direct consequence of modifications in the Hb subunit composition (Kobayashi and Takahashi, 1994; Kimura et al., 1999; Lamkemeyer et al., 2003; Zeis et al., 2003a,b; Gorr et al., 2004; Gerke et al., 2011). Compared to vertebrates, invertebrate Hbs are known to exhibit a much broader variability in their molecular structures (Weber and Vinogradov, 2001), presumably indicating adaptations to the wider ranges of environmental conditions to which these animals are subjected in nature. Studies on the molecular structure of Hb in *Daphnia*

provide evidence that the extracellular protein is composed of multiple 31−37 kilodaltons (kDa) di-domain subunits (Dangott and Terwilliger, 1980; Ilan et al., 1982; Peeters et al., 1990; Kimura et al., 1999; Lamkemeyer et al., 2003; Zeis et al., 2003a,b; Gerke et al., 2011). As previously demonstrated (Tokishita et al., 1997), there is remarkably low similarity between the amino acid sequences of the first and second Hb domains (approximately 24%). This suggests that *Daphnia*'s di-domain Hb subunits derive from an ancient duplication via unequal crossing over of two single-domain globin genes, which has been frequently shown for members of the Hb gene family (see Weber and Vinogradov, 2001). Each subunit consists of two heme-containing globin domains (Dangott and Terwilliger, 1980) and has a characteristic structure of a pre-A segment, eight alpha helices, and five interhelical regions (Tokishita et al., 1997; Kato et al., 2001). So far, 12 (*D. pulex*) to 16 (*D. magna*) subunits are known to aggregate, forming functional macromolecular multimers (Dangott and Terwilliger, 1980; Peeters et al., 1990; Lamkemeyer et al., 2006) that are freely dissolved in the hemolymph (Pirow et al., 2001). Proteomic analyses of *D. pulex* that were acclimated at different temperature and oxygen conditions revealed the differential expression of a set of seven different Hb subunits, each encoded by a separate gene locus (Gerke et al., 2011). Similar results were obtained from studies on *D. magna* (Zeis et al., 2003a,b; Lamkemeyer et al., 2006). As demonstrated by the Gerke et al. (2011), low-oxygen and elevated-temperature acclimation mainly caused similar changes in the Hb subunit composition. Due to the poikilothermic nature of *Daphnia*, the animals' metabolism and consequently its oxygen consumption rate strongly increase at higher temperatures, whereas the solubility of oxygen decreases in warmer water. This mismatch of higher oxygen demand at reduced supply is answered by hemoglobin induction, bridging the gap by increasing the oxygen transport capacity of the hemolymph. Differential subunit expression allows the adjustment of functional quality along with the hemoglobin quantity, increasing the oxygen affinity along with the concentration of the respiratory protein (Zeis et al., 2003a; Gerke et al., 2011).

Although the presence of multiple genes encoding the different Hb subunits provides the basis for alterations in the protein's structural and functional characteristics, the differential gene expression of specific sets of Hb isoforms is regulated via the binding of distinct transcription factors to the promoter regions (Kimura et al., 1999) of the respective *hb* genes. In response to hypoxia, binding of the transcription factor HIF (a protein) to HREs in the upstream regulatory (promoter) regions of genes was shown to have a direct impact on the induction of multiple Hb isoforms in *D. magna* (Gorr et al., 2004). HIF-1 is a heterodimer comprising two bHLH-PAS factors, named HIF-1α and aryl hydrocarbon receptor nuclear translocator (ARNT), which are members of the family of basic helix loop helix-Per/ARNT/Sim (bHLH-PAS) transcription factors (Wang et al., 1995). Stability of HIF-1α is controlled by ambient oxygen conditions, whereas ARNT is a partner of various other bHLH-PAS proteins and is stable regardless of ambient oxygen conditions (Salceda and Caro, 1997). Stability of HIF-1α is regulated by a member of 2-oxoglutarate-dependent dioxygenase superfamily, named HIF prolyl hydroxylase (HIF-PHD, see review in Pouyssegur and Mechta-Gregourio, 2006). A proline residue within the conserved LXXLAP motif (L: leucine, A: alanine, A: any amino acid, P: hydroxylacceptor proline) within the oxygen-dependent degradation domain (ODDD) of HIF-1α is hydroxylated by HIF-PHD in response to increased oxygen concentration in mammals. Hydroxylation of this proline residue results in degradation of HIF-1α mediated by the ubiquitin−proteasome pathway (Jaakkola et al., 2001). HIF-1α and its oxygen-dependent regulation are conserved in various organisms such as

nematodes, insects, fishes, amphibians, and mammals. HIF-1α recognizes *cis*-acting elements called HREs in the regulatory region of target genes; the T/G/CACGTG hexanucleotide is a core sequence of HREs (Wenger et al., 2005). As to crustaceans, cDNAs encoding HIF-1α homologs were isolated from grass shrimp *Palaemonetes pugio*, Dungeness crab *Cancer magister*, and *D. magna* (Li and Brouwer, 2007; Tokishita et al., 2006). These HREs are also found upstream of the *D. pulex hb* genes (Gorr et al., 2004; Colbourne et al., 2011; Gerke et al., 2011), with analog roles for differential isoform expression in response to disturbed oxygen homeostasis. By employing heterologous transfection studies, Gorr et al. (2004) provided evidence that the extent of hypoxia-induced differential gene expression is not only governed by the number of HREs in the intergenic regions (Kimura et al., 1999; Nunes et al., 2005) but may also involve interfering effects of other regulating elements. Thus, the role of HIF binding, the position of binding sites, and possible interactions with other transcription factors still need to be addressed.

It is, therefore, clear that *hb* gene expression is a complex mechanism that includes numerous regulatory components and processes that contribute to a highly fine-tuned cellular response. Besides HIF-1-induced Hb expression, further transcriptional control elements were shown to regulate the Hb concentration in *Daphnia*. Under normoxic conditions, Gorr et al. (2006a) revealed Hb levels in *D. magna* to be predominantly under the regulatory control of methyl farnesoate or related juvenoid hormones, which bind to their respective response elements [i.e., juvenoid response elements (JREs)]. However, Hb transcript and protein levels only poorly correlate in *D. pulex* (Zeis et al., 2013), suggesting that *Daphnia*'s Hbs are also posttranscriptionally regulated. By exploiting the exceptional knowledge available on *Daphnia*'s Hbs, further studies on this multigene family, in particular, will allow the identification of major regulatory networks linked to plastic responses.

The remarkable flexibility of *Daphnia* at responding to altered oxygen availabilities and demands, by adjusting both the quantity and the quality of Hb, is assumed a direct consequence of their exceptional genomic characteristics. Although investigations in the late 1990s (Tokishita et al., 1997; Hebert et al., 1999; Kimura et al., 1999) identified multiple *hb* genes encoding the different Hb subunits in several species of *Daphnia* (i.e., in *D. magna* and *Daphnia exilis*), our understanding has been significantly improved by genome sequencing of *D. pulex* and *D. magna* (Colbourne et al., 2011). The draft *D. pulex* genome sequence assembly and annotation revealed 11 di-domain *hb* genes (*hb1*−*hb11*); eight of these (*hb1*−*hb8*) are found arranged within a tandem gene cluster on chromosome 7 (Colbourne et al., 2011). Gene duplication events are considered important for shaping genomes and are significant sources of evolutionary novelty (for reviews, see Innan and Kondrashov, 2010; Proulx, 2012). *Daphnia*'s di-domain *hb* genes thus provide a vivid illustration of how paralogs can evolve context-dependent functional specifications. Exposing *D. pulex* to eight different environmental conditions and investigating the respective gene expression profiles of all di-domain *hb* genes revealed a significant age-related trend in the functional divergence among the paralogs' recent duplicates. These duplicates are most similar in their gene sequences, generally showed indistinguishable gene expression patterns for the tested conditions, whereas more ancient duplicates within *Daphnia*'s *hb* gene family were more divergent in their expression patterns (Colbourne et al., 2011; Fig. S29). Consequently, duplication followed by functional divergence in the *hb* gene family of *Daphnia* represents an impressive evolutionary mechanism, which enables these animals to express a selective suite of functionally distinct Hb isoforms. These isoforms mediate adequate oxygen transport capacities

to maintain or restore cellular integrity in response to the environmental conditions faced (Tokishita et al., 1997; Zeis et al., 2003a,b; Gerke et al., 2011).

Although many duplicates tend to diverge in sequence and function over time, paralogous genes arranged within tandemly duplicated gene clusters are predisposed to homogenization events by unequal crossing over and unidirectional GC due to their high degree of sequence homology (Hoffmann et al., 2008). In the genus *Daphnia*, GC events are a common feature, and one example of widespread GC is found in the di-domain *hb* genes (Hebert et al., 1999; Sutton and Hebert, 2002; Colbourne et al., 2011). By shuffling nucleotide variation among related genes, these genetic exchanges result in a substantial reduction in divergence among duplicates as their sequence evolution becomes concerted. Multigene families that undergo such a concerted evolution are characterized by a higher than expected sequence similarity among paralogous genes within a species, whereas distinct divergence from the orthologous gene family occurs in other species.

Cladocerans are an attractive target for comparative studies of Hb evolution (Hebert et al., 1999). Several investigations have explored the origin and evolution of duplicated di-domain *hb* genes and the consequences of their structural arrangements in different genera of the Daphniidae (Dewilde et al., 1999; Hebert et al., 1999; Kimura et al., 1999; Sutton and Hebert, 2002; Colbourne et al., 2011), yet virtually nothing is known from other cladoceran families. Comparative analyses of the *hb* gene clusters in two *Daphnia* lineages, *D. pulex* and *D. magna*, revealed nearly identical genomic arrangements within a 23.5-kb interval (Colbourne et al., 2011). However, *D. pulex* possesses an extra *hb* gene (i.e., *hb6*) within the tandem gene cluster, and three additional, nonclustered *hb* genes (i.e., *hb9*—*hb11*) are found in other genomic regions, with *hb9* being located on the antisense strand (Gerke et al., 2011). All clustered *hb* genes, plus *hb9* in *D. pulex*, are composed of seven exons that are separated by six introns. In contrast, *hb10* and *hb11* in *D. pulex* consist of six exons, with the second intron deleted from the ancestral gene structure (Colbourne et al., 2011). Similar intron losses in di-domain *hb* genes have also been reported (Kato et al., 2001) in another cladoceran species, *Moina macrocopa*. Other than the obvious absence of *hb6* from the *D. magna*, cluster, elements in synteny between the two species are seemingly preserved from a duplication history that may predate the split between the *Ctenodaphnia* and *Daphnia* subgenera (Colbourne et al., 2011), including a noncoding RNA gene that interrupts the cluster between *hb4* and *hb5*. Although the *hb* gene clusters in both species are homologs due to ancestral gene duplication events, phylogenetic reconstructions of all di-domain *hb* genes in *D. pulex* and *D. magna* provided evidence that GC tracts have heavily homogenized the protein-coding regions, whereas the intergenic regions of all *hbs* show less divergence between the two *Daphnia* lineages (Colbourne et al., 2011).

In contrast to laboratory studies, investigations into *Daphnia*'s Hb function in natural populations are scarce (but, see Pinkhaus et al., 2007; Schwerin et al., 2010). However, different clones and populations of *Daphnia* have been shown to vary in their ability to synthesize adequate levels of Hb in response to distinct oxygen concentrations in the ambient environment (Carvalho, 1984; Weider and Lampert, 1985; Wiggins and Frappell, 2002; Pinkhaus et al., 2007; Duffy, 2010). Spatial and temporal changes in dissolved oxygen concentration may be an important selective force influencing the clonal (genotypic) composition of natural populations. Investigating Hb variations and tracing the variation of individual globin genes within and among species allows the exploration of regulatory mechanisms that enable animals to adapt to different environmental conditions (see e.g., Pinkhaus et al., 2007; Storz et al., 2007, 2010). As this case study illustrates, organisms can

survive and function under a wide range of conditions in nature owing to a distinct set of adaptive responses. The remarkable plasticity, characterized by differential Hb subunit expression in response to altered oxygen conditions, is not only found in cladocerans but also occurs in other branchiopods such as *Triops* (Notostraca) (e.g., Guadagnoli et al., 2005). As these authors show, Hb subunit expression is not reversed in *Triops* when conditions return to normoxia, which contrasts with hitherto-studied cladocerans. In combination with the differences in Hb domains of the two *Daphnia* subgenera mentioned earlier, this shows that genetic expression mechanisms may differ significantly within the Branchiopoda, and even within the Cladocera, depending on the lineage.

The structural and regulatory polymorphisms of cladoceran *hbs* represent a superb model for studying oxygen-responsive genes and the mechanisms involved in their regulation. Hence, they highlight the relationship between environmental stimuli and downstream functional molecular responses. The detailed knowledge available on Hb structure–function relationships and its key role in oxygen homeostasis consequently promote a deep understanding of the cellular mechanisms that control a divergent and fine-tuned expression of context-dependent suites of molecules under environmental constraints, and ultimately provide genuine insight into the nature of acclimation and adaptation processes.

17.6 HUNTING FOR PHYSIOLOGICALLY RELEVANT GENES AND REGULATORY NETWORKS

17.6.1 Linking Gene-expression Profiles to Physiological Traits and Responses

Genome-wide expression profiling, also known as *functional genomics*, is a powerful tool for understanding how organisms develop, function, and respond to environmental factors. Although posttranscriptional processing of messenger RNA (mRNA) and downstream regulation of protein degradation and translation can modify the effects of gene expression on organism physiology and cell function, gene-expression profiling still provides important information about the genetic control of biological processes and how variation in gene expression affects phenotypic variation among individuals and among taxa (Gibson and Muse, 2009).

In the absence of global gene-expression studies, the expression of candidate genes known or suspected to be involved in the biological process of interest can be assayed by northern blot or quantitative polymerase chain reaction (qPCR) methods. These allow targeted questions about gene function under different environmental conditions, mutational perturbations, or across different taxa (e.g., Scoville and Pfrender, 2010; Schwarzenberger et al., 2012). This has been performed to investigate the expression of candidate innate immune system genes and their role in bacterial resistance in *D. magna* clones (Decaestecker et al., 2011). Differential expression of genes, from which it has been shown that they code for immune effectors in related organisms (McTaggart et al., 2009), was quantified by quantitative reverse transcription PCR (qRT-PCR) following exposure to the bacterial pathogen *Pasteuria ramosa*. Constitutive expression levels differed between host genotypes, and some genes appeared to show correlated expression. However, none of the genes appeared to show a major modification of expression level in response to *Pasteuria* exposure. By applying knowledge from related genetic model organisms (e.g., *Drosophila*) to models for the study of evolutionary ecology and coevolution (i.e., *Daphnia*), the candidate gene approach is temptingly efficient. However, these results showed that detection of only weak patterns is likely if one chooses target genes for

study based on previously identified genome sequences by comparison to homologs from other related organisms.

