ONE

# The Associations between Fishes and Luminescent Bacteria



## Luminescent Bacteria

Luminescent bacteria are the most common and widely distributed of all luminous organisms, typically emitting a continuous blue-green light, peaking around 490 nm. They occur most frequently in the marine environment but are also found in the freshwater and terrestrial environments (Nealson and Hastings, 1990; Meighen, 1991). Currently, twenty-five species of luminescent bacteria, belonging to six genera and three families, have been identified. Bacteria of the genus *Schewanella* (fam. Schewanellaceae) are commonly found free living in the freshwater environment. Members of the genus *Photorhabdus* (fam. Enterobacteriaceae) are terrestrial, gut endosymbionts of nematodes of the genus *Heterorhabditis*. The genera *Photobacterium*, *Vibrio*, *Aliivibrio* and *Photodesmus* are all marine members of the Vibrionaceae, an ecologically diverse group of Gram-negative bacteria often associated with marine animals (Peat and Adams, 2008; Hendry and Dunlap, 2011; Urbanczyk *et al*., 2011).

Luminescent bacteria of the Vibrionaceae are found in all marine environments from the cold polar seas to the warm tropics and from surface waters to great depths. Both geographic, seasonal and depth-related differences in luminescent bacterial abundance and species composition have been documented. The distribution patterns were suggested to be controlled by temperature, salt tolerance, resistance to photooxidation, hydrostatic pressure and the ability to grow in nutrient poor conditions (Yetinson and Shilo, 1979; Shilo and Yetinson, 1979; Herring, 1982; Al Ali *et al*., 2010). Luminous Vibrionacea are found both free living, as saprophytes, and as gut symbionts and symbionts contained in special light organs of fishes and cephalopods. Five species of luminous bacteria, *Aliivibrio fisheri*, *Photobacterium leiognathi*, *P. kishitanii*, *P. mandapamensis* and *Photodesmus katoptron*, and two groups of not yet identified bacteria associated with deep sea anglerfishes and flashlight fishes have been so far described from fish light organs (Table 1.1). The associations of these bacteria with fishes is discussed throughout this chapter. A shift from one niche to another (e.g., *Photobacterium leiognathi* shifting from the nutrient-rich esophageal light organ of ponyfish into the fish's gut and, subsequently, into the water column adapting a free living mode in a starvation/survival habitat) is typical for many of these symbiotic bacteria (Nealson and Hastings, 1990; Urbanczyk *et al*., 2011).

The bacterial light producing reaction is catalyzed by the heterodimeric enzyme luciferase, which consists of two similarly structured α and β subunits with molecular masses of 40 and 37 kDa, respectively. This enzyme oxidizes with atmospheric oxygen ($O_2$), a reduced riboflavin phosphate ($FMNH_2$) and a long chain fatty aldehyde (RCHO) into an electronically excited flavin. With the release of a blue-green light (490 nm), flavin

*Symbiosis in Fishes: The Biology of Interspecific Partnerships*, First Edition. Ilan Karplus.

**Table 1.1** Fish hosts and symbiotic luminescent bacteria.

| Host Fish Order and Family | Depth and Temperature | Luminescent Bacteria Species |
|---|---|---|
| Anguilliformes Congridae | Shallow? Cool? | Not identified |
| Argentiformes Opisthoproctidae | Deep, cold | *Photobacterium kishitanii* |
| Aulopiformes Chlorophthalmidae | Deep, cold | *Photobacterium kishitanii* |
| Gadiformes Macrouridae | Moderate to deep, cold | *Photobacterium kishitanii*<br>*Aliivibrio fisheri* |
| Gadiformes Merculidae | Moderate to deep, cold | *Photobacterium mandapamensis* |
| Gadiformes Moridae | Moderate to deep, cold | *Photobacterium kishitanii*<br>*Photobacterium mandapamensis* |
| Lophiformes 9 families | Deep, pelagic, cold | Not identified |
| Berciformes Anomalopidae | Shallow to moderate, warm to cool | Not identified* |
| Berciformes Monocentridae | Shallow to moderate, warm to cool | *Aliivibrio fisheri* |
| Berciformes Trachichthyidae | Deep, cold | *Photobacterium kishitanii* |
| Perciformes Acropomatidae | Shallow to moderate, cool to cold | *Photobacterium kishitanii*<br>*Photobacterium leiognathi*<br>*Photobacterium mandapamensis* |
| Perciformes Apogonidae | Shallow, warm | *Photobacterium mandapamensis* |
| Perciformes Leiognathidae | Shallow to moderate, warm to cool | *Photobacterium leiognathi*<br>*Photobacteriun mandapamensis*<br>*Vibrio harveyi* (?) |

*Except for *Photodesmus katoptron* (Hendry and Dunlap 2011).
Urbanczyk et al. 2011. Reproduced with permission of John Wiley & Sons.

mononucleotide is produced together with water and a fatty acid (RCOOH):

$$FMNH_2 + RCHO + O_2 \rightarrow FMN + H_2O + RCOOH + hv\ (490\ nm)$$

A fatty acid reductase complex containing three enzymes, a reductase, a transferase and a synthetase, catalyzes the acid with water back into the fatty aldehyde substrate to facilitate the continuous production of light (Nealson and Hastings, 1990; Meighen, 1991). Structural genes coding for enzymes involved in the bioluminescent reaction of bacteria are located in the *lux* operon (Figure 1.1). The genes *lux* A and *lux* B code for the α and β subunits of luciferase, which probably resulted from gene duplication, since there is about 30% identity in the amino acid sequence between the α and β subunits of all bacterial luciferases. The order of the *lux* CDE genes coding for the fatty acid reductase complex is the same in all operons. The *lux* C and *lux* D genes, which code for fatty acid reductase and acyl-transferase polypeptides, respectively, flank the luciferase genes upstream and *lux* E, which code for acyl-protein synthetase being downstream (Figure 1.1). Downstream of *lux* E is located *lux* G which specifies flavin reductase. Upstream of the *lux* operon of *Aliivibrio fisheri* are found the genes *lux* I and *lux* R, which are involved in quorum sensing, as discussed later. Similar content of the bacterial *lux* genes, their organization, and

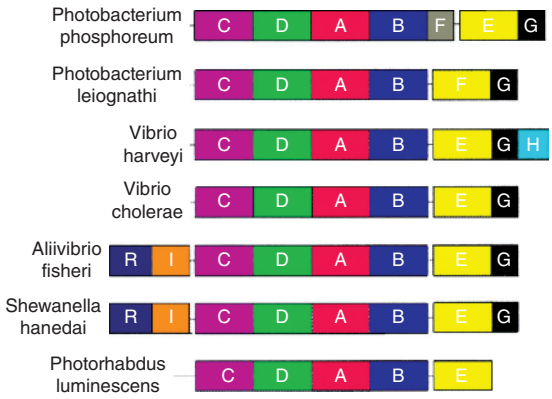


**Figure 1.1** Organization of the *lux* operons of a variety of luminescent marine, freshwater and terrestrial bacteria including two light organ symbionts. (Peat and Adams 2008. Reproduced with permission of Springer Science+Business Media B.V.)

homology, including those of the unculturable *Photodesmus katoptron*, support the view that bacterial luminescence arose apparently once (Meighen, 1991; Dunlap and Ast, 2005; Peat and Adams, 2008; Hendry and Dunlap, 2011).

Bacteria emit light in an energy conserving manner depending on population density, termed quorum sensing. The bacteria release into the growth media a signal molecule, termed an inducer, which accumulates and triggers luminescence only after a critical threshold concentration is crossed. Below a critical density the generated light will probably not be seen by higher organisms and is, therefore, nonbeneficial. The regulatory mechanism of auto induction was first studied in *Aliivibrio fischeri*. This bacteria secrets a highly specific auto inducer purified as β-ketocaproyle homoserine lactone. *A. fischeri* are usually nonluminous in sea water while free living when their concentration is usually below $10^2$/ml. However, when a density of $10^7$/ml is reached the inducer activates the *lux* R regulatory gene, which in a simplistic manner acts as a specific inducer of the *lux* operon, leading to the generation of the enzymes involved in luminescence, as well as adding units of the inducer. In fish light organs, the concentrations of *A. fischeri* (e.g., $10^9$–$10^{10}$/ml) is by far above the threshold and light is continuously generated. The actual mechanism regulating quorum sensing is more complicated, involving more than one inducer and both negative and positive feedback loops (Ruby and Nealson, 1976; Nealson and Hastings, 1990; Meighen, 1991; Dunlap, 1999; Hoff, 2009).

## Symbiotic Luminescent Bacteria in Fish Light Organs

The anatomical, physiological and behavioral expression of luminescence reaches its zenith in fishes, being more diverse and complex than in any other group of organisms. Overall, the majority of luminescent fishes produce their own light in intrinsic numerous photophores, whereas only a minority forms symbiotic associations with luminescent bacteria which they harbor in few light organs. Partnerships with luminous bacteria, the subject of this chapter are only formed by approximately 500 species, constituting of less than 2% of all recognized fish species. However, these species are members of 21 families and 7 orders (Table 1.1). In contrast to the diversity of host fishes (Figure 1.2D, 1.2E, 1.2F), the symbiotic bacteria are few, relatively closely related, and found in three monophyletic groups (Figure 1.3). These bacteria consist of one group of facultative symbionts of the genera *Photobacterium* (Figure 1.2A, 1.2B, 1.2C) and *Aliivibrio* and two groups of not yet identified bacteria apparently occurring obligatorily with flashlight fishes and deep sea anglerfishes. These symbionts were suggested to be unable to reproduce outside the fish light organs due to extreme specialization and metabolic integration with their hosts (Herring and Morin, 1978; Haygood, 1993; Urbanczyk *et al.*, 2011).

Light produced by bioluminescent organisms at the water surface is too dim to be functional in full sunlight or moonlight. In shallow water luminous species of fishes are, therefore, crepuscular or nocturnally active (e.g., flashlight fishes) whereas deep water fishes (e.g., ceratioid anglerfishes) may use light irrespective of the circadian light cycle (Morin, 1981; Haygood, 1993). Luminescence in coastal water fishes is more often of a bacterial than of an endogenous origin. This phenomenon was suggested to be in some way related to the relative abundance of luminescent bacteria in coastal waters, particularly in the tropics and subtropics, and their reduced abundance in oceanic waters. However, some oceanic fishes which occupy the bathypelagic (e.g., Ceratioidei), mesopelagic (e.g., Ophistoproctidae) and benthopelagic (e.g., Macrouridae) zones, do harbor luminescent bacteria in their light organs (Herring, 1977, 1982). There are no fishes with luminescent bacteria in freshwater, except for few marine species such as *Gazza* spp. that enter estuaries and brackish water (Nicol, 1967).

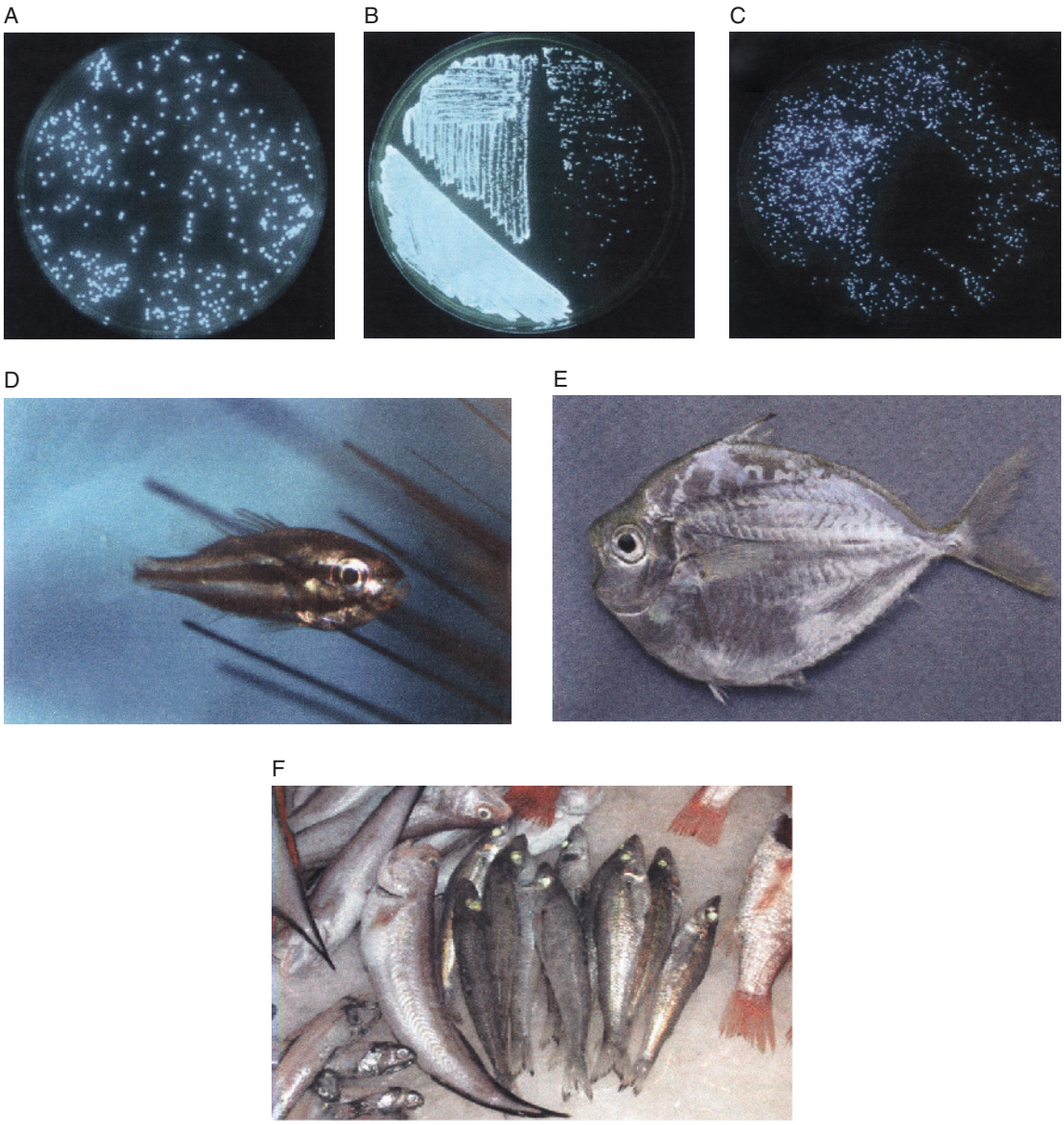


**Figure 1.2** Luminescent bacteria photographed by the light they produce and their host fishes: A. *Photobacterium mandapamensis*; B. *Photobacterium leiognathi*; C. *Photobacterium kishitanii* D. *Siphamia versicolor* host of *P. mandapamensis*; E. *Secutor megalolepis* host of *P. leiognathi*; F. *Chlorophthalmus albatrossis* host of *P. kishitanii* (Urbanczyk *et al.* 2011. Reproduced with permission of John Wiley & Sons).

Luminescent bacteria are contained extra cellularly within the light organs, occupying the sheltered spaces usually formed by parallel tubules or chambers. The bacteria obtain from their host both oxygen and nutrition for growth and luminescence (Harvey, 1952). Light organs may be either external, connected directly via pores or ducts to the surrounding sea water, or internal, opening into the fish's gut being only

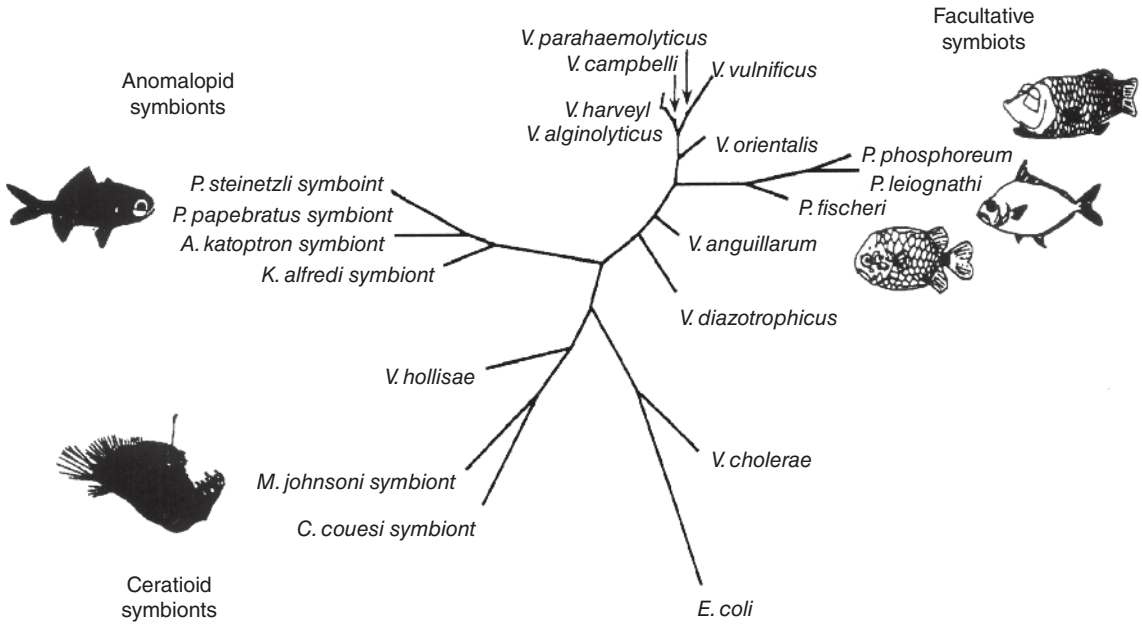


**Figure 1.3** Phylogenetic relationships among luminous bacterial symbionts and other Vibrios based on parsimony analysis of small subunit rRNA sequences. Representative hosts are illustrated next to their respective symbionts. *P. phosphoreum* in the original figure was changed to *P. kishitanii* (Haygood 1993. Reproduced with permission of Taylor & Francis).

indirectly connected to the environment. There is an enormous diversity of both structure and location of light organs (Figure 1.4). For example, light organs of cardinal fishes of the genus *Siphamia* consist of two different types. A single disc-shaped organ with complex masses of tubules located in the body cavity connected to the intestine, and paired gular sacs with a simple chambered structure which protrude into the oral cavity (Fishelson, *et al*., 2005; Thacker and Roje, 2009). Fish light organs are fundamentally different from intrinsic light producing photophores of fishes. Light organs consist usually of one or two organs but never more than of four, whereas a single fish may carry thousands of photophores. Light organs are always directly or indirectly open to the exterior for discharge of surplus bacteria or inoculation, whereas photophores are often closed. Internal light organs are mostly connected to the gut whereas photophores are usually not associated with the digestive tract (Herring, 1977). According to Herring (1977) fishes utilizing luminous bacteria in their light organs must cope with several issues in order to maximize their benefit from this association. Light organs must be infected with the appropriate species of bacteria, which has to be contained exclusively in the light organ, whereas other species should be excluded. Bioluminescent light must be maximized whereas bacterial growth must be strictly curtailed to save resources. Finally, the continuously produced bacterial light has to be controlled in order to effectively serve the fish. Means for light control include structures such as shutters, rotatable light organs and chromatophores which may completely block light passage. Accessory structures, such as differentially reflective swim bladders, translucent tissues and tubes lined with guanine crystals, may serve for light guidance, transmission and diffusion.

In most cases the presumed functions of light emission have their basis in inference from morphological and physiological characters and remain conjectural. Experimental studies involving light emission and direct *in situ* observations are rare. Even the monitoring of light emission in the field with submersibles and remotely operated vehicles is problematic, since many behaviors can only be observed unobtrusively (Widder, 2010). Despite the difficulty of studying bioluminescence in the marine

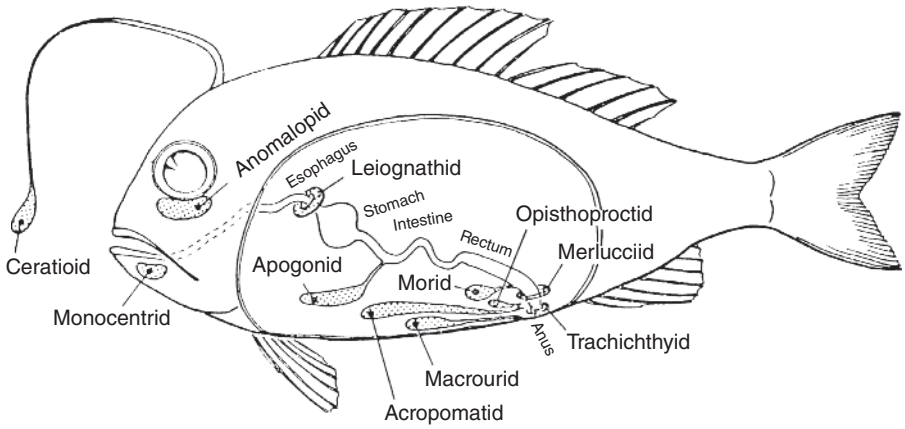


**Figure 1.4** The locations, sizes and openings of the light organs of various groups of luminescent fishes presented in a single diagrammatic fish. (Hastings *et al.* 1987. Reproduced with permission of Springer Science+Business Media B.V.)

environment and the little progress achieved, this topic has remained highly attractive over decades, leading researchers to frequently review the suggested functions for the emission of light (Nicol, 1967; Tett and Kelly, 1973; Herring and Morin, 1978; Morin, 1981, 1983; Young, 1983). One of the best reviews, presented by Morin (1983), included three major function categories of the emitted light, namely, predator evasion, prey capture and intraspecific communication. However, Morin (1983) in his review emphasized that multiple functions of a single light organ is common among fishes. In view of the dearth of behavioral observations on light organ operation and function, the three better known groups of fishes using bacterial light, the flashlight fishes, the ponyfishes and the deep sea anglerfishes are discussed here. These fishes represent both shallow coastal and oceanic deep sea fishes, harboring facultative and probably obligatory bacteria in external and internal light organs.

## Flashlight Fishes

### *Taxonomy and Distribution*

Eight relative small species of flashlight fishes have been described from the tropics and subtropics (Table 1.2). These night active, dark colored fishes, which are quite similar in their general appearance, belong to the Beryciform family Anomalopidae (meaning abnormal eye). The name of the family was derived from the suboccular light organs possessed by all flashlight fishes (Figures 1.5, 1.6, 1.7). The light organs are packed with a monomorphic culture of bacteria that continuously emit a bright blue-green light. The light from a single fish (e.g. *Photoblepharon steinitzi*) can be noticed underwater from a distance of 10–20 meters and from several hundred meters from the shore when watching a large school close to the water surface. Flashlight fishes control the passage of light from their suboccular light organs by either a shutter mechanism (e.g., *Photoblepharon*) or a rotational mechanism (e.g., *Anomalops*) or a combination of the two (e.g., *Kryptophanaron*). In the shutter mechanism a black skin flap is lifted up, completely covering the light organ, thus blocking the passage of light (Figure 1.7E, 1.7F, 1.7G, 1.7H), while in the rotational mechanism the light organ is turned downward facing the black-lined orbit (Figure 1.7A, 1.7B, 1.7C, 1.7D). Most flashlight fishes occur in the Indo-Pacific with only two species found in the New World. *Kryptophanaron alfredi* was collected in different localities in the Western Central Atlantic and *Phthanophaneron harveyi* has so far only been captured in the Eastern Pacific in the Gulf of California (Table 1.2). The relationship among the different flashlight fish genera was suggested on the basis of multiple morphological traits, such as body scale rows, fin spines and number of vertebrae, and traits related to the light occluding mechanism

**Table 1.2** Size, coloration, depth range and distribution of flashlight fishes.

| Species | Maximal standard or total length (mm) | Light organ (% head length) | Coloration | Depth range (meters) | Distribution | References |
|---|---|---|---|---|---|---|
| *Anomalops katoptron* (Bleeker, 1856) | 350[3] (TL) | 35.4[2] | Black, the base of the dorsal anal and pelvic fins white-grey[1] | 0–365[1] | Central Western Pacific Ocean[1] | [1] McCosker and Rosenblatt, 1987<br>[2] Baldwin *et al.*, 1997<br>[3] FishBase |
| *Kryptophanaron alfredi* (Silvester and Fowler, 1926) | 125[3] (TL) | 36.3–44.7[2] | Black, head and fins darkest, white scales at the basis of the second dorsal and anal fins[1] | 27–200[1] | Western Central Atlantic (Jamaica, Puerto Rico, Curacao, Grand Cayman, Bahamas) | [1] Colin *et al.*, 1979<br>[2] Baldwin *et al.*, 1997<br>[3] FishBase |
| *Parmops coruscans* (Rosenblatt and Johnson, 1991) | 66.5[2] (SL) | 35.6[3*] | Black, fins and lower part of head paler than the rest of the body[1] | 350[1]–440[2] | Tahiti[1] and Fiji[2] | [1] Rosenblatt and Johnson, 1991<br>[2] Johnson *et al.*, 2001<br>[3] Baldwin *et al.*, 1997 |
| *Parmops echinatus* (Johnson, Seeto and Rosenblatt, 2001) | 88.5[1] (SL) | 35.6[2*] | Black[1] | 440–550[1] | Fiji[1] | [1] Johnson *et al.*, 2001<br>[2] Baldwin *et al.*, 1997 |
| *Phthanophaneron harveyi* (Rosenblatt and Montgomery, 1976) | 204[2] (SL) | 22.7–31.2[3] | Black, lateral line scales lighter[1] | 32–36[1] | Eastern Pacific (Gulf of California)[1] | [1] Rosenblatt and Montgomery, 1976<br>[2] McCosker and Rosenblatt, 1987<br>[3] Baldwin *et al.*, 1997 |
| *Photoblepharon palpebratus* (Boddaert, 1781) | 120[3] (TL) | 48.6[2**] | Dark brown to black with a conspicuous white spot on the opercule[1] | 0–50[1] | Central and Western Pacific Ocean | [1] McCosker and Rosenblatt,1987<br>[2] Baldwin *et al.*, 1997<br>[3] FishBase |
| *Photoblepharon steinitzi* (Abe and Haneda, 1973) | 110[4] | 48.6[3**] | Dark brown to grey black[4] | 0–200[2] | Red Sea[1]<br>Comoro Island[1]<br>Maldive Islands[4]<br>southern Oman and Somalia[4] | [1] McCosker and Rosenblatt, 1987[1]<br>[2] Heemstra *et al.*, 2006<br>[3] Baldwin *et al.*, 1997<br>[4] FishBase |
| *Protoblepharon rosenblatti* (Baldwin, Johnson and Paxton, 1997) | 229 (SL) | 14.5 | Dark brown to black (in alcohol) | 274 | Cook Islands | Baldwin *et al.*, 1997 |