Assaying genome-wide patterns of gene expression frees researchers from the requirement of a priori genetic knowledge, and allows the discovery of previously unknown components important to physiological functioning. Hierarchical cluster analysis of genome-wide expression patterns reveals the coordination of gene function within pathways and provides insight into the interaction of pathways through regulatory networks. A network perspective makes it possible to infer the functional roles of unannotated genes if they share expression profiles with annotated genes. Principal component analysis of gene-expression data can also reveal underlying similarities and differences in physiological responses to different kinds of perturbations (Gibson and Muse, 2009). Global gene-expression studies often generate hypotheses, identifying genes that can most usefully be subjected to the genetic and biochemical analysis necessary to pinpoint the molecular basis of physiological functioning. Transcriptomics data can be integrated with other omics, such as those generated by comparative phylogenomics, proteomics, epigenomics, and metabolomics, for a systems biology approach that works to understand holistically (1) how organisms develop, function, and respond to their environments and (2) the basis of physiological and phenotypic diversity within and between lineages. These data sets can also yield insights into the emergent properties of gene regulatory networks, thus furthering our understanding of the sorts of gene regulatory network architectures that exist and how different expression patterns have evolved (Scoville and Pfrender, 2010; Latta et al., 2012; Whitehead, 2012).

Work on cladoceran genome-wide gene regulation mostly utilized microarray platforms developed for *D. pulex* or *D. magna*, particularly cDNA or long oligonucleotide microarrays that permit competitive hybridization of fluorescently labeled, amplified cDNA to a set of probes representing expressed genes, predicted genes, and other transcriptionally active genome features. Hybridization of two samples per array (e.g., control vs. treatment, adult vs. neonate) allows a direct comparison of gene expression; many experimental designs also permit indirect comparisons of samples across arrays, e.g., comparisons among different treatments that have each been directly compared to a common reference.

Following the decrease of sequencing costs and the continued development of bioinformatics tools, inferring gene expression from direct sequencing of amplified cDNA fragments via next-generation sequencing (NGS) platforms, or RNA-Seq, has become more attractive. This has the potential to overcome some of the limitations of oligonucleotide microarray platforms, such as incomplete probe sets and a limited ability to capture differential transcription of splice variants and allele-specific transcription. RNA-Seq approaches might allow forgoing the array-optimization step as well, and RNA-Seq data can be used to construct draft assemblies of transcriptomes and/or substantially improve existing gene models (Orsini et al., 2016b).

An early and crucially important step in genome-wide expression studies is developing the experimental design that will best answer the research questions with the limited resources available: which genotypes (e.g., species, populations, mutants, or wild-type individuals), life stages (neonate, juvenile, or reproductive adult), environments (e.g., chemical exposure, chemical concentration, and the duration of exposure, as well as basic culture decisions about light, temperature, and feeding regimes), and whether to collect time-series data as animals undergo physiological changes or to assay organisms in physiological equilibrium. Finally, decisions about replication (technical vs. biological), and, if microarray platforms are used, which samples will be directly compared and which will be compared indirectly, have important implications on the power to detect differential gene

expression (Gibson and Muse, 2009). After exposures, sacrifice of animals, RNA extraction, preparation of labeled cDNA, microarray hybridization, and collection of fluorescence intensity data, the data must be analyzed with appropriate statistical methods to identify those genes with differential expression across comparisons.

Finally, these data must be further analyzed for biological interpretation and visualization of significant genes within pathways and regulatory networks. Functional analysis is based on homology to annotated genes in databases such as the Gene Ontology (GO) database, which assigns each gene to an inferred biological process, molecular function, and cellular component. Other databases infer membership in gene pathways [e.g., the Kyoto Encyclopedia of Genes and Genomes (KEGG)] (reviewed in Tipney et al., 2010). It is worth noting that about 36% of *Daphnia* genes show no homology to other sequenced organisms (Colbourne et al., 2011); these lineage-specific genes are, therefore, excluded from homology-based analyzes. After the categorization of significant genes, a variety of methods exist to test whether sets of significant genes are enriched for GO categories (e.g., Blast2GO), KEGG pathways [Database for Annotation, Visualization and Integrated Discovery (DAVID)], or previously identified gene lists [e.g., Gene Set Enrichment Analysis (GSEA)]. These higher-order analyses allow the biologist to make sense of and visualize the millions of data points that comprise a microarray experiment (Tipney et al., 2010).

Since 2013, ~70 scientific papers have been published about gene expression in *Daphnia*, with a third of them being genome wide. Among them, seven RNA-Seq studies address questions as diverse as the rhythmicity of gene expression (Rund et al., 2016), sex-biased gene expression (Huylmans et al., 2016; Raborn et al., 2016) or the transcriptional response to a combination of factors (Orsini et al., 2016b). Among the most recent important work in functional genomics of cladocerans (see also other sections), Yampolsky et al. (2014) studied the acclimation of *D. pulex* to high temperature, using heat-sensitive and heat-tolerant genotypes exposed to optimal (18°C) and higher temperatures (28°C). Their results showed a strong G×E interaction of a wide number of genes, most pronounced in genes responsible for DNA repair, transcription, and translation, and heat-sensitive clones showing higher plasticity. Rund et al. (2016) focused on the rhythmic physiology in *D. pulex* at the transcriptional genome-wide level and showing that the rhythmic expression of an array of genes (e.g., sensory process and immunity genes) differs from many terrestrial arthropods. Through revealing the rhythmicity of genes in *Daphnia*, the authors illustrate "the power of using a network analysis approach to identify differential gene expression and provide novel functional annotation" (Rund et al., 2016). Such approaches are very important for future annotations, not only in cladocerans but also for other invertebrates containing genes with unknown functions.

17.6.2 Case Studies in Environmental Functional Genomics—Metals and Toxicants

The study of genome-wide responses to environmental conditions is a young but rapidly growing field and many of the early applications involve exposure to environmental stressors and toxicants. Environmental biologists are exploring ways to incorporate the results of genome-wide expression studies in biomonitoring efforts, seeking to identify gene-expression patterns specific to conditions, and using functional genomics to illuminate the physiological mechanisms underlying these responses so that better estimates of individual effects and better predictions about the population and community impacts of exposures can be made. Environmental stressors often do not affect just a single gene or gene pathway, but can instead have cascading effects,

ranging from a highly specific response (e.g., to lowered oxygen concentrations) to general stress responses (e.g., to altered food quality and quantity), while simultaneously involving many aspects of organismal functioning. Therefore, such stressor exposures can provide a suitable model for exploring the intersections of multiple physiological pathways using a systems biology approach (Eads et al., 2008; Shaw et al., 2008). *Daphnia* is often used as a surrogate species to understand the genomic responses to environmental stressors that are important factors in human health and well-being. So far, tolerance limits and regulatory processes that allow organisms (including humans) to cope with pollutants in the short-term span of an individual's lifetime and in the longer time frame of evolutionary change, are still poorly understood. *Daphnia* constitute an excellent model for physiological functional genomics because a long history of environmental and ecological research has provided a wealth of physiological and toxicological information that can be exploited when designing genomics experiments. Its importance as an ecotoxicological model makes it imperative that the research community rapidly and collaboratively use these relatively new genomic approaches to understand *Daphnia*'s baseline physiology and the ways that individuals and populations function in the face of environmental perturbations (Shaw et al., 2008). In recognition of its relevance to understanding basic biological processes and as a sensitive organism for environmental monitoring, *D. pulex* was listed as one of the few selected model organisms for biomedical research at the National Institutes of Health (http://www.nih.gov/science/models/).

David et al. (2011) explored the global transcriptional response to genotoxicants, a study that also compared the sensitivity of gene expression profiling to the commonly used comet assay for DNA damage. *D. magna* neonates and 7-day-old adults were exposed to a mixture of benzo(a)pyrene and sodium dichromate. Gene expression was found to be a more sensitive indicator of genotoxicant effect than the comet assay; even though the comet assay was negative, significant differential gene expression was detected. Gene expression differed in neonate and adult *Daphnia*, and adults showed a stronger transcriptional response to genotoxicants, with three times as many genes showing differential expression (106 genes in adults vs. 34 genes in neonates). Adults had higher constitutive expression of DNA repair and oxidative stress genes than neonates under unexposed conditions, suggesting that adults are better prepared to deal with DNA damage and, perhaps, that adults activate these repair and protective pathways in response to their higher metabolic rates and the associated production of reactive oxygen species.

Exposed adults also showed induction of DNA repair genes, whereas neonates did not. Additionally, in adults oxidative stress genes and *hb* genes were induced, but chitinase and other proteases were downregulated upon exposure. Because these catabolic genes are implicated in molting associated with reproduction, suppressed gene expression is consistent with reproductive deficits and population-level consequences after genotoxicant exposure. Unexposed neonates showed a greater expression of genes involved in polysaccharide binding, pattern binding, and chitin binding than unexposed adults, consistent with greater cuticle formation in developing neonates. Although neonates did not show induction of DNA repair pathways, they did show induction of oxidative stress response genes. This study demonstrated the potential of gene expression-based biomarkers for genotoxicant exposure and showed that gene-expression assays can be more sensitive than the comet assay following nonlethal exposure levels that nevertheless can induce reproductive effects. It also showed that gene-expression profiles can differ dramatically between adult and neonate *Daphnia*. Adults

were more transcriptionally responsive to the compounds tested and may therefore be more appropriate subjects for bioassays than the neonates traditionally used in toxicology testing.

Another functional genomics study explored the effects of ibuprofen on gene expression in *D. magna* (Heckmann et al., 2008a,b). The observed effects of ibuprofen had parallels to its effects on invertebrates, including early disruption of eicosanoid metabolism. In *Daphnia*, there were also effects on the endocrine system, juvenile hormone metabolism, and oogenesis, thus providing strong evidence for a mechanism underlying observed reproductive effects in ibuprofen-exposed daphniids. By comparing the expression profile of ibuprofen exposure to that of exposure to a very different stressor, cadmium (Cd) (Poynton et al., 2007), the authors were also able to identify common transcriptional patterns associated with a general stress response in *D. magna*. These patterns included induction of glycolytic, proteolytic, homeostatic, and heat-shock protein genes, and downregulation of genes involved with energy metabolism and translation. The authors suggested that the ibuprofen-specific transcriptional response could be useful as an early indicator of significant population ibuprofen exposure with risk to *Daphnia* reproductive biology. Moreover, the study is valuable for the identification and evaluation of genes with "housekeeping" properties that can be used as internal standards for gene expression levels (Heckmann et al., 2008).

The *Daphnia* transcriptional response to Cd has also been investigated in *D. pulex*. Shaw et al. (2007) used a first-generation cDNA microarray to identify transcriptional responses in adult animals exposed for 48 h to sublethal concentrations of Cd. The study revealed many Cd-responsive genes associated with molting and metal detoxification, including the metallothionein gene. Importantly, this led to the discovery of additional novel metallothionein genes in the genome, with low homology to any previously known metallothionein genes. This chapter highlights the utility of microarray studies, not only for determining environmental toxicogenomic signatures and exploring physiological pathways involved in stressor response but also for gene discovery. The Cd response in *D. magna* was also studied by combining microarray-based transcriptional profiling with metabolomics data to investigate the role of nutrient uptake, metabolism, and decreased energy reserves in chronic toxicity (Poynton et al., 2011). Mass spectrometry of hemolymph of *Daphnia* exposed for 24 h to sublethal Cd concentrations revealed disruptions in both amino acids and fatty acids, whereas differential gene-expression analysis showed reduced expression of genes encoding digestive enzymes (consistent with a role for impaired nutrient absorption in Cd toxicity), as well as upregulation of genes for cuticle proteins and oxidative stress response. This study focused on the initial phase Cd response but revealed metabolic changes that could lead to decreased fitness during prolonged exposure. Assaying gene transcription and metabolite composition in parallel has allowed researchers to explore the hypothesized roles of gene expression changes and more fully describe the physiological mechanisms of Cd toxicity.

Another study addressed the effects of the algal toxin microcystin on *D. pulex* (Asselman et al., 2012). Microcystins are produced by the harmful cyanobacteria *Microcystis aeruginosa*, an organism associated with toxic algal blooms. Microarray analysis of gene expression supports a characteristic stress response and a role for energy budget disturbances in microcystin toxicity. *Daphnia* fed a diet contaminated with *Microcystis* for 16 days showed differential gene expression of ribosomal genes, oxidative phosphorylation genes, protein export genes, and genes associated with mitochondrial function. Gene-expression patterns suggested that microcystin toxicity may involve upregulation of protein synthesis and energy production pathways. *Microcystis* also affected expression of several paralogous gene clusters, and some paralogous

genes were variably regulated. Taken together, these results suggest physiologically important roles for duplicated genes and indicate that paralogs can assume different functional roles. The authors caution that statistical methods tailored to analyze the overabundance of duplicated genes in *Daphnia* genomes might be necessary to accurately assess functional gene and pathway enrichment in these species. Comparison of single and mixed stressors (Cd and *Microcystis*) using genome-wide transcription profiles have shown responses to be genotype-dependent in *Daphnia* (De Coninck et al., 2014).

17.7 COMPARING PAST TO PRESENT PHYSIOLOGIES

An emerging area of research, with a huge potential for the study of cladoceran physiology, is the field of paleogenetics and paleogenomics. Diapause in the Cladocera allows the study of populations through time and an assessment of historical changes to fitness and genes. Paleogenomics, i.e., the study of extinct genomes, can be directly applied to extinct populations using cladoceran resting stages. The method can be combined with other techniques that have been used to study adaptability of populations through time. For example, the term *resurrection ecology* is widely used (Kerfoot et al., 1999) for the study of reviving specimens from diapausing or dormant stages and comparing them to present populations to examine fitness and gene responses under certain (experimental) conditions. This approach allows an analysis of neutral versus adaptive variation of the genes and an increased understanding of evolutionary processes, particularly those operating on adaptive genetic variation (see Orsini et al., 2013). The ecological significance lies in the fact that microevolutionary changes at the population level can be studied in response to a wide range of human-induced impacts such as climate change, eutrophication, landscape changes, and pollution. As Hairston and De Meester (2008) noted in their review "The animals hatched from decades-old sediments are living organisms with genotypes representative of past populations. Evolutionary changes in plastic characters such as physiology and behavior can thus be reconstructed by comparing the performance of genetic lineages obtained from different sediment layers in a common set of environmental conditions. This approach has successfully been applied for a growing range of traits and environmental changes."

As all branchiopods can produce dormant stages, the genetic responses to environmental change over time can be studied for a wide range of taxa in the form of genetic archives in freshwater lake sediments deposited as egg banks (Brendonck and De Meester, 2003). Yet, despite its wide occurrence over a wide phylogenetic range, nearly all the research on cladoceran resurrection ecology and paleogenetics gravitates around *Daphnia*, in which the dormant embryos are encased in ephippia (as for all Anomopoda, but not for all Cladocera). A few studies exist on other genera, e.g., *Ceriodaphnia*, which is still within the daphniid family (Reinikainen and Åhlén, 2012). The extent of the time dimension in which the genes can be studied through resurrection ecology is determined by the viability of the ephippia, which mostly stretches to decades and rarely to centuries (Frisch et al., 2014). Nevertheless, paleogenomics allows us to examine traits (through genes) even further, to centuries and even millennia (e.g., Mergeay et al., 2007; Morton et al., 2012; Frisch et al., 2014). Besides being ecologically relevant, the importance of such techniques lies in the possibility to examine the genetic adaptivity in plastic responses, the evolution of phenotypic plasticity (physiological and behavioral), and rapid evolutionary changes under strong selection pressures induced by humans or biological interactions (e.g., Decaestecker et al., 2007, 2013; Hairston and De Meester, 2008; Geerts et al., 2015; Roy Chowdury et al., 2015; Orsini

et al., 2016b). Hairston and De Meester (2008) reviewed two case studies on human-induced selection pressures that strongly alter aquatic ecosystems (eutrophication and fish introduction) and elicit rapid genetic responses in *Daphnia*. Such studies provide hard evidence for the evolution of cladoceran physiological traits, as well as the range (plasticity) and limits of acclimation and adaptation (evolutionary constraints).

In Decaestecker et al. (2007, 2013) a unique historical reconstruction of Red Queen dynamics, a key concept in host–parasite interactions, in which the host and the parasite reciprocally adapt with no directional change through time (as the Red Queen in *"Through the looking glass"* of Van Valen: *"Now, here, you see, it takes all the running you can do, to keep in the same place."*) was performed. The Red Queen hypothesis is a crucial concept in evolutionary biology to explain the maintenance of genetic diversity via sexual reproduction. Via cross-infection experiments, *Daphnia* clones were exposed to parasites of different depths isolated out of a sediment core and showed there was continuous short-term adaptation between both antagonists. Integration of host genetic variation in host–parasite coevolution models showed that an increase in host genetic diversity leads to a greater stability of host–parasite coevolutionary dynamics. The accumulation of resistance alleles creates an opportunity for the host to stabilize Red Queen dynamics. It leads to a larger arsenal enhancing the host performance in its coevolution with the parasite in which *"it takes all the running both antagonists can do to keep in the same place."*

Resurrection ecology and paleogenomics provide a powerful tool for understanding the genetic basis of phenotypic plasticity (Orsini et al., 2013; 2016b). Using a combination of these methods, Orsini et al. (2012) examined the genome of *D. magna* in space and time, and correlated the response of genes clusters to three major selection pressures (land use, fish predation, and parasitism) that are known to elicit a rapid (i.e., microevolutionary) genetic and phenotypic response in cladocerans. Such studies illustrate that the genetic background of cladoceran physiology remains a vast terra incognita. "Of the 164 genes identified, only 88 (54%) could be annotated and 86 of these have a putative function based on the homology with other organisms. 109[sic] genes were linked to general stress response; of these, 53 (49%) returned unknown function" (Orsini et al., 2012). The authors found repeated local adaptation by the populations and also identified the mechanisms for rapidly responding to the three selection pressures (through homology) as genes involved in proteolysis, metabolic processes, neuronal development, and transcriptional and translational regulation. This provides hard evidence that even human impact can elicit a strong and rapid genetic response in cladocerans and that genotypes in the industrialized world have gone through this selection and are not representative of the original "wild" genotypes. Similarly, De Meester et al. (2011), Geerts et al. (2015) and Orsini et al. (2016b) have shown that temperature changes can quickly result in genetic adjustments in *Daphnia*, even over very short time spans (months to years), yet with irreversible effects. Human-induced impacts on freshwater ecosystems might (irreversibly) change cladoceran physiology by driving selection. Frisch et al. (2014) were able to hatch live *D. pulicaria* from ephippia derived from a core taken in South Center Lake, Minnesota (US) from as far back as 1418 AD, the oldest resurrected cladoceran to date. The authors compared the genetic structure of populations through time in relation to the lake's eutrophication, using a combination of resurrection ecology, paleogenomics, and historical records to investigate the response of extinct versus extant populations in relation to eutrophication levels (throughout the lake's history). As De Meester et al. (2011) and Geerts et al. (2015) illustrated for temperature, work by Frisch et al. (2014) suggests that

cladocerans can adapt quickly to environmental conditions and that such changes can be irreversible. Loci with a signature of directional selection were related to historic phosphorus levels in the lake and an adaptive shift occurred to higher phosphorus concentrations. Resurrected clones from the 15th century grew faster under low-phosphorus conditions and showed higher phosphorus retention when compared to the 21st century population. Therefore, after agricultural expansion around the lake (around the 1920s), *D. pulicaria* lost the physiological ability to retain phosphorus at low levels due to clonal selection and genetic erosion, in contrast to the "extinct" populations from before eutrophication. This shows that over the course of a few hundred years (which is a very short geological time, considering the age of the lineage), a cladoceran species can undergo significant and rapid genetic shifts that alter its tolerance to the environment. Other important work on functional genomics using past versus present genotypes related to phosphorus supply in *Daphnia* has been carried out by Roy Chowdury et al. (2015) and on the comparison of transcriptomes of contemporary genotypes, assessing phenotypic variation (Roy Chowdury et al., 2014).

The key to using paleogenomics to its full potential lies in identification of the genes that determine ecologically relevant trait variation (Miner et al., 2012). Identifying the large number of unknown genes in cladocerans (*Daphnia*; Colbourne et al., 2011) is a task for the future, and would help to identify the important genes involved in determining plasticity. Other issues also remain challenging, such as the extraction and sequencing of ancient DNA from ephippia, and the use of homologies to identify unknown genes and gene functions.

Furthermore, all resurrection ecology and paleogenomics is currently focused on Daphniidae and therefore on the planktonic filter feeders in the aquatic ecosystem. No studies have yet been carried out on benthic/littoral (e.g., Chydoridae) or predatory cladocerans (e.g., Leptodoridae). Well studied in paleolimnology and better preserved in the sedimentary record (e.g., Frey, 1962), benthic/littoral chydorids have a particular potential for paleogenomics, yet the level of genetic knowledge is nowhere near that of daphniids. In time, our knowledge in all of the aforementioned fields will undoubtedly expand (vide infra). For further reading on paleogenomics and resurrection ecology, we refer the reader to Brendonck and De Meester (2003), Hairston and De Meester (2008), and Orsini et al. (2012, 2013, 2016b).

17.8 FUTURE DIRECTIONS: EXPLORING PHYSIOLOGICAL VARIATION WITH FUNCTIONAL GENOMICS

To understand physiology in an evolutionary and ecological context, the similarities and differences among individuals within and among populations and among species must be understood and exploited. Physiological responses can vary, but little is known about the mechanisms underlying variation or the evolutionary forces generating, maintaining, or constraining such variation. Comparative approaches to identify apparently (i.e., phenotypically) universal physiologies and those that vary among populations and species will be facilitated by the further development of cladocerans as models for physiological genomic research. Importantly, working in species with well-characterized ecologies will allow us to ask how physiologies and genetic mechanisms vary with environmental factors. Comparative functional genomics can test whether or not similar physiologies have been achieved by regulating the same genetic pathways in the same way or through the independent evolution of substantially different mechanisms, and can help to unravel the basis of qualitative and quantitative differences in physiological responses (Whitehead, 2012).

Ultimately, researchers will want to know the genetic and genomic differences responsible for

the physiological variation and associated gene expression variation that we observe. Gaining this knowledge will require the incorporation of evolutionary genetics and genomics [e.g., quantitative trait locus (QTL) mapping, expression-level QTL (eQTL) mapping, genome-wide association studies, and comparative genomics] and molecular genetics [e.g., RNA interference (RNAi)]. Other molecular and bioinformatics methods can identify variation in regulatory elements associated with gene regulation (e.g., experimental identification of transcription factor-binding sites and sites of epigenetic modification, and computational searches for conserved regulatory motifs and their variation within and among populations). The integration of physiology and functional genomics with EEG analysis has the potential to enrich many aspects of our understanding of *Daphnia*.

As outlined in this chapter, the field of (physiological) genomics is rapidly advancing and Cladocera are on the frontline. Studies on *Daphnia* genomics facilitate the identification of ecologically relevant genes that enable context-dependent responses. By linking different research disciplines, such as evolutionary ecology, physiology, molecular biology, and genomics, current research on *Daphnia* aims to increase our understanding of organisms as integrated units of biological organization shaped by the context of the ecological and the history of evolution. Studying adaptive traits using such a systems-level approach will ultimately provide a radically new perspective on the nature of genetic constraints and the evolution of adaptation and speciation. Consequently, unraveling key adaptive responses in *Daphnia* will positively trigger a cascade of subsequent studies (in *Daphnia* and other organisms) that may have a great impact on forecasting the fate of keystone species, and therefore ecosystem stability, under growing environmental challenges. But where do we go from here? There can be no doubt that our knowledge of the genetic mechanisms of cladoceran physiology has rapidly increased since the late 20th century. Because the first cladoceran genome has become available (Colbourne et al., 2011) and more are emerging, we are on the brink of a new era. Because of their ecological and genetic characteristics, cladocerans can play an important role in exploring relevant biological questions. Containing model organisms with well-studied ecologies and a major role in ecosystems, future study of the Cladocera has the potential to "revolutionize genomic research and enable[s] us to focus on a number of outstanding questions" (De Meester et al., 2011). For example, by approaching cladoceran physiology and biology from a functional genomics perspective, we can advance our understanding of gene-evolution processes and the genetic basis of phenotypic plasticity. Research on cladoceran genomics is also relevant in the context of biodiversity and conservation because of the presence of human-induced, irreversible genetic changes in natural populations. As cladocerans play a pivotal ecological role, their system functioning and fitness are important indicators for freshwater ecosystem health (e.g., Rapport et al., 1998). Therefore, genetic methods combined with physiology provide a powerful tool for monitoring (and perhaps forecasting) ecosystem stability.

17.8.1 Trends and Suggestions for Future Research

The application of omics in physiological research may only be starting in crustaceans (Stillman and Hurt, 2015), yet Cladocera are at the forefront. The future looks promising.

1. New state-of-the-art high-throughput techniques such as NGS and RNAi have now become more accessible. Promising new methods include advances in bioinformatics, which allow the manipulation of large data sets, such as gene-expression profiles. RNAi is a powerful tool to investigate gene function by knocking down distinct target genes. In

fact, RNAi has been developed for cladocerans (i.e., the *Dll* gene in *D. magna*) (Kato et al., 2011), and injection (Hiruta et al., 2013) as well as ingestion methods (Schumpert et al., 2015) have been successfully used to knock down genes of interest. Nakanishi et al. (2014) even achieved targeted mutagenesis of a functionally conserved regulator of eye development, *eyeless*, with the CRISPR/Cas method, in *D. magna.* This technique holds great promises to test the inferences made with genome-wide gene expression analysis. Nakanishi et al. (2016) also tested TALEN-mediated knock-in using the same gene (eyeless) producing transgenic *D. magna*, with low success rate (∼3%) (see also Hiruta et al., 2014a,b; Naitou et al., 2015). This important start of genome engineering in *Daphnia* will allow a better understanding of the function(s) of genes by analyzing the mutations produced.

2. Cladocera are promising model systems for *epigenetics* (*D. magna*; Vandegehuchte et al., 2010a,b; Gómez et al., 2015; Harris et al., 2012; Robichaud et al., 2012; Asselman et al., 2017). Sex determination and sexual reproduction in cladocerans may be epigenetically controlled and DNA methylation patterns are responsive to toxicants and heavy metals (Harris et al., 2012). For example, Asselman et al. (2015) studied global cytosine methylation levels in two different genotypes of *D. magna* after exposure to a wide array of biotic and abiotic environmental stressors. The authors observed a significant genotype effect, an environment effect, and a G×E effect. In particular, global cytosine methylation levels were significantly altered after exposure to *Triops* predation cues, *Microcystis*, and sodium chloride compared with control conditions. Significant differences between the two genotypes were observed when animals were exposed to *Triops* predation cues, *Microcystis*, *Cryptomonas*, and sodium chloride. Despite the low global methylation rate under control conditions (0.49−0.52%), global cytosine methylation levels upon exposure to *Triops* demonstrated a five-fold difference between the genotypes (0.21 vs. 1.02%). The authors' results point to the potential role of epigenetic effects under changing environmental conditions such as predation (i.e., *Triops*), diet (i.e., *Cryptomonas and Microcystis*), and salinity and indicate that, despite global cytosine methylation levels being low, epigenetic effects may be important in environmental studies on *Daphnia.* In addition, bisulfite sequencing (a genome-wide methylation approach) of exposed and control *D. magna* individuals highlighted differential methylation patterns in *Daphnia* upon exposure to *Microcystis* primarily in exonic regions. These patterns are enriched for serine/threonine amino acid codons and genes related to protein synthesis, transport, and degradation. Genes with differential methylation corresponded well with genes susceptible to alternative splicing in response to *Microcystis* stress. Overall, these results suggest a complex mechanistic response in *Daphnia* characterized by interactions between DNA methylation and gene regulation mechanisms. These results underscore that DNA methylation is modulated by environmental stress and can be an integral part of the toxicity response in *Daphnia* (Asselman et al., 2017).
3. In other omics (besides genomics), Cladocera research is catching up. Examining protein expression profiles under different environmental conditions falls under the area of *proteomics*, and the *D. pulex* genome provides a major step forward in this area of research as well (Fröhlich et al., 2009; Schwerin et al., 2009; Zeis et al., 2009), for example in studying the mechanisms behind

predator-induced phenotypic plasticity (Otte et al., 2014, 2015). The same can be said for *metabolomics*, which examines cladoceran metabolites in response to different environmental conditions or stressors (e.g., toxins; Taylor et al., 2010). Complete metabolomes of model organisms such as *Daphnia* are considered (Edison et al., 2016) and important for physiological research as the metabolites provide a link between the genome and the environment. For paleogenomics, new techniques are expected to increase the molecular resolution of ancient DNA samples (see Section 17.7).

4. A major challenge for the future is the characterization and exploration of function (and structure) of the c.13,000 (forming approximately 36% of the genome) unknown "*Daphnia*-specific" protein-coding genes (Colbourne et al., 2011). However, as more crustacean genomes have been sequenced (*Eurytemora affinis*, *Hyalella azteca*) within the i5K initiative (Poelchau et al., 2015), and others appear recently (*P. hawaiiensis*; Kao et al., 2016) or are in the making (including other Branchiopoda such as *Artemia franciscana*; De Vos et al., 2013), *comparative genomics* studies will allow elucidating the evolution and the function of these genes. Furthermore, as of January 2017, draft transcriptome assemblies for no less than 131 crustacean species are publicly available on the European Nucleotide Archive (http://www.ebi.ac.uk/ena). This as well will allow finding out whether the *Daphnia* orphan genes are truly unique to the Cladocera lineage or rather frequent within Crustacea. The extensive understanding of phylogenetic diversity, genomics, physiology, and ecology in *Daphnia* is unrivaled among the cladocerans, unfortunately currently at the expense of research in other taxa. Until now, nearly all the genomic research has focused on *Daphnia*, leaving the rest of the Cladocera untouched. In many of these groups, a strong basis of ecological research is lacking, yet other cladoceran lineages will undoubtedly reveal a vast amount of ecological, genetic, and physiological variation, as they take up equally important yet different roles in aquatic ecosystems. Examples are the predatory haplopods and the benthic/littoral chydorids (the most speciose group in the Cladocera; Forró et al., 2008). A revised fossil record of the cladocerans in the context of the evolution of freshwater ecosystems may serve as a time frame for the evolution of the genes and gene families of the Cladocera within the Branchiopoda (Van Damme and Kotov, 2016). Functional genomics studies such as Rund et al. (2016) will also further help the annotation of unknown genes, using *Daphnia* as an important model among arthropods.

In conclusion, the majority of physiological studies in cladocerans have hitherto focused on species acclimation, acclimatization, and adaptation processes in response to (natural or human-induced) environmental stimuli. To understand the mechanistic basis of physiological responses (which include phenotypic plasticity), holistic approaches are needed. This integration can only be achieved by analyzing physiological traits in a functional genomics context, and *Daphnia* is an ideal model to do so.

Glossary Terms

Acclimation Phenotypic change in an individual organism under laboratory conditions as a physiological response to one defined ambient factor.

Acclimatization Phenotypic change in an individual organism as a physiological response to its natural environment, responding to multiple factors.

Adaptation Phenotypic change in a population due to natural selection favoring alleles that confer increased fitness.

Allele Any variant of a gene at a particular locus.