*Computed for the genus *Parmops*
**Computed for the genus *Photoblepharon*

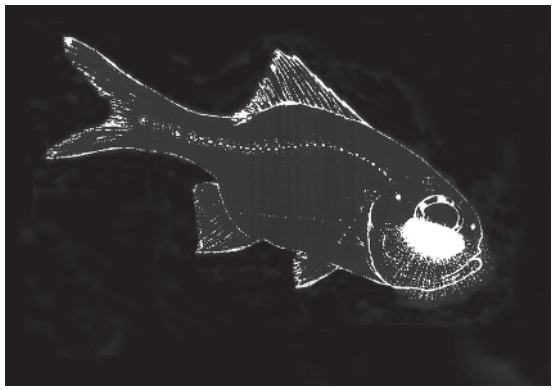

**Figure 1.5** *Photoblepharon steinitzi* (Reproduced with permission of D. Darom).

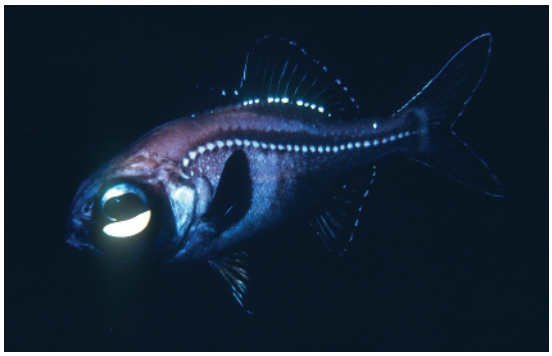

**Figure 1.6** *Kryptophanaron alfredi* (Reproduced with permission of P. Colin).

(Johnson and Rosenblatt, 1988; Rosenblatt and Johnson, 1991; Baldwin *et al*., 1997; Johnson *et al*., 2001). *Photoblepharon* and *Anomalops*, genera with different light occluding mechanisms are maximally separated but also interconnected by several genera that possess both light occluding mechanisms (Figure 1.8).

Several species of flashlight fishes (e.g., *Parmops echinatus*) were only collected from depths of several hundred meters (Table 1.2). Other species (e.g., *Photoblepharon steinitzi*) ascend during moonless nights from deep water in the Comoro Islands (McCosker and Lagios, 1975) or dark caves in relative shallow water in the Red Sea (Fridman, 1972) to feed at the water surface on zooplankton. Some flashlight fishes remained unknown for a long time because of their occurrence at depths not accessible to scuba divers and in areas with hard bottoms that preclude collecting of fishes with nets and trawls. These species were only discovered after being unexpectedly found in the stomach of a grouper (i.e., *Parmops coruscans*) and in a prawn trap (*Parmops echinatus*). Some species of flashlight fishes, such as *Kryptophanaron alfredi*, were believed to be extremely rare, since only a single specimen was collected in 1907 at the water surface off Jamaica (Dahlgren, 1908) and the species was not encountered over the next seventy years. However, after researchers realized that flashlight fish are active during moonless nights, additional specimens were collected from different localities in the Western Central Atlantic (Colin *et al*., 1979). In the future, more flashlight fish species and new records will probably be discovered with aid of manned submarines and scuba divers during dark night dives.

## The Light Organs

Early researchers who examined preserved specimens of flashlight fishes did not consider light organs as such. According to McCosker (1977), Boddaert in 1781 suggested that these structures protect the eyes of the fish from coral branches, while Lacepede in 1803 suggested that they serve for eye protection from solar radiation. Vorderman in1900 (cited in Harvey, 1922) was the first to report seeing light produced by the living fish. Harvey (1921, 1922) provided evidence that the light originates from bacteria which occupy the light organs. He based his suggestions on microscopy of the bacteria and several characteristics of the luminescence, such as continuous light emittance and the inhibiting effect of desiccation and potassium cyanide on light production, which are typical for bacterial light. More recently, bacterial luciferase activity was detected in anomalopid light organ extracts (Leisman *et al*., 1980). The ultimate proof that the bacteria are the source of light requires that these bacteria are grown in a pure culture which luminesces. Our inability to rear these bacteria outside the light organs was suggested to be due to the bacteria's obligate dependence on their host for the supply of factors essential for their growth (Haygood, 1993). Inability to rear these bacteria hindered research related to their relationship with other bacteria, identification of the genes of their luminescent system and bacteria–host interactions.

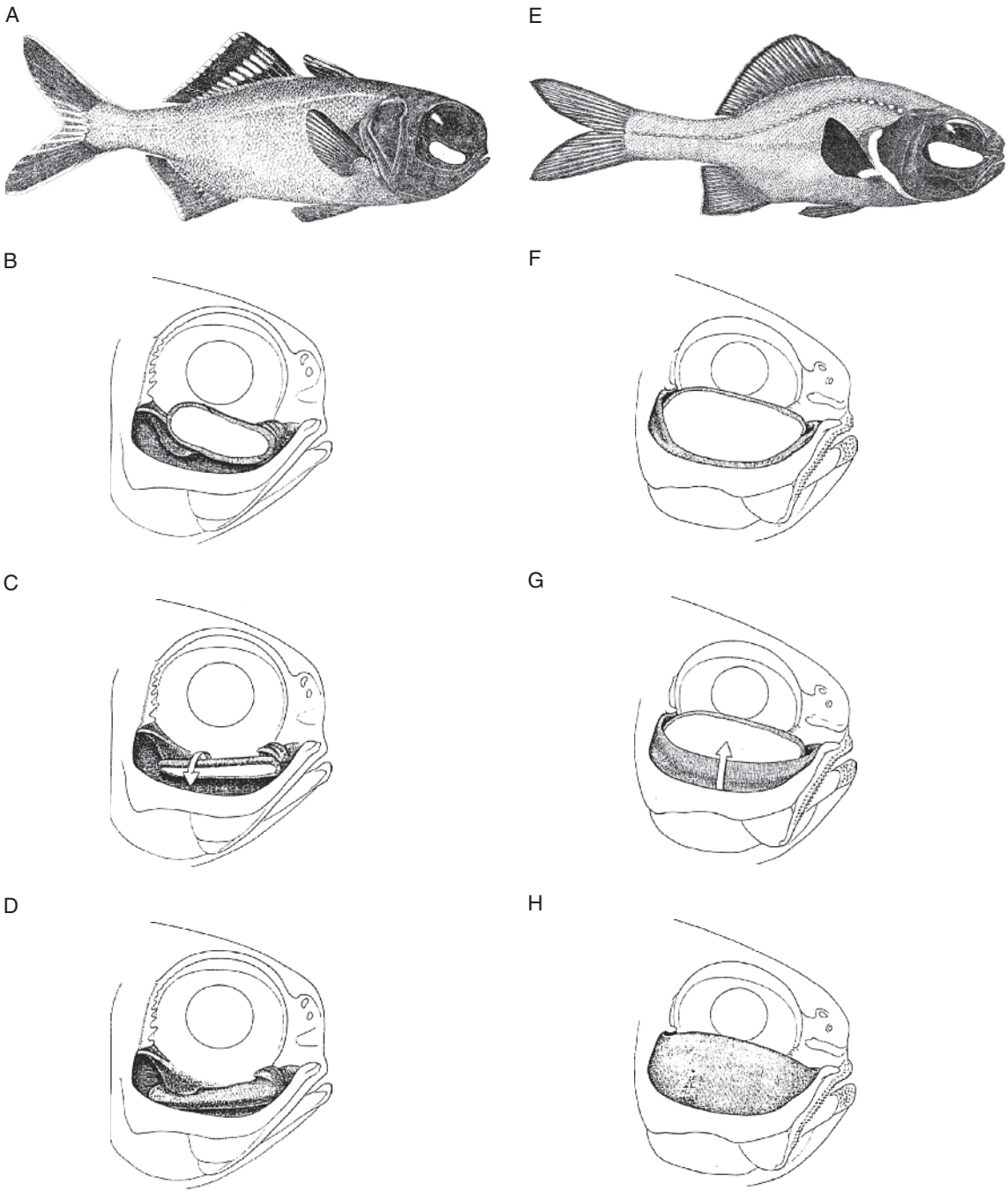


**Figure 1.7** The rotational and shutter light occluding mechanisms of flashlight fishes. Left side: The rotational occluding mechanism of *Anomalops katoptron* (A); the exposed light organ (B); the downward rotation of the organ into a pouch (C); the occluded light organ (D). Right side: The shutter occluding mechanism of *Photoblepharon palpebratus* (E); the exposed light organ (F); the upward lifting of the shutter (G); the occluded light organ (H). (McCosker 1977. Reproduced with permission of Scientific American, Inc.)

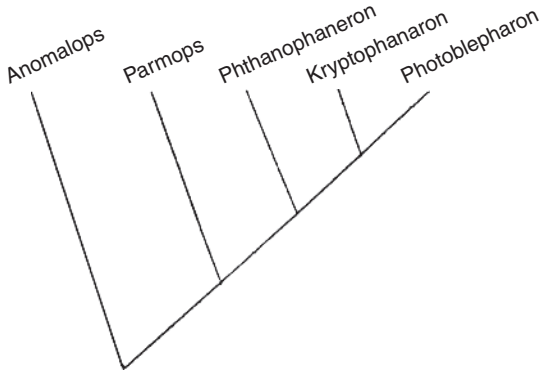


**Figure 1.8** Cladiogram of anomalopid fishes (Rosenblatt and Johnson 1991. Reproduced with permission of Allen Press Publishing Services).

However, recently, culture independent methods, such as PCR amplification of DNA of bacterial cells, which occur at high concentrations in the absence of other bacteria in the light organs have led to new insights (Haygood and Distel, 1993; Hendry and Dunlap, 2011). These exciting new discoveries are discussed in a later section dealing with the evolution of the fish–bacteria symbiosis.

Several early studies addressed the structure of the light organs of flashlight fishes (Dahlgren, 1908; Steche, 1909). A more recent detailed study of this structure in *Anomalops katoptron* was carried out by Bassot (1968) with aid of light microscopy. Subsequently, Kessel (1977) studied the ultrastructure of *Photoblepharon steinitzi* with aid of light, scanning and transmission electron microscopy. The structure of the light organs of these two species were quite similar (Kessel, 1977). Anomalopid light organs are composed of four basic structural and functional elements: black pigment cells which block light passage, guanine crystals that reflect light, epithelial tubules which contain luminescent bacteria in their extra cellular lumen and a translucent epidermis that transmits the generated light. The entire light organ except for its anterior face is covered from the outside by layers of black pigment cells richly supplied by blood vessels and nerves. This layer prevents the passage of light into the fish's eyes and body.

Light reflectance was studied in detail in *Anomalops katoptron* (Watson *et al*., 1978). In this species, two light reflectors differing in structure and function were described. The internal opaque reflector, which is positioned above the pigment cells, contains stacks of hexagonal guanine crystals positioned parallel to the reflectance surface. It reflects about 70% of the visible spectrum. The partly translucent external reflector is positioned at the lower ventral edge of the anterior face of the organ. It consists of a row of thin crystals lying obliquely to the reflecting surface. This reflector according to Watson *et al*. (1978) directs light outward and upwards and dims light emission ventrally. Light emission is augmented by about 35% due to light reflectance. Epithelial tubes of a length of about 0.5–1.0 mm and a diameter of about 30–40 µm lie perpendicular to the sagital plane of the fish. The walls of the tubes, which are triangular with rounded corners in cross-section, consist of a single layer of epithelial cells. These tubes are packed with luminescent bacteria. Five to six tubes are clustered around a capillary forming a rosette-like structure. Whereas the top of the tubes are open, at their base are cells rich with mitochondria (Kessel, 1977), which are further discussed in the section dealing with bacterial light intensity regulation by the fish. The bacteria in the light organ of *Photoblepharon steinitzi* are rod shaped, gram negative measuring 3–6 µm in length and 0.5 µm in width, often possessing 1–3 flagella of the polar sheated type. The anterior face of the light organ is covered by a transparent perforated epidermal layer that transmits the light generated by the bacteria. In this layer are several hundred pores structured like craters about 13–15 µm wide. Several parallel tubes open individually into a common atrium, which itself is connected to the outside by a pore. Surplus bacteria are released from the light organ through these pores into the surrounding water (Nealson *et al*., 1984). The light organ is supported by a fibrocartilaginous cup. This cup articulates at the anterior end of the organ with a fibrocartilaginous stalk that contains blood vessels and nerves. This stalk and several accompanying ligaments form the only connection of the light organ with the rest of the fish's body, being otherwise freely suspended in the lower part of the orbit.