Annotation (of genes/genomes) The process of assigning biological information, such as the identity and function of genes and other genetic elements, to positions in a genome sequence. See also *functional genomics*.

Cas CRISPR-associated endonuclease. See also *CRISPR*.

Complementary DNA (cDNA) DNA synthesized from an mRNA template by reverse transcription of RNA to DNA followed by DNA polymerization.

cDNA library The complete mRNA collection of an organism or sample, which is stored in a host (e.g., bacterial plasmid, yeast, and bacterial artificial chromosomes) as cDNA. See also *transcriptomics*.

CRISPR Clustered Regularly Interspaced Short Palindromic Repeats. See also *cas*.

Enrichment analysis Statistical test of whether a set of genes (e.g., a set of differentially expressed genes) contains more members of a genetic pathway or gene class than would be expected by chance.

Epigenetics Modification to the genome produced through environmental effects (e.g., DNA methylation) that in some cases comprise inheritable factors.

eQTL mapping A quantitative trait loci mapping study for which the continuous variable of interest is the expression level of one or more genes; eQTLs are regions in the genome (loci) that regulate the expression of other areas in the genome. See also *QTL mapping*, *gene expression*.

Expression profiles See *gene expression*.

Functional genomics The study of genomes through the characterization of the effect of sequences on the phenotype at any level (molecular, cellular, tissue, and whole organism). See also *transcriptomics*.

Gene cluster A set of genes located in the same genomic region. See also *synteny*.

Gene conversion (GC) Replacement of one allele by another through nonhomologous recombination.

Gene duplication The duplication of a gene, resulting in the presence of two or more copies in the same genome. It can be caused by errors in replication, by recombination, or by retrotransposons. The *Daphnia* genome is characterized by a high number of gene duplications, resulting in many paralogs of the same gene, as in the *hb* gene cluster for example. See also *paralogs* and *tandemly repeated genes*.

Gene–environment interaction Environmental effects on genetic function that contribute to phenotype.

Gene expression The conversion of the information encoded in genes (as a DNA sequence) into RNA. Examination of gene expression patterns and profiles relates to the study of genes that are differentially regulated and transcribed under certain conditions of interest (e.g., metallothioneins under metal pollution stress in *Daphnia*).

Genetic linkage The probability of two genes being inherited together in a nonrandom fashion due to their physical proximity on a chromosome or patterns of linkage disequilibrium.

Genome All inheritable genetic material that is present in an organism (i.e., DNA).

Genome assembly The process of uniting individual DNA sequences generated by a sequencing project into long (ideally chromosome length) DNA sequences.

Genomics The study of the structure and function of genomes.

Genotoxicants DNA-damaging mutagenic agents (e.g., UV rays and chemicals).

Heterologous transfection Insertion of an extraneous DNA fragment into a genome. Often used to study gene expression and the functional consequences of genetic variants in a nonnative genetic background.

Hypoxia-inducible factor 1 (HIF-1) Oxygen-responsive heterodimeric transcription factor that mediates cellular responses to tissue hypoxia by the regulation of distinct gene-expression events.

Homolog Identity by descent; homologous genes have the same origin but have evolved independently in different lineages through time. See also *paralog*, *ortholog*.

Hypoxia-responsive element (HRE) Located in the regulatory region (i.e., promoter region) of hypoxia-responsive genes; binding to transcription factors such as HIF-1 induces gene expression of distinct target genes.

Intergenic Regions of the genome that fall outside protein-coding regions.

Isoforms Variants of a protein expressed by the same gene or closely related genes. Can be the result of alternative splicing.

Metabolomics The study of all metabolites in an organism and the chemical reactions they are involved in (structures, pathways, etc.).

Microarray Technique for the quantification of whole-genome transcription levels. All nucleic acids extracted from a sample are labeled with fluorescent dyes and hybridized to a microarray chip. The targets for binding on the chip are short oligonucleotides matching unique regions of the genome. Fluorescence levels are proportional to RNA abundance and gene expression.

Messenger RNA (mRNA) Molecules that convey the genetic sequence of an actively transcribed gene from the nucleus to the ribosome, in which it specifies the sequence of amino acids to be synthesized into a protein.

Model organism An organism that is amenable to scientific investigation and of which the biology can provide insights on other species. *Daphnia* can reproduce clonally, is easy to culture, and is a model organism for the study of genotype–environment interactions, epigenetics, phenotypic plasticity, and ecological adaptation.

Next-generation sequencing (NGS) A variety of methods of high-throughput, low-cost sequencing, which works by generating thousands of short sequencing reactions in parallel. These methods produce a high number of short (length depends on the platform, e.g., 50–400 bp) sequences (or "reads").

Ortholog (or orthologous gene) Genes that share a common ancestor and are present in different species (not in the same genome). See also *homolog*, *paralog*.

Paleogenomics The study of ancient/extinct genomes. In the case of cladocerans, paleogenomics is carried out on embryos from ephippia that are stored in lake sediments.

Paralog (or paralogous gene) Genes that share a common ancestor and are present in the same genome. The result of gene duplication events. See also *gene duplication*, *homolog*, *ortholog*.

Phenotypic plasticity Condition-dependent development that allows an organism to respond to environmental change by altering its phenotype.

Phylogeny A hypothesis about the evolutionary relationships among species or populations.

Polygenic trait (quantitative trait) A trait that is affected by several to many genes.

Proteomics The study of the complete set of proteins present in a given sample (cell, tissue, organism).

Quantitative polymerase chain reaction (qPCR) Quantitative or real-time PCR is a technique that allows the quantification of the abundance of selected DNA sequences in a sample. Can be used on cDNA to quantify gene-expression levels and on genomic DNA to measure gene copy number variations.

QTL mapping Quantitative trait loci mapping, a statistical association of genetic polymorphisms (markers) in a genome and variation of a quantitative phenotypic trait. It allows quantification of the relative genetic contribution of each locus to the total observed variation in the trait. See *eQTL*.

Quantitative genetics The study of the effect of genetic variation on quantitative or continuous traits (e.g., body size). See also *QTL mapping*.

Regulatory elements DNA elements that regulate or modify the expression of distinct genes or gene expression events. Includes transcription factors, promotors, and transcription factor binding sites.

Resurrection ecology The study of the ecology of populations or species revived from the past by breaking dormancy (e.g., resurrection ecology of *Daphnia* by hatching populations from ephippia that are stored in lake sediments). See *paleogenomics*.

RNA interference (RNAi) RNA-directed posttranscriptional mechanism for gene downregulation. Short (approximately 20-nt long) RNA fragments guide a multiprotein complex toward specific transcripts, causing their degradation.

Synteny Conservation of the position of genes along the chromosome in different lineages of organisms. For example, in multiple *Daphnia* species, genes in the *hb* gene cluster are arranged in the same order. See *gene cluster*.

Tandem gene cluster Gene cluster generated by a series of tandem duplications. For example the *hb* genes in *Daphnia*. See *tandemly repeated genes*, *gene cluster*.

Tandemly repeated genes Duplicated genes located in adjacent positions in the genome. Typically the result of small local duplications. See also *gene duplication*.

Transcriptionally active regions Portions of the genome that show evidence of being transcribed under certain conditions. Can be associated with genes in the genome or with transcription of noncoding sequences (e.g., regions encoding short and long RNAs elements).

Transcriptomics The study of gene expression through the measurement of all RNA species [e.g., mRNA, small interfering RNA (siRNA), and microRNA, i.e., the transcriptome] in a given sample. See also *microarray* and *RNA sequencing*.

CHAPTER

18

Notes on the Physiology of Embryogenesis

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18.1 SHORT HISTORY

Investigation of the embryonic development of Cladocera began in the 19th century and first half of the 20th century (Grobben, 1879; Lebedinsky, 1891; Samassa, 1893a,b; Kühn, 1913; Kühnemund, 1929; Baldass, 1937, 1941). Most investigations dealt mainly with early embryogenesis, which in the Crustacea results in the formation of a germ band (Ivanova-Kazas, 1979). Then interest in such studies was lost until the end of the 20th century. Therefore limited progress only was made in the study of some aspects of embryonic physiology in the Cladocera since that time, although some valuable observations were made for other aspects (Hoshi, 1950a,b; Fox, 1948; Green, 1956a, 1965).

However, a new period of embryological study has started. Two main directions of such studies are drawn: (1) investigations of morphological changes during late embryogenesis with the aim of elucidating phylogenetic relationships between different cladocerans and different "large" branchiopods using in vitro observations (Kotov and Boikova, 1998, 2001; Boikova, 2008) and/or the scanning electron microscope study of separate stages (Olesen, 2003; Olesen et al., 2003), and (2) investigations of earlier embryogenesis and segmentation using sophisticated modern approaches of molecular genetics, evo-devo methods, etc. (Sagawa et al., 2005; Kato et al., 2008, 2010, 2011a,b; Alwes and Scholtz, 2014). The main contemporary progress in understanding cladoceran physiology is related to the latter approach.

At the same time, during many years of cladoceran studies, different authors published many papers with systematic (Hoshi, 1950a,b; Fox, 1948; Green, 1956a, 1965) or occasional observations of miscellaneous aspects of embryology, including embryophysiology, which need to be systematized. Observations made by toxicologists of many chemicals stopping or influencing embryogenesis were especially numerous (Abe et al., 2001; Kast-Hutcheson et al., 2001; Sobral et al., 2001), but sometimes it is very difficult to systematize and summarize such data.

18.2 GENERAL INFORMATION AND STAGING

Resting eggs of the cladocerans are developed in the environment, being laid by the female at the gastrulation stage. In contrast, embryonic development in the case of a parthenogenetic female always takes place in the mother's brood

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pouch (Claus, 1876, 1877; Smirnov, 1975; Dumont and Negrea, 2002), a chamber that is surrounded by two valves of the carapace from the dorsal and posterodorsal side and ventrally by the dorsal face of the postcephalic body (the latter is located within the valves) (Fig. 18.1). In the majority of cladocerans the brood pouch is opened from the posteroventral side to the environment; the brood pouch is only an enclosure for the developing embryos and has no feeding support from the mother (Ramułt, 1925; Rammner, 1933). In such cladocerans, epibiotic bacteria could live on the egg membrane (Kotov, 1997b). In contrast, in Polyphemidae, Podonidae, Cercopagidae (order Onychopoda), and Moinidae (order Anomopoda) the brood pouch is closed, and the embryos have feeding support from the mother through its placenta or marsupium. Usually, eggs of the cladocerans with an opened brood pouch are filled by yolk, while taxa with a closed brood pouch have minimal yolk content (Rivier, 1998).

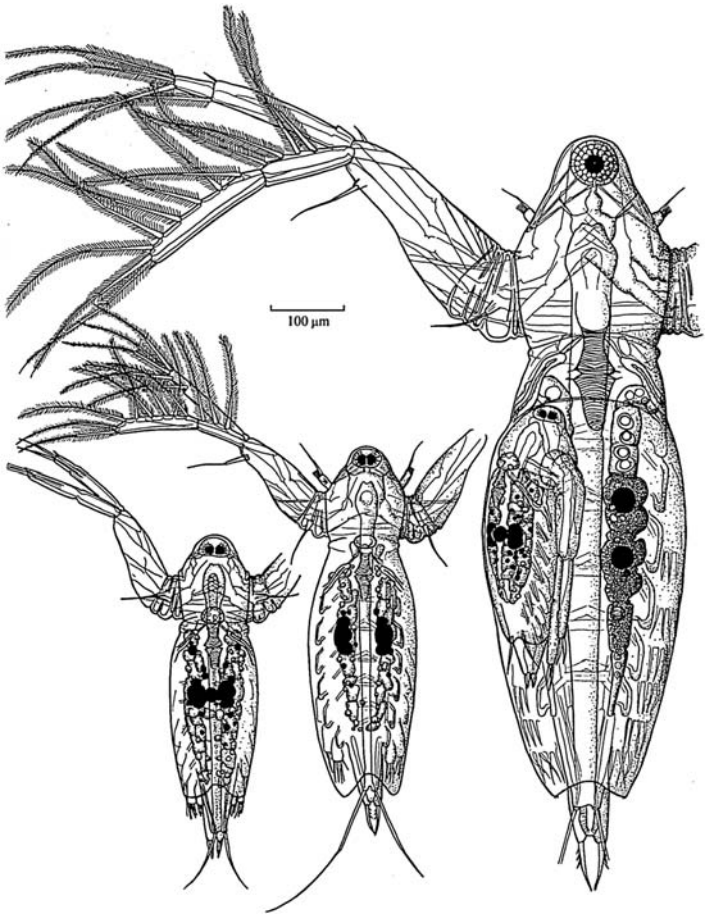

FIGURE 18.1 *Diaphanosoma brachyurum* late embryo released from the brood pouch (*left*), after molting shortly after release (*middle*), and an adult female (*right*) with a single late embryo in the brood pouch and forming an egg of the next brood in the ovary—see the accumulating yolk granules and oil drops. *After Kotov, A.A., Boikova, O.S., 1998. Comparative analysis of the late embryogenesis of* Sida crystallina *(O. F. Müller, 1776) and* Diaphanosoma brachyurum *(Lievin, 1848) (Crustacea: Branchiopoda: Ctenopoda). Hydrobiologia 380, 103–125.*

It is important that the first stage of embryogenesis—unsegmented egg—is a unicellular structure. This means that its life follows the main cytological rules. But in the course of embryonic development we observe differentiation of a multicellular organism whose physiology is regulated somewhat (or absolutely) by other laws. Cells in each system of organs are differentiated, and their functioning is quite different from each other. We need to describe both changes in some groups of cells and formation of specific tissues with their own functions in the course of embryogenesis.

We do not describe the processes of spermatogenesis, oogenesis, and egg laying; see Weismann (1876–1879), Makrushin (1966), Zaffagnini and Sabelli (1972), Smirnov (1975), Zaffagnini (1987), and Dumont and Negrea (2002). The egg mass enters the brood pouch of a cladoceran like "toothpaste from a tube" (Green, 1956a). Each egg is initially sausage shaped, and only after about half an hour it reaches its final elliptical shape.

The embryonic development of *Sida*, *Diaphanosoma* (Ctenopoda: Sididae), *Daphnia* (Anomopoda: Daphniidae), and *Leptododa* (Haplopoda: Leptodoridae) has been investigated by observing living embryos removed from the female brood pouches (Kotov and Boikova, 1998, 2001; Boikova, 2008). During embryogenesis four membranes are cast off. The growth in length of embryos began only after the shedding of the second membrane (=external egg envelope). An outline of the cladoceran embryogenesis proposed by Kotov and Boikova (1998) includes four stages (=instars) demarcated by the shedding of membranes (=molts), as is commonly accepted for juvenile and mature animals in any crustacean (and other arthropod) group. Four instars can be found in the course of the development of the Ctenopoda embryo in the mother brood pouch: two of them are passed within the egg membranes, and the two next instars occur after hatching from egg membranes within the mother's brood pouch

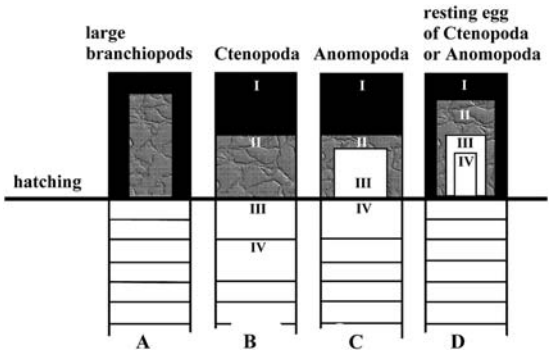

FIGURE 18.2 Comparative instar analysis of the development of Anomopoda, Ctenopoda, and large Branchiopoda. Instars are marked by *Roman numbers*. *After Kotov, A.A., Boikova, O.S., 2001. Study of the late embryogenesis of* Daphnia *(Anomopoda, 'Cladocera', Branchiopoda) and a comparison of development in Anomopoda and Ctenopoda. Hydrobiologia 442, 127–143.*

(Fig. 18.2). In reality, these two last stages can be regarded as already belonging to postembryonic (larval) development (Boikova, 2012).

The sequence of different morphological changes is basically similar in Anomopoda and Ctenopoda, but the sequence of shedding of the embryonic membranes is somewhat different. The ctenopod embryo hatching from the second egg membrane is covered by the third membrane, which will be cast some hours later. The anomopod embryo hatches from the second egg membrane approximately simultaneously with the shedding of the third membrane (Fig. 18.2), and is covered already by the fourth membrane after the shedding of the second egg membrane. Therefore in anomopods the third instar occurs within the second egg membrane; one is incorporated into the egg. Thus the development of the Anomopoda is shortened in comparison with the Ctenopoda.

Boikova (2008) conducted a study of *Leptodora* development and found that "development of *Leptodora* is more like that of Ctenopoda than of Anomopoda." Kotov et al. (2013) found that in *Dunhevedia* (Anomopoda: Chydoridae), in comparison with other cladocerans, the relative

durations of the embryonic instars I and II + III are markedly short, while that of instar IV is long, and the size of the embryo increases quicker during embryogenesis. This could be the result of a family-specific evolution of embryogenesis.

Subsequently, a number of new schemes of embryogenesis periodization were suggested. Mittmann et al. (2014) proposed a staging scheme of *Daphnia* embryogenesis based on observation of morphological events using modern evo-devo techniques; Boikova (2012) subdivided the whole period of embryogenesis into four stages based also on morphological events. Such schemes are attractive for morphological analysis, but have all the disadvantages of "event staging": they are based on the tracing of morphological changes, which is not fully objective because the value of different morphological events for staging is a priori or a posteriori elucidated by the investigator according to his or her subjective ideas on such values.

Toyota et al. (2016) presented photos of each second hour of male and female embryo development in *Daphnia pulex* and *Daphnia magna*. Basically, stages of male and female development are morphologically the same.

18.3 EARLIER DEVELOPMENT: CLEAVAGE, BLASTULATION, START OF SEGMENTATION, AND START OF NEUROGENESIS

Cleavage of the parthenogenetic egg starts several hours after the egg is deposited into the brood pouch. Among the cladocerans, different types of cleavage are described: holoblastic (full), superficial, and mixed (Anderson, 1973; Ivanova-Kazas, 1979; Alwes and Scholtz, 2014). The cleavage type of a cladoceran genus is not correlated with its phylogenetic position, different types of cleavage could be found within the same order: cleavage is superficial in *Leptodora* (Haplopoda) and *Simocephalus* (Anomopoda), holoblastic or mixed—in the majority of the cladocerans of the orders Anomopoda, Ctenopoda, and Haplopoda (Kühn, 1913; Kühnemund, 1929; Cannon, 1921; Baldass, 1937, 1941). Alwes and Scholtz (2014) concluded that the common ancestor of at least three orders of the Cladocera (Anomopoda, Ctenopoda, and Onychopoda) has: (1) the first two cleavages approximately equal and meridional; (2) among four blastomeres appearing after the second cleavage, one that is smaller and division delayed during subsequent divisions—a precursor of the germ line; and (3) a clonal pattern where the cells derived from nonsister cells at the four-cell stage preserve a shared contact zone.

In *Bythotrephes* and *Polyphemus* the cleavage is mixed; it starts as superficial, but is then (since the fourth to fifth cleavage) shifted to a complete one. The first two cleavages are meridional and equal, followed by the equatorial and unequal third cleavage, which results in four micro- and four macromeres (Kühn, 1913; Alwes and Scholtz, 2014) (Fig. 18.3). In *Leptodora* with superficial cleavage the first to seventh divisions are synchronous, then different cells are divided into different rhythms: at the four-cell stage, four energids migrate to the surface; up to the fifth division no cell membranes are observable, but at the sixth division the membranes separate energids from each other and from the yolk; at the 64-cell and 128-cell stage a blastoderm on the surface of the egg begins to form. In *Daphnia* and *Holopedium* the cells are separated at 32-cell and 64-cell stages; they also fuse into a blastoderm (Baldass, 1937, 1941). From the eight division, all cells are separated into (1) prospective ectoderm, continuing superficial divisions, (2) prospective yolk cell, stopping their division, and (3) prospective mesoderm cells dividing perpendicularly to surface.

Gastrulation is twophased: the mesoderm is proliferated first, followed by the endoderm (Baldass, 1937, 1941). In *Leptodora*, gastrulation

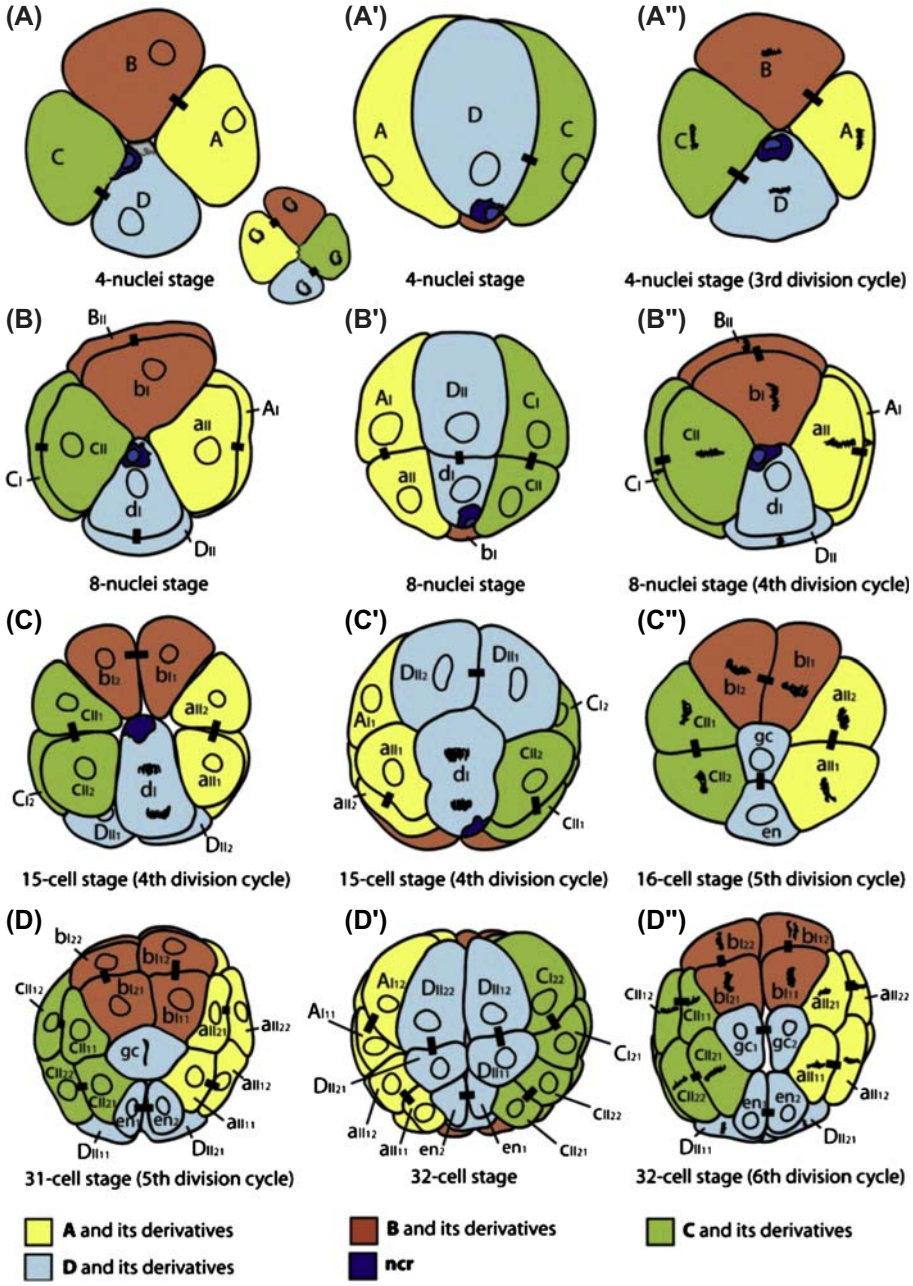

FIGURE 18.3 Early division pattern of *Bythotrephes longimanus* until the 32-cell stage. *Lines* represent the cleavage furrows as they appear at the surface and do not penetrate the complete egg until the fourth cleavage. *Short lines* mark the sister cell relationship according to the previous division. The four colors represent the quadrants of the four-nuclei stage, with A—yellow, B—red, C—green, D—blue. Left column (A–D): View of the vegetal pole (except in A in which the view of the animal pole is added). Middle column (A′–D′): View of the D derivatives, vegetal pole faces down. Right column (A″–D″): View of the vegetal pole during the division cycle. *After Alwes, F., Scholtz, G., 2014. The early development of the onychopod cladoceran* Bythotrephes longimanus *(Crustacea, Branchiopoda). Frontiers in Zoology 11 (10).* http://www.frontiersinzoology.com/content/11/1/10.

takes place as a proliferation of the ectoderm through the mesoderm and yolk cells (Gerberding, 1997; Mittmann et al., 2014), and the germ band is formed on the dorsal side of the embryo. Its subsequent elongation happens without differentiation and segmentation, which could be a result of cell divisions or cell divisions plus migration. Simultaneously, special large cells appear in the center of the gastrulation zone, which correspond to the germ line cells. A paired structure ("Scheitelplatte," a term used by Kühnemund, 1929) appears anterior to the gastrulation zone; these structures together with surrounding ectoderm cells form the brain and the mandibular neuromere (Mittmann et al., 2014).

According to the mistaken opinion of Gerberding (1997), segmentation starts in *Leptododa* with simultaneous differentiation of segments of head and thoracic appendages; only thoracic segments V and VI appear somewhat later. Subsequently, Boikova (2008) demonstrated by in vitro observations that the segments are differentiated in *Leptodora* one after another from anterior to posterior end of the embryo in different anomopods and ctenopods (Kotov and Boikova, 1998, 2001; Mittmann et al., 2014).

The neuroectoderm differentiates as a group of large ectoderm cells located along the median axis. Proliferation of the neuroectoderm results in production of several layers of neuronal daughter cells. After several divisions of all neuroblasts, the ganglion primordia begin to form (Gerberding, 1997).

As a result of the centrifuging of *Daphnia* eggs before cleavage, the content of an egg is rearranged according to its density, which results in very strong alteration of cell division axes, etc., but then some regulatory mechanisms switch on, and the embryos complete their development, although they have certain abnormalities (Kaudewitz, 1950).

18.4 *HOX* GENES AND EXPRESSION OF OTHER GENES

The *Hox* genes are regulators of fundamental development processes in all Metazoa (Abzhanov and Kaufman, 2000; Deutsch and Mouchel-Vielh, 2003; Heffer et al., 2010). The ancestral arthropod had 10 such genes. All these genes are present in *Daphnia* and located on a single genomic scaffold, in contrast to other arthropods (Pace et al., 2016). The *Hox* genes of *Daphnia* are arranged in the same transcriptional orientation as in the majority of arthropods (Fig. 18.4). At the same time, the *Hox* gene cluster of *D. pulex* is the smallest among all arthropods observed to date (0.34 Mb), which could be partly observed by reduction of intronic regions (Pace, 2015; Pace et al., 2016). Although many genes in the *Daphnia* genome are duplicated (Colbourne et al., 2011), no duplications of *Hox* genes were reported.

For some *Hox* genes a dynamic expression pattern was found during embryogenesis of *D. pulex*. *DpuHox3* in early embryogenesis is expressed in a complex pattern, with the most anterior boundary of expression lying at the anterior limit of the second antennal segment as well as a ring of expression around the embryo; in later embryos, expression takes place in the limb buds. *Dpuftz* in early embryogenesis is expressed in a ring around the embryo following the posterior limit of the mandibular segment; in later embryos, expression is restricted to the posterior portion of the mandibular segment (Papillon and Telford, 2007).

Certain other genes also have specific patterns of expression: pair-rule homologs *odd-skipped-like* (*odl*) and *pairedA/B* (*prdA/B*) are expressed shortly before segmentation, but their expression is never revealed in the "postnaupliar formation zone" (Eriksson et al., 2013); *Dam snail* is expressed in transverse stripes during segmentation (Ungerer et al., 2011) (Fig. 18.5). *Notch* signaling is involved in segmentation in all

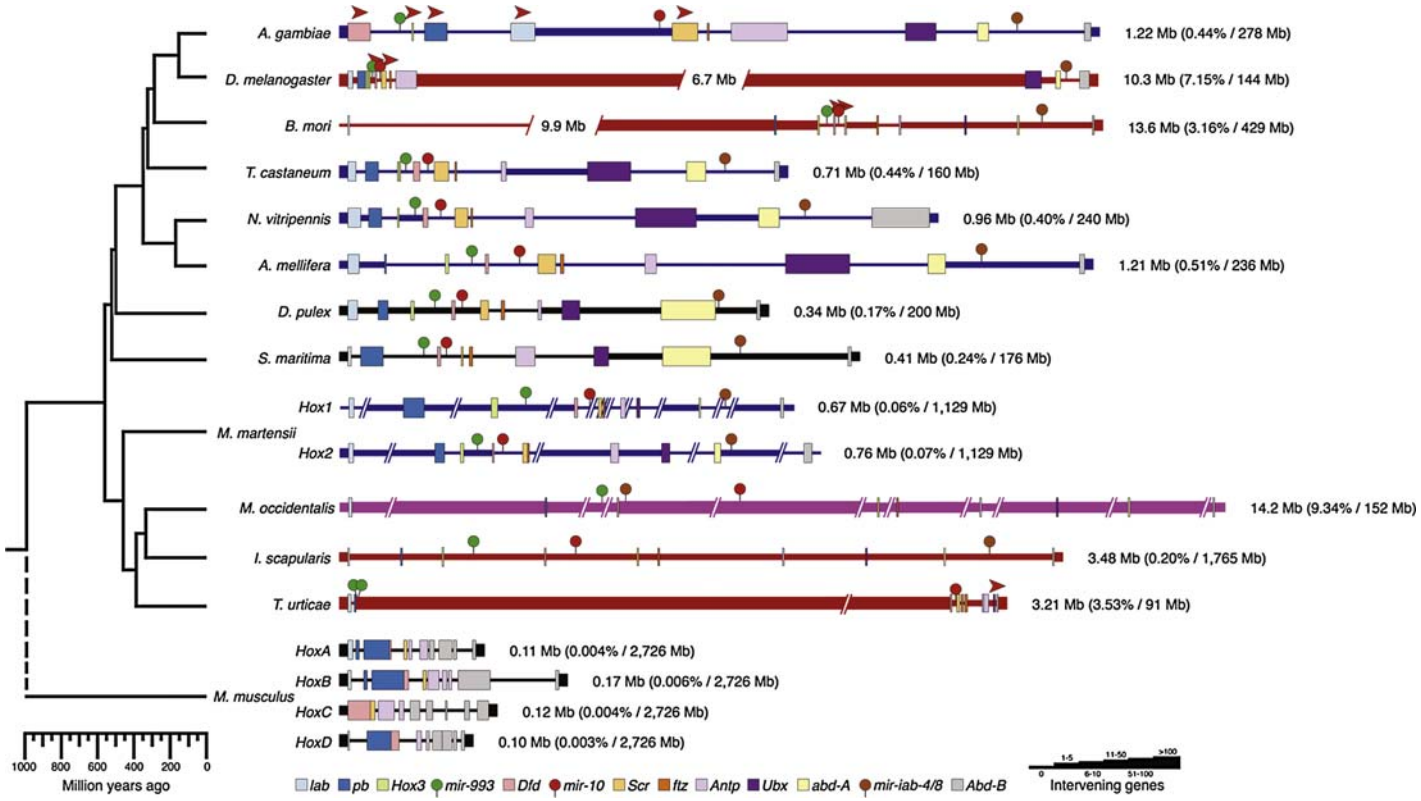

FIGURE 18.4 The overall size and genomic organization of arthropod *Hox* genes vary. On the left is a representative phylogenetic tree depicting relationships between the arthropod taxa; *Mus musculus* is used as an outgroup. *Colored boxes* represent *Hox* genes (and mice homologs) and miRNAs, with numbers to the right of the *black line* indicating approximate size of the genomic region displayed for individual taxa. All *Hox* genes are depicted in the same transcriptional orientation, except where indicated with a *red arrowhead*. The number of intervening protein coding genes between *Hox* genes is indicated by *horizontal line* thickness. Numbers to the right indicate the respective length of the *Hox* clusters in the genome in megabase pairs (Mb), as calculated from the transcriptional start of the most 3′ *Hox* gene to the transcriptional stop of the most 5′ *Hox* gene, and the proportion of the genome that contains the *Hox* cluster is indicated as a percentage along with the genome size in parentheses, respectively. *After Pace, R.M., Grbić, M., Nagy, L.M., 2016. Composition and genomic organization of arthropod Hox clusters. EvoDevo 7 (1), 1.*