Newton Harvey, a pioneer of bioluminescence research (Harvey, 1952), raised the question of the different mechanisms controlling light passage (i.e., rotational vs shutter) in two related fishes, *Anomalops katoptron* and *Photoblepharon palpebratus*. In his own words "why these two similar in most respects and especially in the general structure of the luminous organ, should have developed

such totally different means of extinguishing the light is a mystery" (Harvey, 1922). This enigma was solved by Johnson and Rosenblatt (1988) in a detailed study of the functional anatomy of the light occluding mechanisms of all anomalopid fishes known at that time. Following their study, light occluding mechanisms were also described in newly discovered species of flashlight fishes (Rosenblatt and Johnson, 1991; Baldwin *et al*., 1997; Johnson *et al*., 2001). In their study Johnson and Rosenblatt (1988) discovered that the light occluding mechanisms are only superficially different whereas in reality they are similar in many aspects. The power for both mechanisms involves the Adductor Mandibulae muscles transmitted through the same complex biomechanical linkage, which includes the Ethmomaxillary and Diogenes ligaments, the latter present only in flashlight fishes. The ligament of Diogenes inserts in *Anomalops* at the ventral lateral (outer) corner of the cartilogenous cup, unlike *Photoblepharon*, where this ligament inserts at the ventral medial corner. Due to the differential attachment of this ligament a pull on it results in rotation of the organ in *Anomalops* but not in *Photoblepharon*. Only *Anomalops* possess at the floor of its orbit a fibrocartilaginous rotation pad. The cartilogenous stalk of *Photoblepharon* possess a hook that is firmly attached to a moveable cartilaginous knob. Both these structures, which are essential for lifting the shutter, are missing in *Anomalops* but are found in less developed forms in other species such as *Kryptophaneron*, which possess both light occluding mechanisms.

According to Rosenblatt and Montgomery (1976) the light organs probably evolved in the twilight zone habitat. Johnson and Rosenblatt (1988) stated that the ancestral flashlight fish probably controlled light passage through a forced rotational mechanism similar to that of *Kryptophaneron*, possessing also a skin flap at the base of the light organ which may have been erectable or not. The light occluding mechanisms diverged from the ancestral form into two lines, namely the flipping rotational and the shutter mechanisms (Figure 1.7). Whereas *Anomalops* diverged from the early ancestral flashlight fish about sixty million years ago, extant flashlight fishes with a shutter mechanism evolved only within the last four to five million years (Wolf and Haygood, 1991). The series of five genera, which starts with *Protoblepharon* followed by *Parmops, Phthanphanaron, Kryptophaneron* and culminates with *Photoblepharon*, provides "a rare illustration of the gradual evolutionary elaboration of a functional complex in which each genera exhibits a slightly more intricate and integrated linkage system to effect the shutter erection" concomitantly with a gradual increase in the relative size of the light organ (Rosenblatt and Johnson, 1991; Baldwin *et al*., 1997). The unnecessary complexity of the shutter mechanisms is according to Johnson and Rosenblatt (1988) possibly a result of functional-morphological constraints imposed on the system by the pre-existence of a rotational mechanism in the ancestral flashlight fish. Finally, actual blinking in flashlight fishes is more complicated than usually assumed. In *Anomalops* lateral and anterior movement of the stalk causes the entire organ to swing out and forward (Johnson and Rosenblatt, 1988). Moreover, the light from each organ can be independently controlled and also "squinted" by partially occluding the organ (Morin unpublished in Herring and Morin, 1978).

To better understand the relationship between flashlight fishes and the bacteria which occupy their light organs, Meyer-Rochow (1976a) carried out a starvation experiment with seven *Anomalops katoptron*. These fish were deprived for four weeks of food and concomitantly the luminescence of the bacteria was monitored. Following one week of starvation light became dimer. After two weeks a black central spot appeared in the light organ and finally after three weeks the light seemed to be extinguished to the human eye. Four weeks after the start of the experiment, the fish showed no signs of malnutrition. The light organs were dissected and were found to contain fewer bacteria compared with fresh light organs. Meyer-Rochow (1976a) concluded that the fish transfers via the blood capillaries either nutrients essential for bacterial growth or substances directly related to light production such as long chain aldehydes. Flashlight fishes lose luminescence not only in response to starvation and malnutrition but also in response to an environmental temperature stress (Haygood, 1993), low ambient oxygen and prolonged exposure to light (Herring and Morin, 1978). There have been reports of flashlight fishes with extinguished light organs that regained luminescence 3–6 months following maintenance in isolation. Moreover, fish with extinguished lights maintained together with several fish with bright lights failed to do so (Haygood, 1993). Haygood (1993) suggested on the basis of these facts that regaining luminescence is probably related to resumption of light production by surviving bacteria and not due to new colonization of the organ. Flashlight fishes with extinguished lights were successfully maintained

for many months in captivity, provided they were fed under dim light conditions. This fact suggests that the major benefit for the fish from the bacteria, at least under captive conditions is the light they produce (Haygood, 1993).

## The Eye and the Light Organ

An histological study of the eyes of two flashlight fishes *Photoblepharon palpebratus* and *Anomalops katoptron* by Meyer-Rochow *et al.* (1982) revealed in both species structural dim light adaptations, such as relative large eyes, pupils and lenses, and the presence of only rods in the retina. On the basis of the ratio of nuclei in the various retinal layers of these two species, Meyer-Rochow *et al.* (1982) suggested that *Photoblepharon* has a greater light sensitivity, whereas *Anomalops* a better visual acuity. These suggestions are in accord with his own field observations in the Banda Islands that the former fish is more photophobic confining its activity to nights with very low levels of light.

The light emitted by the bacteria residing in the light organ of *Photoblepharon steinitzi* was found to correspond with the light to which this fish's eye is most sensitive – λ max 496 nm (Girsch, 1976). This finding clearly supports the suggestion of Meyer-Rochow *et al.* (1982) that the eye and the light organ form a well-adjusted pair of organs in flashlight fishes. According to Morin (unpublished, cited in Herring and Morin, 1978) the light organ of *Photoblepharon* is flush with the eye emitting a discrete beam laterally and somewhat anteriorly and downward. In *Anomalops* the exposed light organ, which is flared out, partially overlapping with the eye, emits a more anteriorly-laterally directed beam. The angular distribution of light radiating from the organs of freshly killed *Photoblepharon palpebratus* and *Anomalops katoptron* was monitored in the laboratory (Herring, 1982). *Photoblepharon* had a broad and uniform angular distribution whereas *Anomalops* had a more limited dorsal illumination (Figure 1.9). However, live *Anomalops* were suggested to possess some ability to alter the direction of the emitted light (Herring and Morin, 1978). According to Morin (1981) the position of the light organ close to the eye reduces the parallax between the eye and the light source, allowing the fish to detect the tapetal reflection of its crustacean prey. Thanks to the bacterial light beam the fish is able to feed on smaller zooplankton (e.g., crustaceans in the range 1–3 mm) than most nocturnal fishes (Morin and Harrington, unpublished, cited in Morin, 1981).

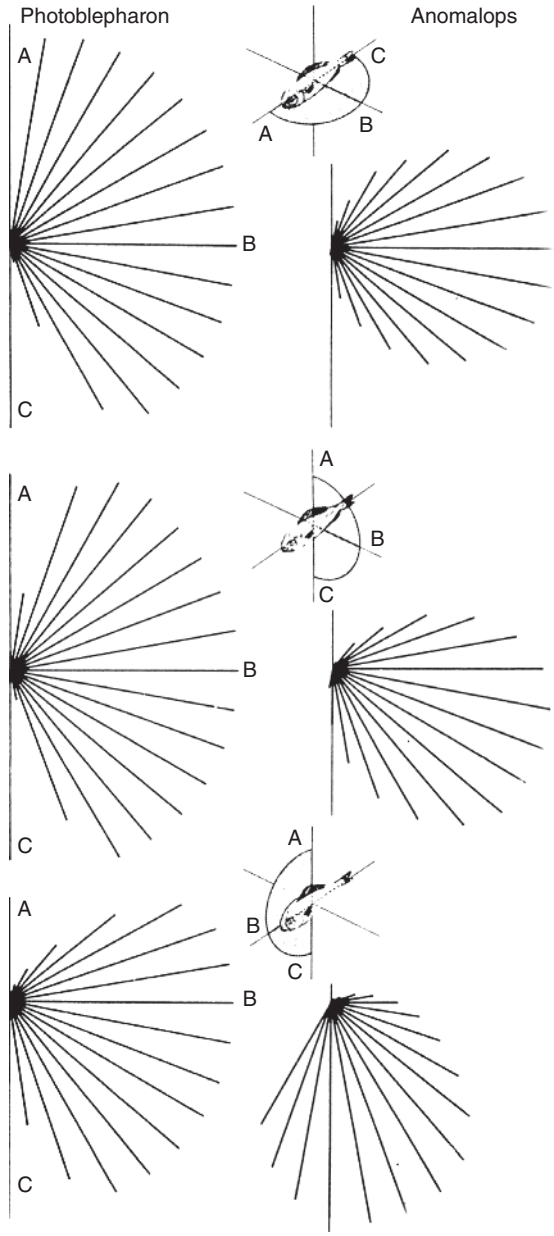


**Figure 1.9** The angular distribution of light from the light organs of *Photoblepharon palpebratus* (left) and *Anomalops katoptron* (right). The plane in which each pair of measurements was made is indicated by the central diagram; the relative light output is indicated by the length of the line in each direction; the longest line indicates the direction of the maximum intensity and has been normalized to the same length in each angular diagram (Herring 1982. Reproduced with permission of Taylor & Francis).

Howland *et al.* (1992) suggested that the spatial relationship of the light organ to the pupil of the flashlight fish is similar to that of some photoretinoscopes, facilitating the detection of fishes due to their tapetal eye shine. *Anomalops katoptron* makes a retinoscope-like movement when rotating the light organ away from its pupil. According to these authors, at a four meter distance the eyeshine of a conspecific will be four orders of magnitude brighter than that of reflections from adjacent surfaces.

## *Reproduction, Larval and Light Organ Development*

Knowledge of the breeding season and reproductive behavior of flashlight fishes is mostly missing, fragmentary and mainly restricted to the genus *Photoblepharon*. In the Red Sea, the breeding season of *P. steinitzi* occurs between July and September. Following daily exposure of a pair to 14 hours of light during the winter, sexual maturation was attained in January (Sagi, 1978). In the Banda Islands, mature eggs were striped from *P. palpebratus* and *Anomalops katoptron* in October (Harvey, 1922) and, in the case of the former species, captured pairs also spawned in April (Meyer-Rochow, 1976b). Finally, ripe *Kryptophanaron alfredi* females were collected in Puerto Rico in April and in January in the Cayman Islands, with the former group shedding infertile eggs three days after capture (Colin *et al.*, 1979).

Spawning of flashlight fishes has never been witnessed either in the field or captivity. Sexual dimorphism has only been reported for the genus *Photoblepharon*. Females of *P. palpebratus* possess more rounded tail fin edges than males (Meyer-Rochow, 1976b). In established pairs of *P. steinitzi* (Morin *et al.*, 1975) and *P. palpebratus* (Meyer-Rochow, 1976b) females are larger than males. Pairs of *P. palpebratus* captured in shallow water in the Banda Islands successfully spawned at night in small aquaria. In the case of a single pair kept with a male or two pairs kept together, the male and female of each pair were closely associated, swimming one after the other in circles or figures of eight with the female leading most of the time. The proximity of additional individuals was avoided by the pair but no aggressive interactions were witnessed. This is in contrast to *P. steinitzi*, where pairs in shallow water defended territories from conspecific intruders (Morin *et al.*, 1975). Finally, a group of nine *Kryptophanaron alfredi* was once observed close to the substrate and to one another at a depth of 36 meters off Puerto Rico within less than 1 $m^3$. The fish were "blinking" their lights rapidly and swimming around one another, suggesting a form of courtship or spawning aggregation (Colin *et al.*, 1979).

A single *Photoblepharon palpebratus* female can produce in a breeding season up to 1000 transparent spherical positively buoyant eggs 1.2 mm in diameter. After a short planktonic phase of 5–10 hours, these eggs become negatively buoyant and adhere to the substrate with the aid of a sticky adhesive substance that covers the vitaline membrane. Dissection of the ovary of a mature female revealed the presence of eggs of three size classes: large ripe eggs about 1 mm in diameter, medium sized eggs (diameter of 0.2–0.6 mm) and tiny eggs (diameter below 0.2 mm) (Meyer-Rochow, 1976b). A similar structure of mature eggs has also been reported for *Anomalops katoptron* (Harvey, 1922; Colin, 1988). According to Meyer-Rochow (1976b), the short planktonic phase of the eggs may explain the limited distribution of *Photoblepharon palpebratus* occurring in the Banda Islands but not in other localities in the South Moluccan Sea despite presence of habitats suitable for these fish.