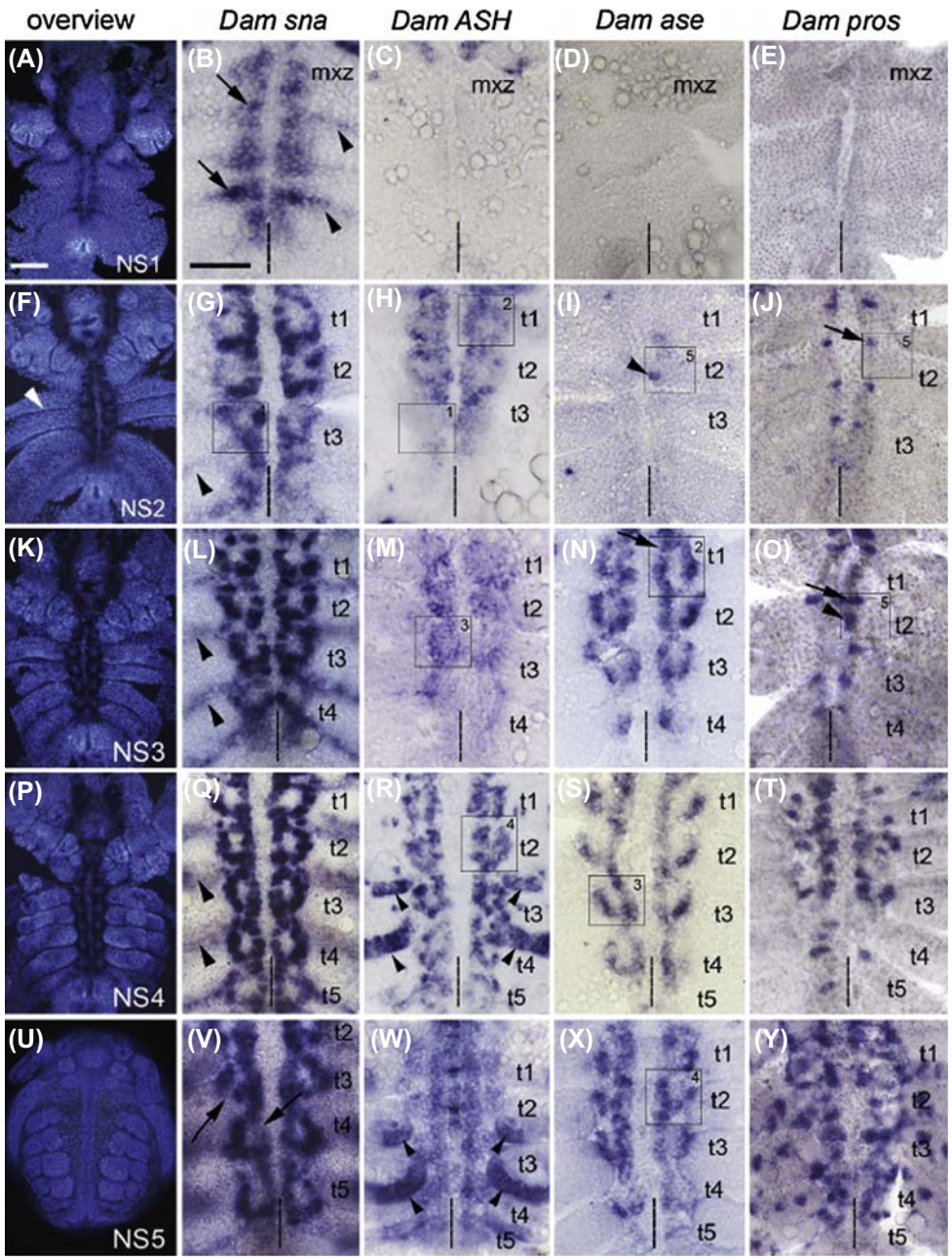

FIGURE 18.5 Temporal and spatial expression patterns of neural genes in the ventral neuroectoderm of *Daphnia magna* embryo at subsequent studies of its development. Confocal and light micrographs of flat preparations of *D. magna* embryos stained with *DIG*-labeled RNA probes of *Dam sna*, *Dam ASH*, *Dam ase*, and *Dam pros*, respectively. The embryos in A, F, K, and P were double-stained with a *DIG*-labeled *Dam sna* probe and the nuclei marker Hoechst; U is a single Hoechst staining. The *dashed vertical lines* indicate the ventral midline. *After Ungerer, P., Eriksson, B.J., Stollewerk, A., 2011. Neurogenesis in the water flea* Daphnia magna *(Crustacea, Branchiopoda) suggests different mechanisms of neuroblast formation in insects and crustaceans. Developmental Biology 357, 42–52.*

arthropods, including *Daphnia* (Eriksson et al., 2013); it is expressed as stripes that encircle the embryo in all nascent segments except for antenna I to the mandible. Eriksson et al. (2013) suggested two possible scenarios of its functioning in segmentation: (1) a segmentation clock involving *Notch* signaling, and (2) integration of *Notch* signaling into a hierarchical segmentation cascade.

Based simply on molecular marker genes, Ungerer et al. (2011) identified neuroblasts in *D. magna* cell division pattern. In contrast to insects, in the branchiopods the neuroblasts are not selected from protoneural clusters, and *Snail* is the first gene to be expressed in them instead of *ASH* in insects; the latter is already involved in maintaining neuroblasts in the neuroepithelium. These facts confirm a different mechanism of neuroblast formation in insects and crustaceans (Fig. 18.6). *Notch* signaling alone is required in neural stem cells for regulating the time of neural precursor production and for cell fate decision in the neuroectoderm (Ungerer et al., 2012). In general, expression and function of neural genes in *Daphnia* have several significant differences from those in insects, although in general the genetic modules controlling the earlier neurogenesis are conservative in different arthropods (Hartenstein and Stollewerk, 2015).

Distalless (*Dss*) is a well-known regulator of segmentation and appendage formation. It is expressed in *Daphnia* (Kato et al., 2011b) in the same pattern as in other arthropods. Probably,

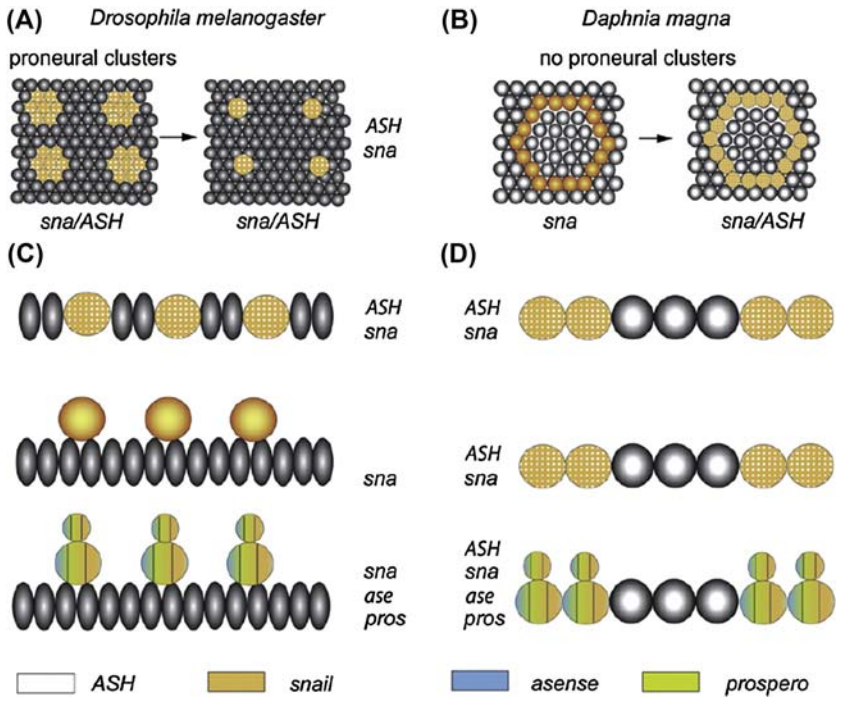

FIGURE 18.6 Comparison of the expression of neural genes in *Drosophila melanogaster* and *Daphnia magna*. Schematic drawings of horizontal (A, B) and transverse (C, D) views of the ventral neuroectoderm and neuroblasts. *After Ungerer, P., Eriksson, B.J., Stollewerk, A., 2011. Neurogenesis in the water flea* Daphnia magna *(Crustacea, Branchiopoda) suggests different mechanisms of neuroblast formation in insects and crustaceans. Developmental Biology 357, 42–52.*

differences in *Daphnia* limb patterns correlate with a change of *Dss* expression: its expression starts early in limb development and "persists until a late stage when each limb primordium acquires branched morphology characteristics of each segment, allowing for the correlation of each domain of DLL expression to specific limb branches" (Shiga et al., 2006).

Shiga et al. (2006) detected expression of *UBx* and *Antp* in *Daphnia*. Expression of *Antp* is capable of repressing *Dss* expression and limb development, and specifying limb morphology. This gene is expressed in the embryos, but the adults contain much lower concentrations of its mRNA. At the same time, eggs threatened by juvenile hormone showed lower expression of *Antp* than did normal eggs. This means that *Antp* expression is involved in the molecular pathways, inducing the male phenotype of *Daphnia* (Schwarzenberger and Von Elert, 2016).

Doublesex (*Dsx*) genes are found to be a main regulator of male phenotype (Kato et al., 2011a; Xu et al., 2014). Indeed, in situ hybridization in late embryos using *DIG*-labeled probes corresponding to *Dsx1* and *Dsx2* reveals their expression in rudiments of antenna I and limb I of male embryos, while in female embryos no such expression was detected, and it is these body parts that demonstrate strong morphological differences between the sexes. In addition, knockdown of *Dsx1* in male embryos resulted in the production of female traits including ovarian maturation. In contrast, injection of mRNA of *Dsx1* to the female embryo lead to elongation of its antenna I, while a long antenna I is a male, not female, character.

In insects, special transformer genes (*tra*) are also involved in sex determination, but Kato et al. (2008) determined a *tra* gene from *D. magna* and showed that its embryo and gonadal expression are not sexually dimorphic. Same gene plays an important role in regulating growth and development, and in switching the mode of reproduction in adult females (Zhang et al., 2014). Expression of *Fushi tarazu factor-1* starts shortly after gastrulation of *D. magna* embryos, has a different pattern in male and female embryos, and stops 30−48 h after ovulation (Mohamad Ishak et al., 2016).

In *Drosophila*, two homeodomain proteins have been identified as important for regulating r-photoreceptor differentiation, *Orthodenticle* (*Otd*) and transcription factor *PvuII-PstI homology 13* (*Pph13*). Two *Otd* homologs were identified in the genome of both *D. pulex* and *D. magna*, as well as the homolog of *Pph13*, and their expression was detected in the developing photoreceptors of *Daphnia* embryos (Mahato et al., 2014).

18.5 NERVOUS SYSTEM AND SENSORY ORGAN FORMATION

Already at the 32-cell stage of *Polyphemus*, 8 cells are differentiated and then form "Scheiteplatten" (Kühnemund, 1929). These cells are separated into two clusters of 4 cells, 10 cells dividing many times together with a few surrounding ectodermal cells forming the brain, optic ganglion, and eye (Mittmann et al., 2014). Expression of the gene *Dam atonal*, which undoubtedly takes place in the developing nervous system, is detected in these cells as well as in the surrounding area of the "Head-V," a specific cell pattern observed in *Daphnia* embryogenesis (Mittmann et al., 2014).

The eye is formed in the embryo as a structure with traces of its paired origin (Löpmann, 1937). First, two spots of eye pigmentation appear in the embryo, which grow and fuse together only at the time that embryogenesis finishes, or even at the first postembryonic instar (Kotov and Boikova, 1998). The eye itself (in contrast to paired pigment spots) is differentiated as a single "curved, disc-shaped layer of cells situated anteriorly in the embryo, beneath the outer layer of ectoderm" (Flaster et al., 1982), but the paired nature of the eye rudiment is observable in some stages of its development

(Kotov and Boikova, 1998). Immediately after the eye primordium, the optic ganglion is located. Then an extracellular sinus separates the eye from the primordium of the optic lamina; "a palisade of glial cells" forms in the midplan of the embryo in the eye, extends posteriorly toward the optic lamina, the ommatidia differentiate, and formation of photoreceptors in each ommatidium takes place (Flaster et al., 1982) (Fig. 18.7). As a result, an adult *Daphnia* has 22 ommatidia, each consisting of eight photoreceptors and a lens (Flaster et al., 1982), while other cladocerans have other numbers of ommatidia (Korovchinsky, 2004).

At the same time, a formation of the eye-lamina projection takes place because of growth of optic axons from the ommatidia, see Flaster et al. (1982). Eight photoreceptors from each ganglion project their axons to be jointed with five cells of the optic lamina to form a unit structure, an optic cartridge. Differentiation of the optic lamina cells is triggered by contact with growing axons of the photoreceptors (Macagno, 1981). Projection from eye to optic ganglion is retinotopic at the level of single cells (Macagno et al., 1973; Flaster and Macagno, 1984), and this retinotopy is a consequence of cellular interactions (Macagno, 1978, 1984).