Developmental series of flashlight fishes encompassing the ontogeny of the light organs are missing (Baldwin and Johnson, 1995). Moreover, there are only few data on larval flashlight fishes. Colin (1988) described early pre-flexion *Anomalops katoptron* larvae from hatching (2.6–3.3 mm NL -notocord length) to an age of 132 hours post hatch (4.2 mm NL) based on larvae reared in the laboratory. The larvae hatch with a fairly large yolk sac, unpigmented eyes, and undeveloped mouth. Distinctive features of these pre-flexion larvae are slender body, long straight gut, large pelvic fins and heavy pigmentation (Figure 1.10A, 1.10B, 12.10C). There was no sign of luminescent bacteria in these larvae. A 5.8-mm (SL) post-flexion *Anomalops katoptron* was collected in the Western North Pacific (Konishi and Okiyama, 1997). The head of the larva was large, its length occupying nearly one-half of the body length. An unpigmented crescent-shaped tissue lay beneath the eye, possibly representing the incipient luminous organ (Figure 1.10D). Regretfully, the presence of bacteria inside this organ was not examined. A 6.2-mm (NL) larva of *Kryptophanaron alfredi* in an advanced state of Notochordal flexion was collected with a mid-water trawl off the Bahamas from a water depth ranging between 400 meter and the water surface. The larva possessed an anteriorly-directed rod-like projection on each side of the snout (Figure 1.11A, 1.11B).

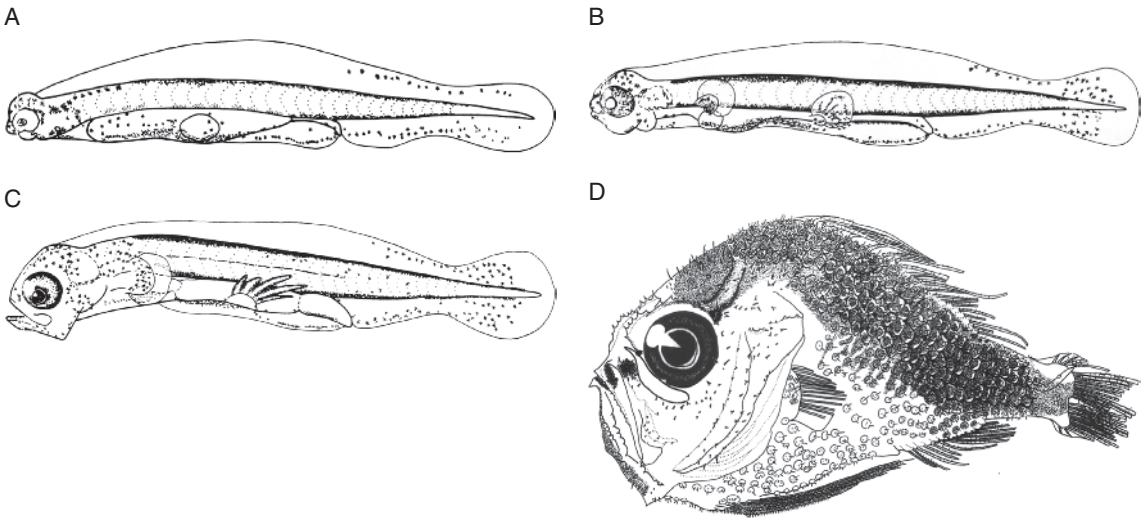


**Figure 1.10** Reared and captured larvae of *Anomalops katoptron*: A. 3.3-mm NL (12 h post hatch); B. 3.5-mm NL (38 h post hatch); C. 3.8-mm NL (62 h post hatch); D. 5.8-mm SL (captured larva). (A–C: Colin *et al.* 1988. Reproduced with permission of University of Hawaii Press; D: Konishi and Okiyama, 1997. Reproduced with permission of the Rosenstiel School of Marine and Atmospheric Science.)

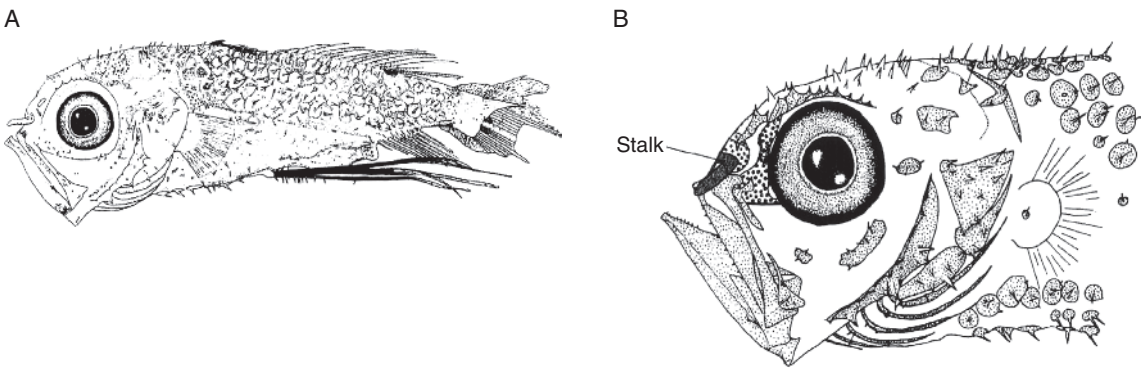


**Figure 1.11** Larva of *Kryptophanaron alfredi:* A. 6.2 mm NL larva; B. Enlarged head with stalk. (Baldwin and Johnson 1995. Reproduced with permission of the Rosenstiel School of Marine and Atmospheric Science.)

These projections may represent the anlage of the fibrocartilaginous cup, stalk as well as the light organ. Examination of the projections with light and electron microscopy revealed invaginations of epidermal tissue reminiscent of those characterizing the light organs of adults (Haygood personal communication cited in Baldwin and Johnson, 1995). According to Baldwin and Johnson (1995), the ultrastructure of the rod as well as the lack of any anlage below the eye for the development of the light organ and supporting structures, supports the notion that, indeed, these structures develop from the rostral rod projections. The light organs are fully developed and functional in small juveniles (e.g., *Photoblepharon steinitzi* about 15 mm SL (Sagi, 1978), *Kryptophanaron alfredi* 25 mm SL (McCosker, 1982), *Phthanophanaron harveyi* 20 mm SL (Baldwin and Johnson, 1995)), indicating that the flashlight fish are infected sometime before this stage. According to McCosker (1982), a pronounced

negative allometry was found between the length of the light organ of *Kryptophanaron alfredi* (Figure 1.6) and the head length. A relatively larger light organ is advantageous in accommodating enough bacteria to provide sufficient light for a small fish. A similar but less significant trend was also found in fishes of the genus *Photoblepharon* which among all flashlight fishes possess the largest light organs (Table 1.2).

The mechanism by which anamalopid bacteria transfer between host generations is not known (Hendry and Dunlap, 2011). However, Baldwin and Johnson (1995) suggested that the rarity of planktonic flashlight fish larvae may be due to the fact that the few captured larvae were stray individuals and most of the larvae are not planktonic as suggested by McCosker (1982). According to Baldwin and Johnson (1995), the short positive egg buoyancy and the egg stickiness facilitate adherence to substrates in the adult's caves where the eggs are spawned. The larvae were suggested to normally develop inside the caves and cracks occupied by the adults during the day and there they also become infested by the symbiotic bacteria expelled from adult light organs. Baldwin and Johnson's (1995) suggestions for the transfer of bacteria between generations have so far not been either confirmed or refuted.

### *The Photophobic Response*

Flashlight fishes are extremely sensitive to strong illumination. Researchers have taken advantage of this trait, approaching the fish in the darkness and then stunning them by rapid exposure to strong diving lights, which allows their easy capture with hand nets (McCosker and Lagios, 1975; McCosker, 1977; Colin *et al*., 1979). A longer exposure of the fish to lights may result in the darkening of the light organs (Herring and Morin, 1978). Sagi (1978) reported an unfortunate long term exposure of ten *Photoblepharon steinitzi* to lights that resulted in the darkening of their light organs and their subsequent death. In the laboratory *Anomalops katoptron* exposed to white and black substrates in differently illuminated aquaria consistently occupied the darker areas. Under similar levels of illumination these fish equally frequent occupied the dark and light substrates (Meyer-Rochow, 1976b).

The nocturnal activity and particularly, the avoidance of nights with a bright moon by all studied flashlight fishes have already been mentioned several times in this text (Haneda and Tsuji, 1971; Morin *et al*., 1975; Meyer-Rochow, 1976a, 1976b; Colin *et al*., 1979). In the case of *Photoblepharon steinitzi* nocturnal activity outside the shelter was restricted in time between one hour after sunset and one and a half hours before dawn (Herring and Morin, 1978). Almost nothing is known of the behavior of flashlight fishes in the field during the day, since these fishes occupy inaccessible caves and crevices. Our only daytime observations in the field are those carried out on *Photoblepharon steinitzi* off the Grand Comoro Island with aid of a manned submarine (Heemstra *et al*., 2006). Flashlight fish were reported to be common during the day inside and outside of Coelacanth caves at depths of several hundred meters. According to McCosker (1977), the restricted foraging of *Photoblepharon steinitzi* due to avoidance of bright nights was probably counterbalanced by the accumulation of large fat reserves in the coelom of these fish. The resulting increase in fish buoyancy was suggested to be compensated by a decrease in the size of their swim bladder compared with related similarly sized fishes. A small swim bladder is also advantageous for a fish carrying out a large daily vertical migration. Based on this information, Studer and Wirz (1984), of the Basel Zoo, successfully maintained a group of *Photoblepharon steinitzi* for several years in captivity, without providing the fish with food for one week around the time of the full moon. Food was withheld to avoid surplus feeding possibly leading to obesity.

In contrast to most other organisms that periodically emit light in flashes, flashlight fishes are blinking, namely blocking the light which is continuously produced by the symbiotic bacteria. The duration of light occlusion by either a shutter or by the rotation of the light organ depends on the blinking rate and the length of the light-off period. During the night in the Red Sea, active nondisturbed *Photoblepharon steinitzi* emit light almost continuously with only brief interruptions caused by the short lifting of the shutter. The blinking rate of the fish was estimated from laboratory measurements with a photomultiplier to be about 2.9 blinks per minute with a short light-off period of about 260 ms (Morin *et al*., 1975). During the day, the fish in illuminated aquaria shelter inside inverted flower pots with occluded light organs (Sagi, 1978). A different blinking pattern was observed in *Anomalops katoptron* during the night on the reefs of the Banda Islands, resulting in the occlusion of the light organ for about a quarter of the time. Again, on the basis of laboratory measurements

with photomultipliers, the blinking rates were estimated to be about 65–70 per minute, with a mean light-off period of about 250 ms (Morin and Harrington, unpublished, cited in Herring and Morin, 1978). The longer occlusion time of *A. katoptron* was suggested by Rosenblatt and Montgomery (1976) as an antipredatory strategy of this elongated open water fish, which forms schools of up to 200 individuals. According to these authors, the relative long light-off periods, combined with the fish movement hinder the focusing of piscivores on a specific target. In the case of *Photoblepharon steinitzi*, the continuous light emission is less hazardous due to the close proximity of these fish to sheltering rocks and corals. A circadian blinking rhythm was maintained by several isolated *P. steinitzi* under conditions of continuous darkness shortly after their capture on the reef (Morin *et al*., 1975). During the night the fish emitted light almost continuously as observed on the reef, whereas during the day the light organs were occluded about half of the time. *P. steinitzi* that were isolated under similar conditions for periods of several months lost their circadian blinking rhythm. These fish displayed varied mean daily blinking rates ranging between 7 and 63 blinks per minute with long light-off periods (Sagi, 1978).

## The Use of Light by Flashlight Fishes

Scarcity of knowledge concerning the use of light by fishes is mainly due to paucity of *in situ* observations on light production, the fragility of many of these fishes when captured and the difficulty in maintaining them in captivity often after removal from deep water. All this leads to excessive speculation and nonrigorous anthropomorphic interpretations of the role of light in the lives of fishes (Herring, 1990). The anomalopid fishes are one of the exceptions. The functions of light were better studied in flashlight fishes due to their predictable occurrence in shallow water and the ease of their maintenance in captivity.

Early researchers suggested, without evidence, that light was used by flashlight fishes to see (Steche, 1909; Haneda and Tsuji, 1971) and to attract prey (Harvey, 1922; Haneda and Tsuji, 1971; Fridman, 1972). Most of our current knowledge on the use of light by these fishes is based on Morin *et al*.'s (1975) study addressing the multifunctional use of light by *Photoblepharon steinitzi* and a combined field and laboratory study, carried out subsequently on the same species by Sagi (1978). This study is regretfully only available as a nonpublished MSc dissertation written in Hebrew.

According to Morin *et al*. (1975) light is used in three different ways in coping with predators, namely by predator detection, confusion and evasion. *P. steinitzi* uses a special pattern of light emission combined with movement to effectively outmaneuver predators called "blink and run". A fish moves slowly while emitting light but then when the light is occluded it accelerates its speed and moves into an unpredicted direction, resuming light emission from a different position. This pattern is performed in the field whenever the fish moves in hazardous exposed areas or when disturbed. Likewise, in the laboratory, disturbed fish practice "blink and run" at a rate of 75 blinks per minute with 160 ms light-off periods. Piscivores have difficulty in focusing on a specific target when facing large diurnal schools or aggregations of fishes (Neil and Cullen, 1974). Morin *et al. (*1975) suggested that a similar confusion effect may benefit at night schools of flashlight fishes. A persistent disturbance of schools of *P. steinitzi* in shallow water, which initially led to the performance of "blink and run", eventually led to the dispersal of the schools and the sheltering of fish near rocks and corals with occluded light organs (Sagi, 1978). McCosker (1977) suggested that a piscivore which captured a flashlight fish could be startled by the sudden emission of light. However, handling of flashlight fishes did not evoque any change in light emission (Steche, 1909; Haneda and Tsuji, 1971) and likewise close proximity in the laboratory to a medium sized piscivore –*Pterois* sp. (Sagi, 1978).