18.6 GONAD FORMATION

Sagawa et al. (2005) used the antibodies to VASA proteins, which are expressed in the germ line cells, to trace their fate in *Daphnia*. Dma-VAS was first detected at the eight-cell stage in a subcellular component located adjacent to the nucleus, but at the stage of 16

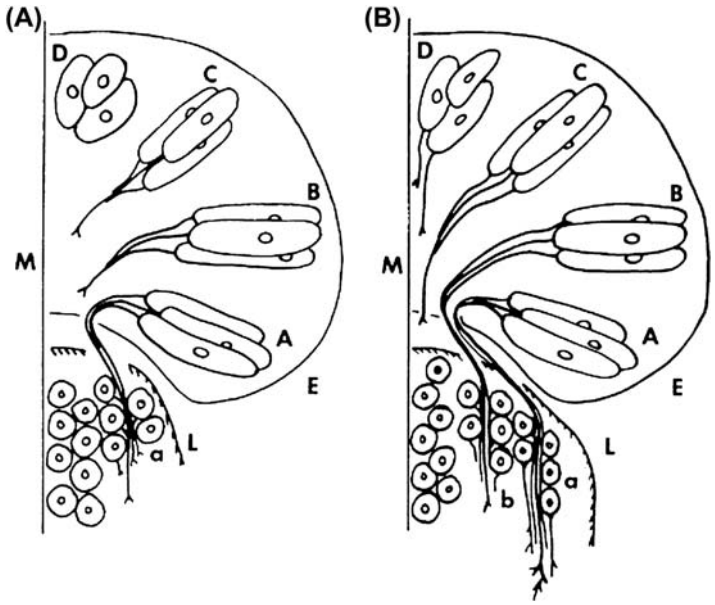

FIGURE 18.7 A scheme of the development of the eye-lamina projection at an earlier stage in its development (A) and several hours later (B). *E*, developing eye; *L*, laminar rudiment, *A*–*D*, ommartidia, *a and b*, laminar cartridges. The axon bundle from ommatidium A has grown posteriorly along the midline glial palisade, has reached the lamina, and recruited presumptive laminar neurons, forming a cartridge *a*. As other axons grow, cartridges are formed and displaced laterally. *After Flaster, M.S., Macagno, E.R., Schehr, R.S., 1982. Mechanisms for the formation of synaptic connections in the isogenic nervous system of* Daphnia magna. *In: Spitzer, C. (Ed.), Neuronal Development. Plenum Publishing Corporation, New York, pp. 267–296.*

blastomers VAS lost association with this region, and such signal was absent in a portion occupied with yolk granules. Mittmann et al. (2014) observed at the 16-cell stage a single cell that was noticeably smaller than the others, which could be a germ line precursor. During cleavage, the number of blastomers with VAS signal was continuously increasing, but they divided slowly as compared with other blastomers: two VAS blastomers are present at the 32-cell stage, four at the 64-cell stage, and eight at the 128-cell stage. During the blastodermal stage, several tens of such cells form a cluster. Then the cluster is divided into two halves, each half migrating dorsoanteriorly and then dorsoposteriorly, also becoming more elongated in shape. Finally, the clusters occupy positions at both sides of the midgut.

18.7 HORMONAL REGULATION OF EMBRYONIC MOLTS

Expression of ecdysteroids (molting hormones) starts at very early stages of embryonic development (Asada et al., 2014).

Special efforts were made to study hormonal regulation of embryonic molts in the Cladocera. Ecdysteroids are known as molting hormones; the ecdysteroid synthesis and signaling pathways are highly conserved in the arthropods, and are similar in daphnids and better-studied insects (Rewitz and Gilbert, 2008; Kato et al., 2010; Sumiya et al., 2014). In general, the titer of the ecdysteroids is increasing in the course of embryo development in *Daphnia* (Mu and LeBlanc, 2004). It is shown that fenarimol (an inhibitor of ecdysteroidogenesis) induces some abnormalities in the embryos of *Daphnia*, and even arrests embryonic development (Mu and LeBlanc, 2002b).

Sumiya et al. (2014) demonstrated that decline of the ecdysteroid's titer is required for subsequent molting during a parthenogenetic reproductive cycle of *D. magna* (Sumiya et al., 2014). Sumiya et al. (2016) identified genes involved in ecdysteroid synthesis (*neverland 1*, *neverland 2*, *shade*) and degradation (*Cyp18a1*) (Fig. 18.8). They demonstrated that *neverland*, expressing in embryonic gut epithelium, regulates the embryonic molting through the regulation of ecdysteroid synthesis. *Neverland* is identified as a target for chemicals that disrupt the molting, like some pesticides.

18.8 KAIROMONES AND EMBRYOS

Many organisms are able to change their morphology and/or behavior in response to the threats of some predators (so-called "predator-induced polyphenism"), and cladoceran *Daphnia* is a model for such studies (Miyakawa et al., 2010). Because of the high interest of hydrobiologists and zoologists in the induction of the appearance and enlargements of so-called defensive structures (caudal needle, helmet, neckteeth) in *Daphnia* by kairomones of some predators (Chaoboridae, *Leptodora*), special efforts were made to investigate the embryonic aspects of such induction.

In the presence of the kairomones, a complicated series of processes is begun, including neuronal components and endocrine signals, which induce changes in the expression of morphogenetic factors (Barry, 2002; Miyakawa et al., 2010). It is found that the physostigmine (inhibitor of the enzyme acetylcholinesterase) modulates the response of kairomone stimulation (Barry, 2002). A lot of different genes and gene families (i.e., zinc-metalloproteinases and vitellogenin) are included in such induction (Rozenberg et al., 2015; Weiss et al., 2015). In the case of *Chaoborus*, the predator cue is a small (>500 Da), heat-stable molecule carrying a carboxyl group (Tollrian and von Elert, 1994).

Embryos of *Daphnia* showed the high efficiency of neckteeth induction (Krueger and Dodson, 1981; Parejko, 1992; Imai et al.,

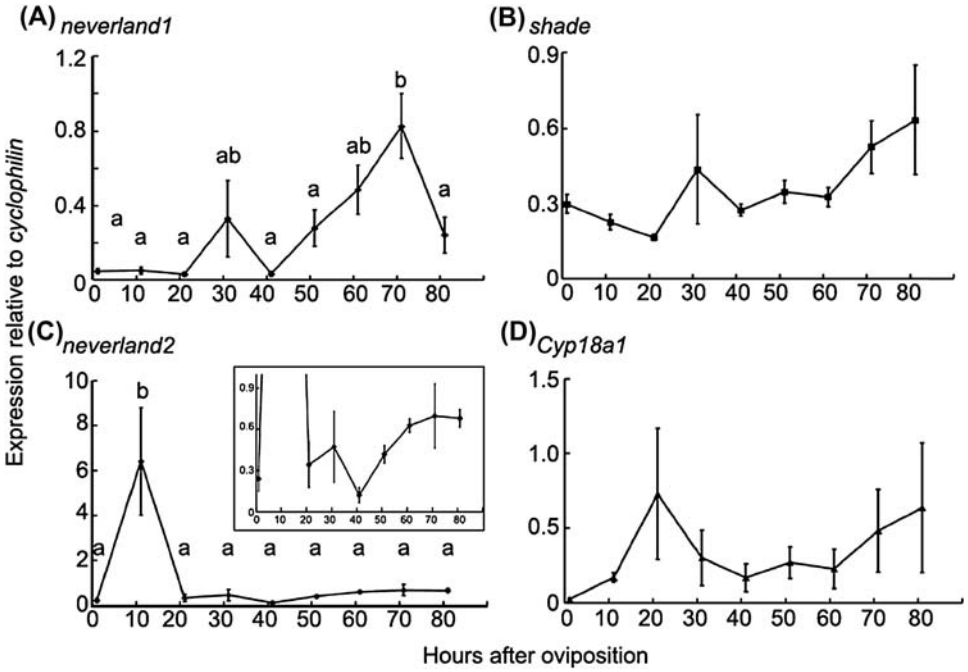

FIGURE 18.8 Gene expression profiles during embryonic development of *Daphnia magna*. (A) *neverland1*, (B) *shade*, (C) *neverland2*, (D) *Cyp18a1*. Hours after oviposition refers to the time after the eggs were oviposited into a brood pouch. *Bars* represent standard errors. *After Sumiya, E., Ogino, Y., Toyota, K., Miyakawa, H., Miyagawa, S., Iguchi, T., 2016. Neverland regulates embryonic moltings through the regulation of ecdysteroid synthesis in the water flea* Daphnia magna*, and may thus act as a target for chemical disruption of molting. Journal of Applied Toxicology.* http://dx.doi.org/10.1002/jat.3306.

2009). In spite of the long period of embryogenesis of *Daphnia*, the kairomone-sensitive period is short, covering only later embryonic stages to the first postembryonic instar. Weiss et al. (2016) concluded that sensitivity to kairomones starts "when compound eye spots begin to fuse and egg membranes are shed," but the neckteeth start to develop after a time lag. Maybe it is a consequence of the fact that the induction has a neuronal regulation, but the nervous system is not functioning at earlier stages.

In induced embryos, a proliferation of cells and their changes in structure leads to necktooth formation and appearance of the head crest (Naraki, 2014), and just at the last embryological stages, significant differences between induced and noninduced embryos of some *Daphnia* take place: the necktooth pedestal appears in *D. pulex* and a helmet precursor on *Daphnia cucullata* (Laforsch and Tollrian, 2004b). The latter authors correlated the ability of embryos to react to the presence of kairomone with shedding of the third embryonic membrane, after which chemosensillae are able to recognize such a signal.

However, no precursors of defensive structures are found at this stage in induced embryos of *Daphnia lumholtzi*, *Daphnia longicephala*, and *Daphnia ambigua* (Laforsch and Tollrian, 2004a). In some species of *Daphnia* the development of defensive structures takes place at postembryonic stages.

Miyakawa et al. (2010) demonstrated that, among many tested candidate genes, morphogenetic factors (*Hox3*, *extradenticle*, and *escargot*) are

involved in the regulation of *Daphnia* morphological response to the predator kairomones in embryogenesis, while juvenile hormone pathway genes (*JHAMT* and *Met*) and the insulin signaling pathway genes (*InR* and *IRS-1*) regulate such response during the first postembryonic instar. It is interesting that LiCl affected only the crest development in the embryos of *Daphnia* rather than necktooth formation itself. The authors found that the influence of LiCl on crest formation suggested that this process may be mediated by *GSK-3β*, because it is known that LiCl inhibits *GSK-3*, allowing β-catenin to accumulate and affect the transcription of *Wnt* target genes, although the expression of the latter was not revealed by the authors by real-time polymerase chain reaction (Naraki et al., 2013).

Undoubtedly, induction of defensive structure formation in embryos has a complicated regulation; see the scheme outlined by Weiss et al. (2015). Dopamine, as a mitogen and sclerotization agent, is actively involved in such processes; it is produced in special polyploidy cells located in the dorsal portion of the head, which are innervated by the brain, and their neurosecretory function is confirmed by staining (Weiss, 2011). The development of neckteeth in *D. pulex* is enhanced by physostigmine and picrotoxin and suppressed by atropine (Barry, 2002). The development of helmet is regulated by terpenoid hormone or its analog in *D. galeata* (Oda et al., 2011).

18.9 OSMOREGULATION IN EMBRYOS

Embryos in many cladocerans have osmoregulatory organs different from those in adults (Gicklhorn and Keller, 1925; Aladin, 1996). The dorsal organ (neck organ, nuchal organ) is present in embryos of all the cladocerans from the end stage of thoracic limb formation (Mittmann et al., 2014) to the first or even second postembryonic instar (as in *Daphnia*), the last embryonic instar (as in *Simocephalus*), or even the previous embryonic instar(s?) (as in at least some Chydoridae; see photos of late chydorid embryos with already formed head pores in the position of the dorsal organ in Kotov, 2013). But the dorsal organ is present in adults in a minor portion of the cladoceran taxa only (all Haplopoda, all Onychopoda, a few Ctenopoda, and a few Anomopoda) (see Gicklhorn and Keller, 1925; Aladin, 1996; Rivier, 1998; Kotov, 2013). In the ctenopods and anomopods that have the dorsal organ, it is frequently morphologically specialized in adults and even in late embryos (Kotov, 2013; see p. 366 and Fig. 279), and its full functional homology with the embryonic organ must be specially confirmed.

The in-transporting role of the dorsal organ is directly demonstrated by the so-called chloride test (Aladin, 1996) and ultrastructure studies using transmission electron microscopy for adult and juvenile onychopods (Meurice and Goffinet, 1983), as well as for *Daphnia* of the first postembryonic instar (Halcrow, 1982). Such an organ could be a subject of experiments with vital staining by different dyes (Gicklhorn, 1931c).

There is a special mechanism of osmotic regulation of the pouch contents by the mother (Aladin, 1996; Aladin and Potts, 1995). For example, the ctenopod *Penilia* with a closed brood pouch occurs in the sea, where the water is saline. During earlier stages of embryo development, the animal demonstrates a hypoosmotic regulation of the brood pouch liquid, but after the development of epipodites in embryos (organs of its own osmoregulation), the depression of the liquid in the brood pouch is equal to that of the external environment. In different cladocerans with closed brood pouches, cases of both hyperosmotic (in fresh water) and hypoosmotic (in saline water) regulation of the brood pouch liquid were recorded (Aladin, 1996; Aladin and Potts, 1995) (Fig. 18.9).

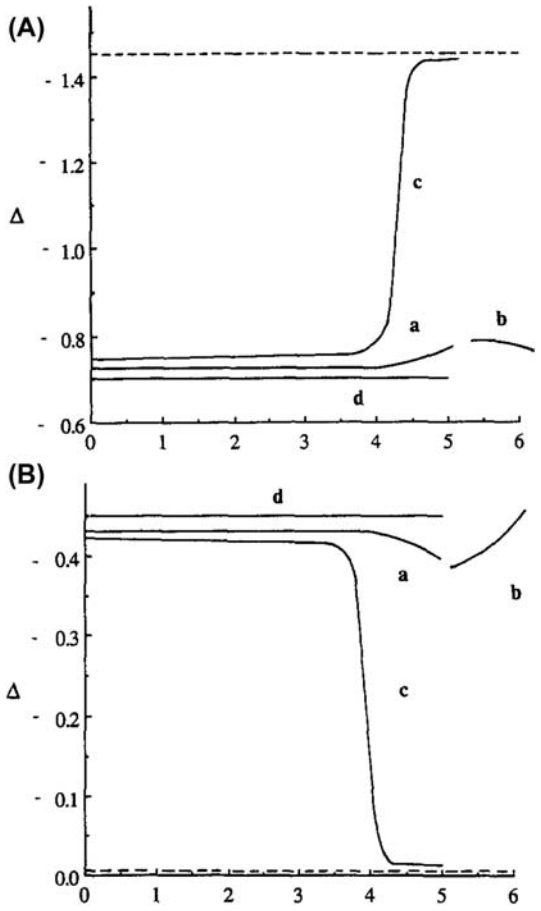

FIGURE 18.9 Osmotic concentrations of liquid from embryos and newborn individuals of cladocerans with closed brood pouch. (A) under hyperhaline conditions; (B) under freshwater conditions. Abscissa—developmental stage; ordinate—osmotic concentration, depression of freezing point °C. *a*, embryo; *b*, newborn individual; *c*, marsupial liquid of parthenogenetic female; *d*, hemolymph of parthenogenetic female. *Dotted line* indicates osmotic concentration of the surrounding water, 0. *After Aladin, N.V., Potts, W.T.W., 1995. Osmoregulatory capacity of the Cladocera. Journal of Comparatrive Physiology, Series B 164, 671–683.*

18.10 RESPIRATION

Hoshi (1950a) and then Green (1965) found that oxygen consumption per dry weight is 6–8 times higher in older embryos as compared with earlier ones.

Hemoglobin was detected in the cladoceran eggs many years ago (Teissier, 1932) and since that time it has been intensively studied (Fox, 1948; Green, 1955, 1956a, 1965). The hemoglobin is initially drained from the mother's blood into the forming eggs (lying in the ovary) where it forms globules in the fat cells; the amount of hemoglobin in the egg depends on the nutrition and oxygen conditions of the mother's habitat (Green, 1955).

Michiyori and Yukio (1994) demonstrated that the concentration of hemoglobin in early embryos of *D. magna* is higher than that in the hemolymph of adults. In addition, a longer time is required for oxygenation and deoxygenation of embryonic hemaglobin as compared with adult hemaglobin (Fig. 18.10); the latter is helpful in case of very numerous and densely packed embryos in a single mother brood pouch. *Daphnia* resolves the problem of oxygen deficit in the brood pouch by creation of a special ventilatory mechanism for their brood. At the earlier stages of embryo development, such current is absent under normal conditions, but is induced by hypoxic conditions (Seidl et al., 2002). The oxygen consumption rate increases considerably in later stages (Glazier, 1991), and regular cardiac activity and an intact circulation develop during these stages (Seidl et al., 2002).

The gut of a late embryo of *Daphnia* contains a hemochromogen named daphniarubin (Fox, 1948), which is absent in egg or early embryo. Therefore this pigment appears during embryogenesis. As the hemoglobin content is decreasing during embryogenesis, Fox (1948) and then Phear (1955) concluded that at least a part of the hemochromogen originated from the hemoglobin initially present in the cladoceran egg.

During the course of experiments with embryos treated with carbon monoxide (to inactivate hemoglobin) their rate of oxygen consumption does not differ significantly from that of embryos in normal conditions (Hoshi, 1957).

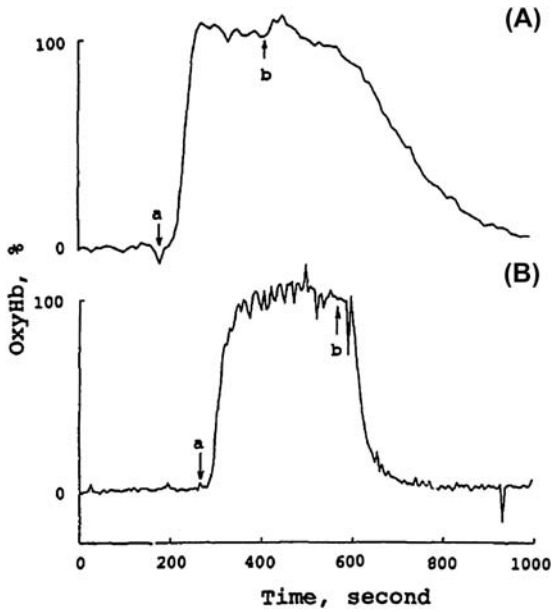

FIGURE 18.10 Time courses for a change in oxygen saturation for early embryos (A) from hemaglobin-poor *Daphnia* and that for adult animals 2 mm in body size (B). The embryos were initially exposed to anoxic water, then to air-saturated water (*arrow* a), and finally to anoxic water (*arrow* b). *After Michiyori, K., Yukio, T., 1994. In vivo oxygenation of hemoglobin in early embryos of* Daphnia magna. *Comparative Biochemistry and Physiology Part A: Physiology 107 (1), 127–131.*

But the former embryos take a longer time to complete their development. Hoshi (1957) and then Fox (1948) concluded that hemoglobin "serves a function in accelerating development, particularly in poorly aerated water." The difference in the duration of development could be partly explained by a slower rate of decomposition of carboxyhemoglobin in such conditions. The decrease in hemoglobin during the course of development supports the view that its function is to supply protein for embryogenesis.

18.11 YOLK AND GLYCOGEN

The sources of material for the development of cladoceran eggs are the yolk and oil (see Chapter 11). Makrushin (1991) proposed that the so-called euphyllopod yolk of resting eggs, containing numerous fat drops, appeared in some cladocerans secondarily as an adaptation to life in temporary continental water bodies. But some phylogenetic evidence suggests that, in contrast, euphyllopod yolk (in both parthenogenetic and resting eggs) is plesiomorphic, while a homogeneous yolk is apomorphic (Kotov, 2013).

Yolk is accumulated in the egg during late stages of its formation in the mother's ovary (before its release to the brood pouch). It is used as an energetic source during embryogenesis and is even present in the cladocerans of the first postembryonic instar. It is demonstrated that the survival of the latter depends on the volume of the postembryonic yolk (Cowgill et al., 1984; Goulden et al., 1987). In general, females of the first postembryonic instar of larger cladocerans (with larger eggs) have a greater relative amount of postembryonic yolk than first instar females of smaller species (Goulden et al., 1987).

Hoshi (1950a) found that at early stages of development the fat and lipids are predominantly utilized for supplying the embryo with energy, but the carbohydrates are the main source of energy in the later stages.

In well-aerated conditions the glycogen is formed at the early stages of embryogenesis and then utilized during later embryogenesis in a small amount (Hoshi, 1951a,b, 1954). Glycogen is present in the cytoplasm of the embryo, but not in yolk. In anaerobic conditions the embryos of *Simocephalus vetulus* die when about 28% of the total glycogen has been utilized. At higher oxygen concentrations less glycogen is used and the metabolism of the embryo is more like that of embryos in well-aerated water.

18.12 MISCELLANEOUS OBSERVATIONS

Different pigments appear in the cladoceran embryos during their development. Green

(1957a) detected carotenoids in many littoral and planktonic species and concluded that these pigments are not utilized by the embryos. The pigment biliverdin is detected in the forming eye of *Polyphemus*; it is visible at earlier stages but then is masked by a high concentration of an ommochrome, which finally makes the eye black (Green, 1965). It is important that an increase of the carotenoid's synthesis takes place in *Daphnia* embryos exposed to a strong light, which is understandable if these pigments are protectors from solar radiation (Green, 1957a). No bile pigments were detected in both embryos and adults of *Daphnia* (Fox, 1955), which possibly indicates that the breakdown of hemoglobin in *Daphnia* involves a coupled oxidation with unsaturated fatty acids (Green, 1957a).

Although embryos require calcium for their development and molting, they do not equilibrate with environmental calcium levels (Giardini et al., 2015).

Berril and Henderson (1972) found in *Daphnia* embryos that the antennae II became active intermittently from the time of their first occurrence until they became continuously active 2−3 h before the end of embryonic development. The bouts of antennae II start as rare, pseudorhythmical movements, which become more frequent in the course of embryo development. After the casting off of the second egg membrane, the embryo is able to respond to tactile stimulation by starting on a prolonged bout of antennal activity that keeps it swimming for about 2−3 min. See the review of the influence of extremal conditions on cladoceran eggs, and information on the abortion and resorption of eggs in Chapter 11.

In conclusion, we need to underline the strong nonproportionality of the studies of different aspects of the embryo physiology of the Cladocera, as our knowledge of certain aspects has not been updated since the 19th century. At the same time, we can expect a rapid progress in the embryology of Cladocera, because this group is now a model for evo-devo studies.

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Conclusions: Special Traits of Cladoceran Physiology

Some representatives of the Cladocera occur in enormous quantities, but other species are rare and confined to narrow ecological niches, potentially partly due to their physiological adaptations. Cladocera consume small algae, bacteria, and detritus, thus forming a link between primary production and the predators that eat them. A high intensity of feeding is characteristic of Cladocera: the ingested food stays in the intestine for several minutes up to about half an hour. During this time, the Cladocera extract sufficient material to support their intensive reproduction. They also synthesize abundant chitin.

Particular body constituents of Cladocera are dynamic in relation to the season, the chemical composition of their food and of the environment, and starvation. Data are now available on quantities of glycogen, chitin, lipids, slimes, and pigments [e.g., carotenoids, melanin, hemoglobin (Hb)], their metabolism, and on the dynamics of particular compounds. Cladocera may accumulate physiologically important substances, those of no such importance, and toxicants.

Cladocera consume foods that have low quantities of nitrogen and phosphorus and an excess of carbon. Thus, they have to get rid of excess carbon. Only a very small amount of the consumed lipids are transformed. With excessive food consumption (i.e., *luxury consumption*), the proportion of ingested food that is digested may be low. Cladocera consume a lot of chlorophyll; during digestion, only a little is slightly reduced to pheopigments and it is not used in the construction of Hb. Starvation is accompanied by profound changes in the chemical composition of the body.

Respiration occurs through the body surface of Cladocera. Littoral, and especially bottom-living species, exist under oxygen deficiency but are surrounded by abundant food resources, whereas planktonic species usually enjoy a good oxygen environment, but their food resources are periodically either abundant or scarce. Cladocera frequently contain Hb but can manage well without it. Studies on the impact of xenobiotics on respiration have been reviewed.

Two kinds of blood cells have now been identified (with reference to *Daphnia*), one of which performs phagocytosis. The heart rate is similar for all Cladocera species, except for *Ilyocryptus* and *Moinodaphnia*, in which the heart rate is 2—3 times lower. The heart rate noticeably increases with various disturbances and drops only before death. Heart arrest occasionally occurs without harming the individual and it can resume beating without an obvious reason. Adhesion of blood cells (which normally drift in the blood) to the surface of organs may occur for unknown physiological reasons.

Excretion is principally carried out by paired maxillary glands. The main final product of protein metabolism is ammonia (NH_3), accompanied by smaller amounts of urea. Depending on their structure, xenobiotics are transformed within the body and may be passed into the next generations. Within certain limits, Cladocera can support homeostasis of various processes within a dynamic environment, including that of osmotic pressure.

Cladocera live from 1 week up to several months, depending on the species and the environmental conditions. Body length increments occur between molts. Mechanical damage is repaired by regeneration, which produces either normal or abnormal structures. The status of investigations into senescence and mortality is described.

The trajectories of littoral and pelagic species differ greatly but are inadequately studied. The complete muscle system has only once been described, with reference to *Daphnia*. Therefore, comparative investigations of the muscles of littoral species are urgently needed. Published studies are available on immobilization, fatigue, stress, and the impact of xenobiotics on locomotion.

Cladocera can discern polarized and colored light, can orient them in space, and manifest endogenous rhythms. Cladocera also exhibit complex behavior, which differs in particular species; they manifest migration, swarming, akinesis, and escape behavior. Published data are available on disturbances to their behavior by xenobiotics.

Ecological aspects considered include the physiological background of limits to physiological factors, synergism and antagonism among factors, lipid pathways from algae via Cladocera to fish, and environmental conditioning by Cladocera. In Cladocera, the absolute value of particular parameters is not species specific but depends on clonal composition (Pietrzak, 2011) and on previous acclimation.

Seemingly chaotic (although within certain limits) combinations of dozens of Cladocera species live under combinations of hundreds of dynamic multidirectional factors. Each specimen simultaneously perceives all of these factors with its sense organs. Due to such a dynamic environment and their physiological individuality, particular species can form resultant population peaks, the position in time and the absolute value for which may differ greatly in different years.

Physiological characteristics belong to reasons that control spatial and temporal distribution of Cladocera, as well as dominance or rarity of particular species. Unfortunately, the actual values of physiological characteristics are not known for most species. Initially, toxic and inhibitory pollutants are diluted and may stimulate various vital processes. Their combination with sex hormone-like pesticides can cause multidirectional effects and lead to disturbances in the natural balance.

Cladocera are useful as an experimental and educational object.

The available data on cladoceran physiology mostly concern daphnids living in open water; bottom-living and littoral Cladocera have been little studied. The latter species live under conditions of hypoxia and anoxia with an excess of food (organic debris at all stages of decomposition); thus, their investigation may reveal specific adaptations that differ from those characteristic of open-water species. In addition, some issues of cladoceran physiology are well studied (e.g., some aspects of respiration, osmoregulation, and neurosecretion), whereas others are still awaiting investigation.

The available information indicates that:

1. Though morphologically simplified, Cladocera cannot be considered physiologically primitive;
2. Some functions are periodic in compliance with the molting cycle. Cladocera are remarkable for their predominantly parthenogenetic reproduction, which sometimes is interchanged with bisexual reproduction. The appearance of males depends on environmental conditions, food composition, and hormonal regulation;
3. In Cladocera, some functions are facultative, such as the presence and formation of Hb, beating of the heart, and vision by means of their eyes (in vertebrates the role of Hb is obligate, with the exception of one group of fish: the Chaenichtyids);

4. The heart is myogenic and acetylcholine is the transmitter substance (Postmes et al., 1989). It was shown that cholinoreceptors of *Daphnia* are analogous to those in rats (Tonkopiy et al., 1994a,b) which may indicate their potential greater role in medical investigations;
5. Cladocera exhibit physiological radiation, as seen in their various functions. Planktonic Cladocera are normoxic; bottom-living ones prosper in hypoxic or anoxic conditions. Sometimes, physiological radiation is formed at the background of conservative morphology, for example, in rather morphologically uniform species of the genus *Moina*, some of which are confined to freshwaters, and some live in saline lakes.

Let everything that has breath praise the Lord. ***Psalm 150.6.***

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Index of Latin Names of Cladocera

'*Note*: Page numbers followed by "f" indicate figures and "t" indicate tables.'

Subject Index

'*Note*: Page numbers followed by "f" indicate figure and those followed by "t" indicate table.'

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P

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Second Edition

Physiology of the CLADOCERA

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A. N. Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, Moscow, Russia

With Additional Contributions

Physiology of the Cladocera, second edition, is a much-needed summary of foundational information on these increasingly important model organisms. This unique and valuable review is based on the world's literature, including Russian research not previously widely available, and offers systematically arranged data on the physiology of Cladocera, assisting with explanation of their life and distribution. It features the addition of new sections and a vast amount of new information, such as the latest data on feeding, nutrition, pathological physiology, chemical composition, neurosecretion, and behavior, as well as hormonal regulation, antioxidants, and the biochemical background of effects of natural and anthropogenic factors. Additional expertly updated contributions in genetics and cytology, and a new chapter in embryology, round out the physiological chapters, and provide comprehensive insight into the state of knowledge of Cladocera and their underlying mechanisms.

Cladocera crustaceans have become globally studied for many purposes, including genetic, molecular, ecological, environmental, water quality, systematics, and evolutionary biology research. Since the genome of *Daphnia* was sequenced and published, that system has gained much wider exposure, also leading to a rapidly growing awareness of the importance of understanding physiological processes as they relate to evolutionary and ecological genomics as well as ecogenomic toxicology. However, the physiological background on Cladocera has been fragmentary (including on the other 700 known species besides *Daphnia*), despite the extensive literature on species identification and morphology. This work addresses this issue by collecting and synthesizing from the literature the state of knowledge of cladoceran physiology, including discussion on both adequately and inadequately investigated fields, and thus directions of future research.

KEY FEATURES

- Summarizes fundamental information obtained in recent years, including on steroids, antioxidants, hormones, nanoparticles, and impact of wastewater of pharmaceutical industries
- Provides the foundational information needed for scientists and practitioners from a variety of fields, including conservation and evolutionary biology, genomics, ecology, ecotoxicology, comparative physiology, limnology, zoology–carcinology, and water quality assessment
- Features coverage of both Daphniids and representatives of other families, with attention drawn to little-studied aspects of their physiology, especially of those living in the littoral zone
- Includes guidance to the literature on cladoceran physiology in four languages
- Discusses advantages and shortcomings of Cladocera as experimental animals and indicators of water quality

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