According to Morin *et al. (*1975) light may assist *P. steinitzi* in both seeing and attracting prey. Flashlight fishes feed mainly on zooplankton and, particularly, on crustaceans. Stomach content analysis of *P. palpebratus* revealed presence of small crustaceans, polychaetes and a few tiny fish scales (Meyer-Rochow, 1976b). Sagi (1978) reported finding in *P. steinitzi* stomachs planktonic as well as benthic crustaceans, fish larvae, polychaetes and hydromedusae. Three specimens of the Atlantic flashlight fish *Kryptophanaron alfredi* contained in their stomachs mainly shrimp and copepods (Colin *et al*., 1979). Morin *et al. (*1975) described the active capture of adult *Artemia* by *P. steinitzi* by the light of their luminous organs. Furthermore, Sagi (1978) found a significant 30% reduction in the rate of

blinking resulting in a longer light-on condition when presented with live *Artemia*. Finally, McCosker (1977) reported of a group of *Anomalops katoptron* with extinguished light organs that were unable to feed on live *Artemia*. However, when light was provided at a level of intensity similar to that produced by the symbiotic bacteria the fish were able to feed on the crustaceans.

Morin *et al.* (1975) suggested that *P. steinitzi* attract their prey with the lights they control due to the positive phototactic response of many crustaceans. Large schools of these fish provide a bright area of considerable size that may be particularly effective in this respect. Sagi (1978) tested and confirmed this hypothesis. In a field experiment he contrasted the structure of the planktonic community present close to a school of about one hundred *P. steinitzi*, white and blue-green lights produced by underwater diving lights and a control of a nonilluminated area. The plankton community in the nonilluminated controls consisted mainly of copepods (88%), few isopods, amphipods, mysids and cumaceans (1–3%), and similarly few nematods and fish larvae (2–3%). The areas illuminated by the diving lights and the fish school contained a relative smaller fraction of copepods (66%) but about four times more of the larger crustaceans such as amphipods and mysids. Analysis of the stomach contents of *P. steinitzi* reported within the frame of this study revealed selective predation of large crustaceans, with amphipods, mysids and cumaceans contributing about (65%) of the ingested items, whereas the much smaller copepods made up only 10%. The total ingested biomass is clearly much more in favor of the former group. Possibly *P. steinitzi* in schools feeds mainly on large crustaceans attracted to the lights of the group whereas individuals foraging by themselves or in pairs may ingest mainly small crustaceans such as copepods.

Man has taken advantage of the attraction of fishes such as groupers to the lights produced by flashlight fishes. For generations, the Banda Islands fisherman removed the light organs and attached them above their hooks as an effective lure that emits lights for 8–10 hours (Harvey, 1922; McCosker, 1977). In a more sustainable approach, flashlight fishes are introduced by fisherman into small perforated bamboo cages and reused over and over again during night fishing by suspending these cages below their canoes (McCosker and Lagios, 1975; McCosker, 1977).

Intraspecific communication in the field with light generated by symbiotic bacteria was reported in the context of school formation, territorial defense and sexual signaling.

### *School Formation*

Schools of *Photoblepharon steinitzi* are not fixed formations according to Sagi (personal communication) but are reformed at night by individuals that have left their shelters and joined together. The school assembly signal is transmitted by individuals that ascend very close to the water surface and blink rapidly. The water–air interface serves as an efficient light reflector, transmitting the signal over a large distance. In the Red Sea the schools consist of up to 100 adult fish. Small individuals of about 15 mm total length with fully developed light organs remain close to large *Acropora* corals. The school movements are usually slow, with the fish changing their position in the school and relative to their neighbors. The route taken by the school is repeated every night with the distance covered ranging between tenths and hundreds of meters depending on the locality. They probably return before dawn to the same shelters. In the northern Red Sea, at Dahab, three schools and four groups of scattered individuals were sighted at the same specific areas on the reef at a three month interval (Sagi, 1978).

### *Territorial Defense*

In the intertidal zone at high tide pairs of *P. steinitzi* were observed occupying small spaces. Invariably the larger female effectively defended these territories against intruders. Upon the approach of a conspecific the female would rapidly swim back and forth. Subsequently, the female would occlude the light organ, approach the intruder very closely and then turn on the light, invariably driving the intruder away (Morin *et al.*, 1975).

### *Sexual Signaling*

According to Morin (1983) blinking patterns exchanged between males and females in pairs of *P. steinitzi* and *Anomalops katoptron*, often segregated from conspecifics in separate territories, show "distinct sexual dimorphism and an intricate interplay between the partners". No additional information is currently available.

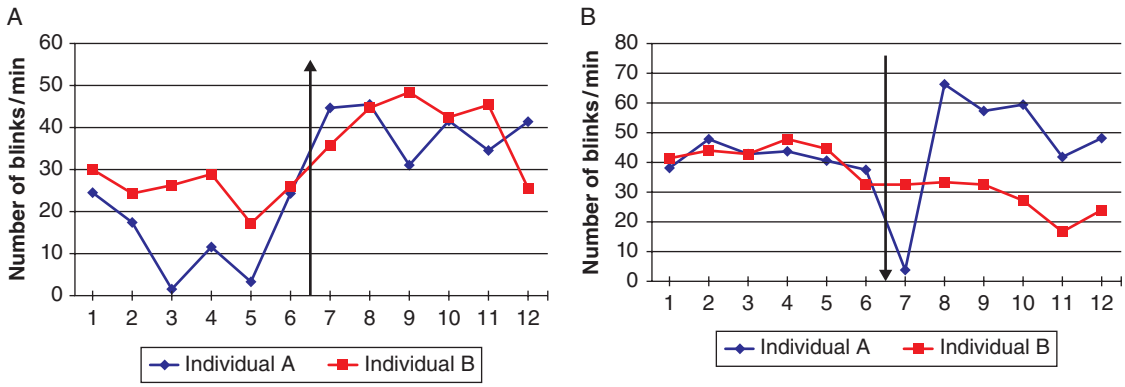


**Figure 1.12** The blinking rates of two *Photoblepharon steinitzi* kept together or in isolation: A. The blinking rates of two individuals monitored at 15 min intervals prior and following partition removal (upward arrow); B. The blinking rates of two individuals monitored at 15 min intervals prior and following partition introduction (downward arrow). (Data from Sagi 1978.)

Laboratory studies have contributed to our understanding of the exchange of signals between interacting *P. Steinitzi*. Morin *et al. (*1975) reported of two fish that were placed into adjacent aquaria, separated by an opaque partition. The two fish occluded their light organs at different rates, the one blinking 10 times per minute and the other 50 times per minute. Following removal of the partition the fish could see one another. Both fish increased their blinking rates to a rate of 40 and 60 blinks per minute, respectively. An increase in the blinking rates was also observed in isolated *P. steinitzi* exposed to their own image in a mirror. These preliminary observations by Morin *et al. (*1975) were corroborated and expanded by Sagi (1978).

The exposure of an isolated individual to a conspecific invariably resulted in an initial increase in blinking rates; however, continued observations revealed similar blinking rates in the two fish. This pattern was observed when the two fish were maintained in the same aquarium, separated by an opaque partition which was subsequently removed (Figures 1.12A and 1.13A). Moreover, two fish that displayed a similar blinking rate while occupying the same aquarium diverged in their blinking rates after being separated by an opaque partition (Figures 1.12B and 1.13B). The impact of blinking by a conspecific on flashlight fish behavior was further investigated by Sagi (1978) with the aid of a model flashlight fish constructed by S. Girsch. This model resembled a flashlight fish in size, color and structure and also possessed a light organ which emitted a blue-green light at the typical intensity of a flashlight fish. Four different patterns of light emission could be programmed for this model (i.e., 18 blinks per minute and a 0.3 s time-off; 90 blinks per minute and a 0.3 s time-off; 10 blinks per minute and a 3 s time-off; 18 blinks per minute and a 3 s time-off). Exposure to each of the four blinking rates of the model resulted in an increase in the blinking rate of previously isolated test fish; however, the rates of blinking did not match those of the model. In contrast, the time-off closely matched that of the model, with test fish displaying only between 2 to 6% time-off periods longer than 0.5 s when exposed to a model with a short 0.3 s time-off. Exposure of test fish to a model with a long 3 s time off resulted in 46–86% time-off periods longer than 0.5 s. According to Sagi (1978) the blinking rates of *P. steinitzi* that belong to the same school are similar in contrast to the blinking rates of members of different schools. The similar blinking rates of members of the same school may increase group cohesiveness. The matching of blinking rates of two previously isolated individuals may be an important step in group formation.

The attraction of five *P. steinitzi* to a blinking male or female conspecific was tested in an elongated one meter long aquarium that was

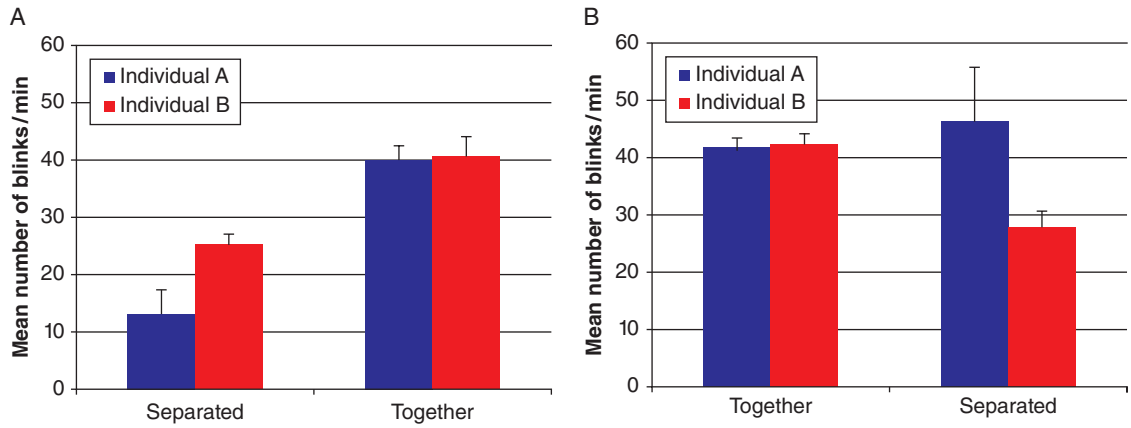


**Figure 1.13** The mean number of blinks of two *Photoblepharon steinitzi* kept together or in isolation: A. The mean blinking rates prior and following partition removal; B. The mean blinking rates prior to and following partition introduction. (Data from Sagi 1978.)

subdivided into four equally sized sections by lines marked on the aquarium front panel (Sagi, 1978). The tested fish spent only about 5% of the time in the quarter of the aquarium which contained an empty cage. The same fish spent tenfold more time in this part of the aquarium (i.e., 78% of the time) when the cage contained a conspecific. Statistical analysis (i.e., paired t-tests carried out on arc sin transformed data) of the data provided by Sagi (1978) demonstrated that the attraction to a blinking fish was indeed significant irrespective of its gender. There was a lack of any attraction to a model flashlight fish irrespective of its blinking rates or time-off periods. This absence of attraction to the model may be due to its fixed blinking pattern and the lack of any response by it to the test fish behavior.

## Deep Sea Ceratioid Anglerfishes

### *Structure, Diversity and Distribution*

Deep sea anglerfishes (Ceratioidei) form the most diverse, distinct and derived suborder of the five that constitute the order Lophiformes. Most fishes contained within this order share a unique feeding strategy characterized by the possession of an angling device, a transformed first dorsal fin ray (the illicium) which bears at its end a lure (esca). This lure can be waved in front of the mouth to attract prey. The monophyly of the pelagic deep sea anglerfishes is revealed in the distinct differences from the benthic and mostly shallow water lophiformes (i.e., members of the suborders Liophioidei, Antennarioidei, Chaunacoidei and Ogcocephaloidei). Only the Ceratioidei possess an extreme sexual dimorphism of dwarfed males lacking a luring device but possess elaborate visual and olfactory sensory systems for the location of their mates. These males attach themselves temporarily or permanently to the relative gigantic females with the aid of pincer-like denticular jaws that replace their original teeth and jaws. Only the females possess lures that contain in their escae luminescent bacteria.

Following metamorphosis, all deep sea anglerfishes lack pelvic fins and their pectoral fins are displaced. Deep sea anglerfishes share reduced ossification and musculature but increased lipid infusion (Bertelsen, 1951; Pietsch and Orr, 2007). These fishes range from small to medium size (i.e., the females of the largest species *Ceratias holboelli*, may attain a total length of 1200 mm; Penrith, 1967), are spheroid to elongated in shape and of a black to dark brown coloration. There are over 160 species (with new ones being continuously discovered) which belong to 35 genera and 11 families.

At metamorphosis anglerfishes descend into deeper water from the epipelagic zone that they occupy as larvae. Juveniles and adults are found below 300 m during the day, mainly in the mesopelagic (200–1000 m), bathypelagic and bathyabysal

zones (1000–4000 m). They constitute by far the most diverse vertebrate taxon in the meso and bathypelagic zones and are of great ecological significance since they are top predators (Bertelsen, 1951; Pietsch, 2005; Pietsch and Kenaley, 2007; Pietsch and Orr, 2007). Deep sea anglerfishes are present in all oceans from the Arctic to the southern seas mainly between 65° N and 65° S (Bertelsen, 1986). Some species, such as *Ceratias holboelli*, which are present in the Indian, Pacific and Atlantic Oceans, have an almost cosmopolitan distribution (Pietsch, 1986). Other species, such as *Acentrophryne longidens*, have a restricted distribution, being limited to the eastern Pacific (Carnevale and Pietsch, 2009).

### *Reproductive Strategies*

Deep sea anglerfishes are gonochoristic and oviparous, shedding into the water mucoid egg rafts (veils) that contain large numbers of minute eggs (i.e., less than 1 mm in diameter) and serve mainly to broadcast the eggs over great distances. A spring and summer spawning pattern was revealed in the north Atlantic, which was extensively sampled for anglerfishes throughout the year. The larvae which occupy the epipelagic zone are usually most common in the upper 50 meters. These larvae feed for several months, mainly on copepods and chaetognaths, until they descend to deeper waters at the start of metamorphosis. It is in these deeper waters that solitary anglerfishes, which are relatively rare, face the major difficulty in completing their life cycle, namely locating their mates (Mead *et al*., 1964). Bertelsen (1951) suggested on the basis of extensive fishing efforts in the north Atlantic at depths of 1000–2000 m that individual anglerfishes irrespective of species and gender are separated at least by 30 m. Males and females of the same species are, therefore, much more spaced and their meeting is a formidable task. Anglerfishes alone among all vertebrates have overcome this difficulty by a reproductive strategy of male dwarfism, temporary or permanent attachment and sexual parasitism (Regan, 1926; Bertelsen, 1951; Pietsch, 2005).

The size differences between adult males and females are very large. In case of the largest anglerfish species, *Ceratias holboelli*, the size difference was the most extreme, the female being 60 times longer than the male and about half a million times heavier (Pietsch, 2005). Males lack a lure but possess a torpedo-shaped body (Figure 1.14), which increases swimming speed. All males possess two pincer-like denticles that are used for attachment to the female. These denticles, which replaced the larva jaws and teeth, are formed by fusion of dermal spinules located on the snout. The upper denticle of the male is attached to a basal bone, which is homologous to the pterygiophore that supports the illicium in the female (Parr, 1930). In some species males possess both developed visual and olfactory sensory systems which may assist in mate location (Figure 1.14A, 1.14B, 1.14C, 1.14D). Alternatively, in other species the males possess only one of the sensory systems in a developed state and the other in a degenerated condition (Figure 1.14E, 1.14F, 1.14G, 1.14H). Moreover, some species possess both systems in a degenerated state and the way they locate their mate is completely unknown (Pietsch, 2005).

A developed visual sensory system found in ceratioid anglerfishes includes large bowl-shaped eyes directed laterally, short axis with pupils much larger than the lens (Pietsch, 1986). An olfactory developed sensory system in free living males consists of large nostrils and olfactory chambers that contain broad stacks of lamellae as well as large olfactory nerves, olfactory bulbs and forebrains (Marshall, 1967). According to Marshall (1967) anglerfish females typically possess reduced olfactory organs, minute olfactory bulbs and forebrains. These females are adapted for living in deep waters in a nutrient poor environment by minimal energy expenditure, relying much on lethargic drifting as documented in videos taken by unmanned submarines (Moore, 2002; Luck and Pietsch, 2008). Females also rely on an energy conserving sit and wait feeding strategy by attracting prey to their lures. This feeding strategy is particularly well suited for conserving energy, since sit and wait predators on average devote just 2% of their energy intake to swimming activities (Kitchell, 1983). Anglerfish females probably attract males from a distance thanks to released pheromones. This olfactory attraction is enhanced through reduced female movements and reduced current speeds at great depths (Marshall, 1967). At close range the species specific escal structure was suggested to assist in species recognition based on visual cues (Bertlesen, 1951).

Anglerfishes were suggested to engage in three reproductive strategies: obligatory sexual

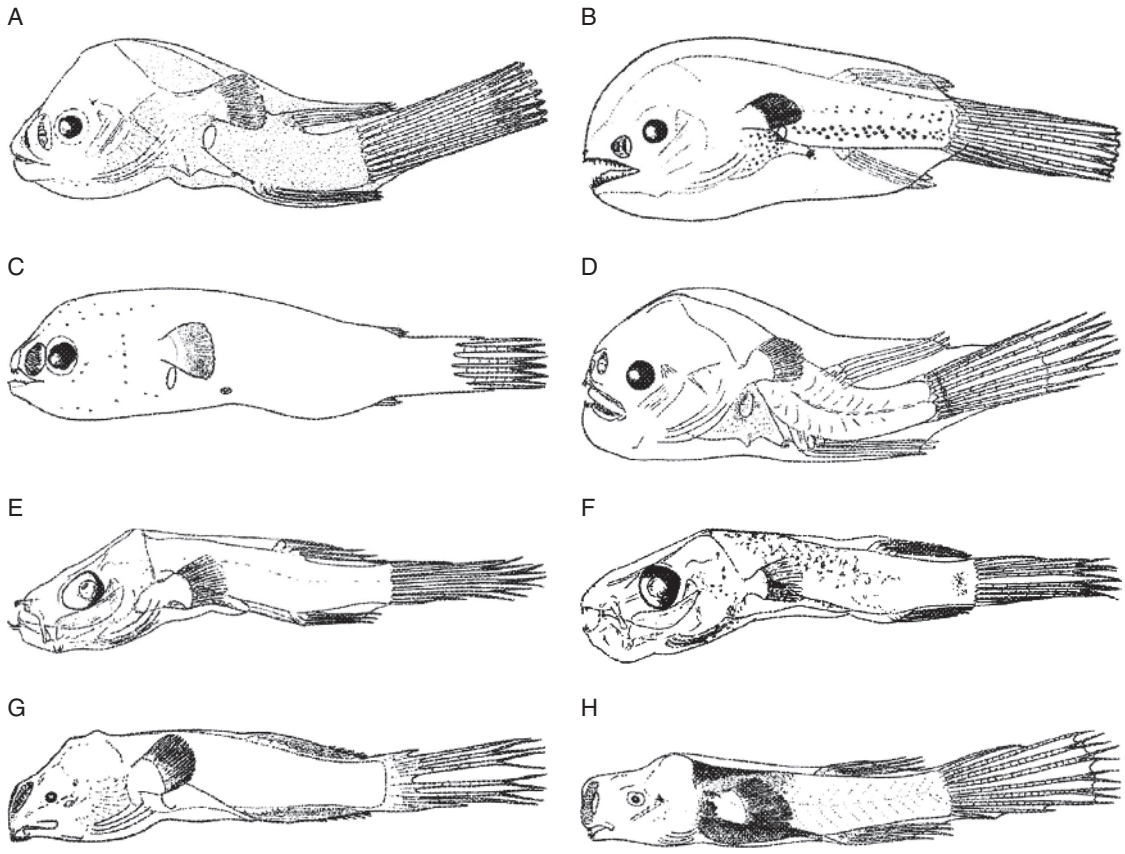


**Figure 1.14** Free living males of Ceratioids. ***Males with well developed eyes and nostrils***: A. *Borophryne apogon*; B. *Haplophryne mollis*; C. *Linophryne* sp.; D. *Photocoryns spiniceps*. ***Males with well developed eyes and small nostrils***: E. *Ceratias* sp.; F. *Cryptopsaras couesi*. ***Males with developed nostrils and small eyes***: G. *Gigantactis* sp.; H. *Rhynchactis* sp. (Bertelsen 1951. Reproduced with permission of Carlsbergfondet).

parasitism, temporary associations and facultative sexual parasitism (Pietsch, 1976, 2005).

### *Obligatory Sexual Parasitism*

Anglerfish species belonging to the families Ceratiidae and Linophrynidae practice this reproductive strategy (Figure 1.15). These fishes mature sexually only after attachment. The changes in the tissues and organs following attachment have been studied in several species of anglerfishes with the aid of light and electron microscopy (Ollson, 1974; Munk, 2000). There is a complete fusion of epithelial and dermal tissues (Figure 1.16D) and the male and female blood systems seem to become continuous; thus, the male becomes entirely dependent on the female for its nourishment. However, Munk (2000) stressed that a definite proof of functional continuity of the blood systems requires the injection of a low viscocity medium into the female and its detection in the male. Indirect proof of nutrient transfer from female to male is the male dramatic growth following attachment compared with the size of free living males, which possess jaws unsuitable for prey capture and an undeveloped alimentary system.

Growth is achieved in the attached males despite the fact that their mouth is blocked by a protruding papilla of the female and their

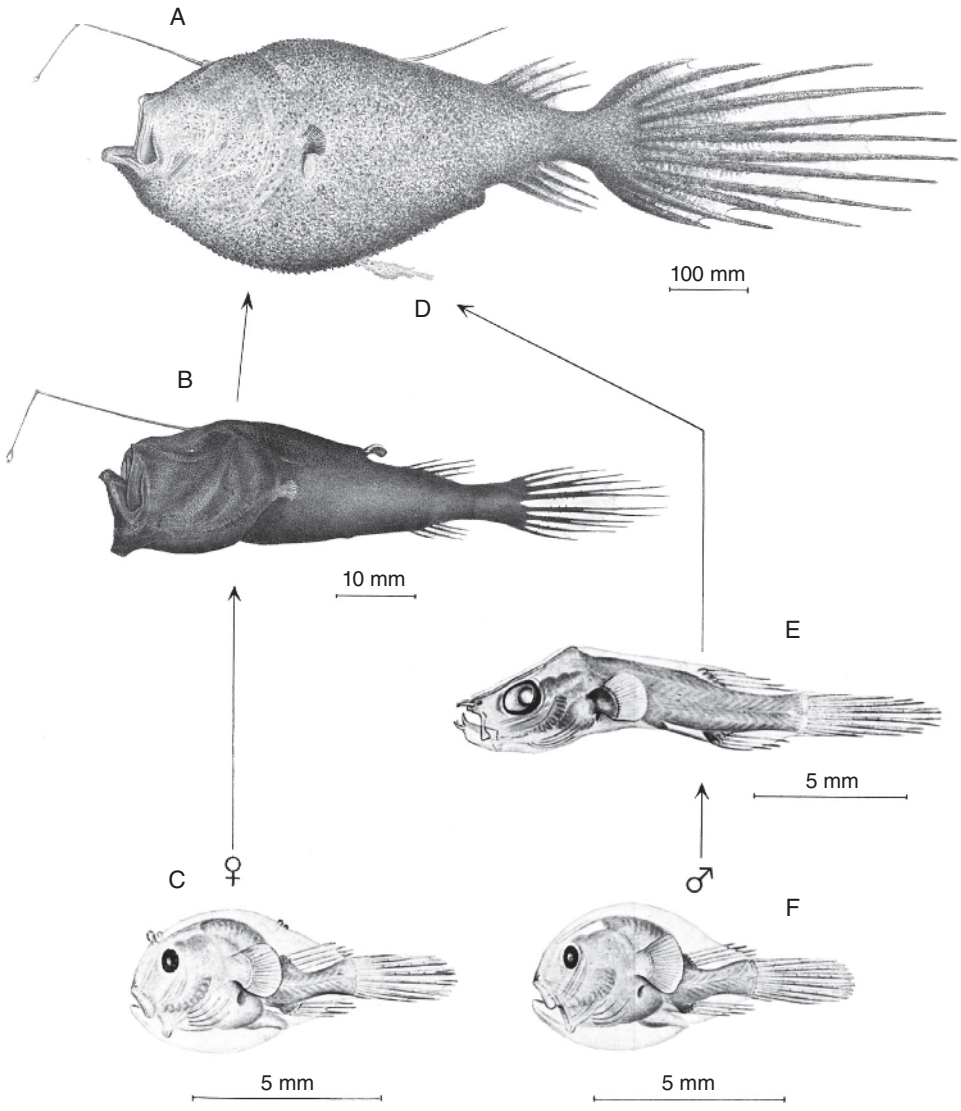


**Figure 1.15** Ontogeny and sexual dimorphism of *Ceratias holboelli*. A male (F) and female (C) larvae are depicted in the lower part of the figure; an adolescent male (E) and female (B) in the middle section and an adult female (A) with a small attached male (D) in the upper section of the figure. Note the primordia of the luring device in the larval female; the denticles of the adolescent male and the backwards protruding pterygiophore in the adult female (Bertelsen 1951. Reproduced with permission of Carlsbergfondet).

digestive system is empty (Figure 1.16D-s). The attached male gills seem to be functional, with water entering the gill cavity either through the openings in the corners of the attached mouth or with the water being both inhaled and expelled through an opening in the operculum. There is a marked degeneration of the sensory systems and the brain but a dramatic increase in the size of the testis (Figure 1.16D-t) – the male becoming a reproductive organ attached to the female, which is turned into a functional hermaphrodite (Mead *et al.*, 1964). Spawning synchrony of the two

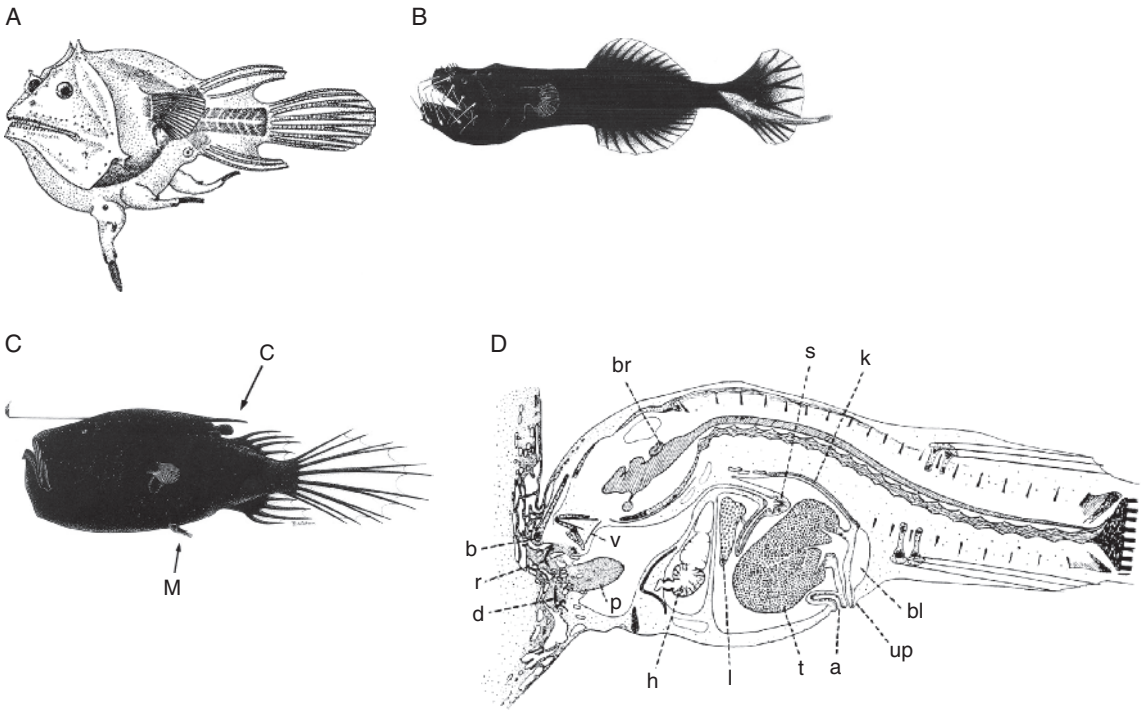


**Figure 1.16** Ceratioid females with attached parasitic males: A. *Haplophryne mollis* with three attached males (Regan and Trewavas 1932); B. Female *Neoceratias spinifer* with male attached to caudal peduncle (Bertelsen 1951); C. *Cryptopsaras couesii* with attached male indicated by lower arrow, upper arrow indicates position of caruncle (Bertelsen 1951); D. Sagittal section of male *Haplophryne schmidti* attached to female (Regan and Trewavas 1932). [a – anus; b – basal bone; bl – urinary bladder; br – brain; d – dentary with tooth; h – heart; k – kidney; l – liver; p – papilla of female tissue in mouth of male; r – rostral denticle; s – stomach; t – testis; up – urinary papilla; v – vomer]. (Regan and Trewavas 1932; Bertelsen 1951. Reproduced with permission of Carlsbergfondet.)

genders may be facilitated through the continuous blood system. Attached males were suggested to live as long as the female and to participate in several spawning episodes (Bertelsen, 1951). Very rarely, small permanently attached males and females have been collected (Pietsch, 1975). The extreme case is that of a 9.8 mm long *Cryptopsaras couesii* male attached shortly after metamorphosis to a 15.5 mm female.

An increase in the incidence of parasitism with increase in size was found for this species. However, the very precocious attachment indicates that female *C. couesii* can trigger a search response in a conspecific male as well as cues for specific identification at a very early stage. This parasitic attachment can take place at any time after metamorphosis, increasing the chances for mate location. Despite all this, the actual level of females with attached males is extremely low (e.g., 6% in *Cryptopsaras*, 11% in *Ceratias*, 16% in *Photocorynus*, 33% in *Haplophrysa* and 40% in *Borophryne*). The reasons for the low levels of parasitized females, the only one that can participate in spawning, is unknown (Pietsch, 2005).

### *Temporary Associations*

Male anglerfishes of the families Melanocetidae, Himantolophidae, Diceratiidae, Gigantactinidae and most oneirodid genera have never been observed permanently attached to females. In these species males and females mature sexually without being attached. The males are capable of feeding by themselves and their denticles are suited for prey

capture. Small zooplankton such as copepods and chaetognaths were found in the stomachs of male melanocetid and himantolophids (Bertelsen, 1951). Species belonging to this group ranged in size between 7 and 12 mm following metamorphosis, most likely due to food ingestion. The low incident of capturing males temporarily attached to females is probably due to self-detachment of the males following capture. Spawning of anglerfishes has never been witnessed but some insight may be gained from observations of males temporarily attached to females in a shallow water tetraodontid, a group which is closely related to the lophiformes (Miya *et al*., 2003, 2005). *Tetraodon schoutedeni* males attempted in aquaria to attach themselves to females by biting into their skin. The females managed to detach some of the males by swallowing large quantities of water, increasing in size and stretching their skin, so pulling it out from the grip of the males. Some males however, remained attached and were transported by the females for some time. At spawning, the female ascended to the water surface and extruded her eggs. The males turned their bellies towards the female and dispersed the eggs with their caudal fins (Wickler, 1961). According to Wickler (1961), this is probably the time that the eggs were fertilized. Only observations on spawning of deep sea anglerfishes will reveal the level of similarity to that activity carried out by their shallow water relatives.

### *Facultative Sexual Parasitism*

Male anglerfishes of the families Caulophrynidae and the oneirodid genera *Leptacanthichthys* and *Bertella* are probably facultative sexual parasites. Males and females of this group mature irrespective of attachment. Males attach to females irrespective of female maturation. Males that attach to a mature female detach after spawning and search for another mate. In case of attachment to a female that is not mature, the bond becomes of long duration and permanent, and the relationship is turned into a parasitic one. Some of the deep sea anglerfishes are so-poorly known that they cannot be classified according to their reproductive strategy (Pietsch, 2005).

Sexual parasitism within the deep sea anglerfishes evolved several times according to phylogenies based on morphological (Pietsch and Orr, 2007; Figure 1.17) and molecular (Sheldlock *et al*., 2004) data. These findings are further supported by the precise nature of the male attachment to females (e.g., number of attached males and the relative position of the male body to that of the female; Figure 1.16A, 1.16B, 1.16C) in the different groups. Presently it is not known whether obligatory sexual parasitism evolved from temporary associations and facultative parasitism or the two later were derived from obligatory sexual parasitism (Pietsch, 2005).

## *Light Organ Structure and Development: Light and the Mechanisms Controlling its Emission*

A comprehensive review of anglerfish light organ structure and function has been published by Munk (1999). Much more is known about light organ structure and development than about the emitted light and the mechanisms controlling light emission because many of the deep sea anglerfishes are only known from dead or dying specimens. The vast majority of female anglerfish possess a luminescent luring device. Exceptions are all members of the family Neoceratidae (Figure 1.17), as well three species of the gigantactinid genus *Rhynchactis* and five members of the family Caulophrynidae (Pietsch and Kenaley, 2007). There is considerable variation in the length and the spatial position of the illicium. Members of the genus *Gigantactis* possess an illicium which is several times as long as their bodies, whereas in members of the genus *Haplophryne* the escae are almost sessile (Herring and Morin, 1978). Ontogenetic changes may also occur in the relative size and position of the illicium and esca. The relatively large illicia of juvenile females of the genus *Thaumatichthys* are located on the head, similarly to other anglerfishes; however, in the adults the esca becomes sessile and shifts to the roof of the mouth. One of the members of this benthic group, *T. axeli*, collected from depths of about 3500 m was aptly described as a "living mouse trap with bait" by Anton Brunn, leader of the Galathea deep sea expedition (Bertelsen and Struhsaker, 1977).

The ultrastructure of the escae of about 20 species of anglerfishes was studied with aid of light and electron transmission microscopy (Munk, 1999). The escal photophore has a basic structure of a small light proof cup (Figure 1.18A), consisting of an inner reflecting and outer pigmented layer. This cup contains bacteria that continuously transmit their light

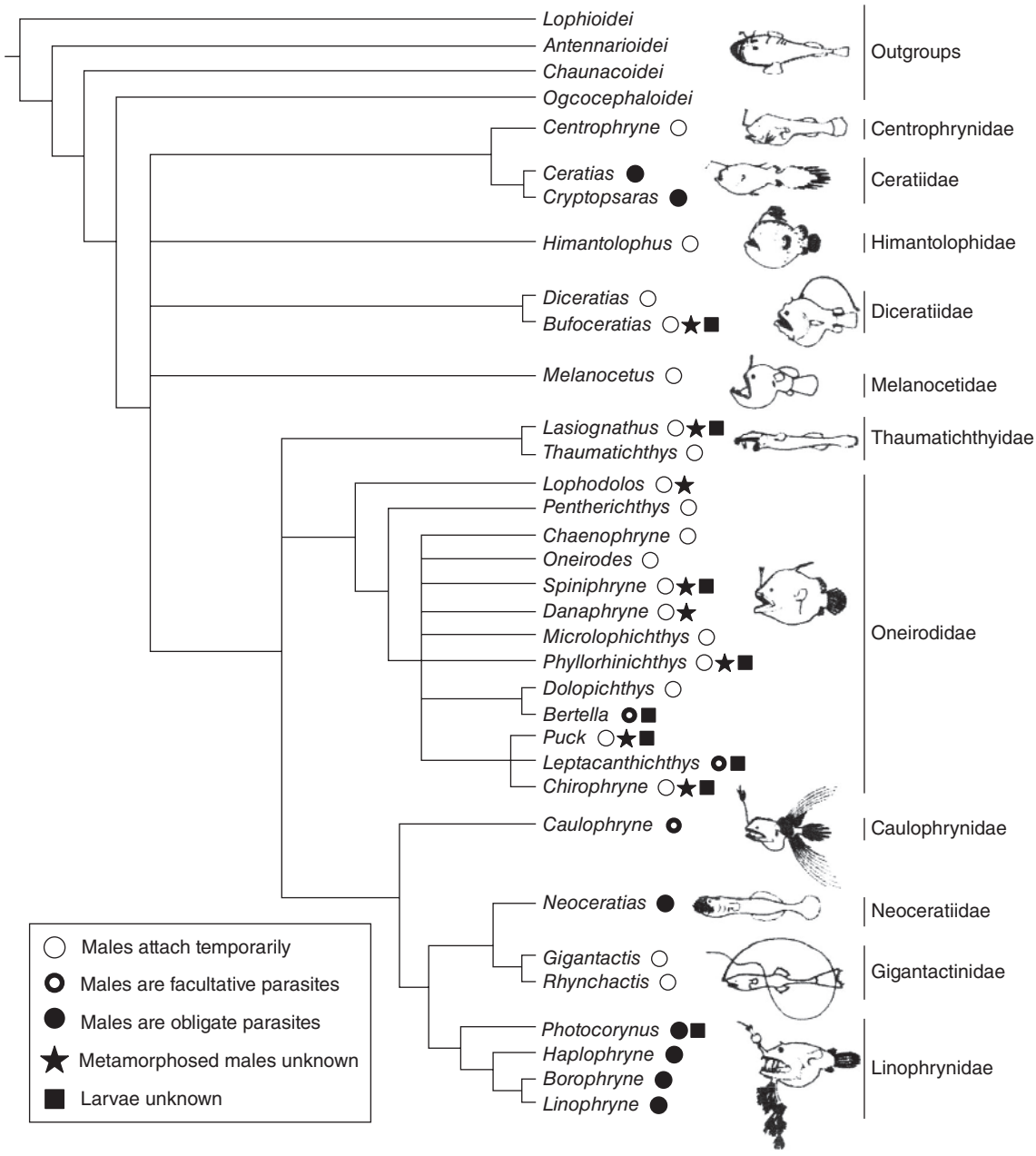


**Figure 1.17** Ceratioid phylogeny based on morphological characters including four lophiiform outgroups. Reproductive modes are plotted and those genera of which metamorphosed males and/or larvae are unknown are indicated (Pietsch and Orr 2007. Reproduced with permission of ASIH).

through a nonpigmented window (Figure 1.18A-w) located on top of the organ. The cup reflecting layer contains guanine crystals and the pigmented layer melanin granules. The major portion of the esca is composed of glandular cells in the form of radially distributed tubules. The blind end of each tubule is located at the periphery close to the light reflecting layer and the open ends lie in the center. The central

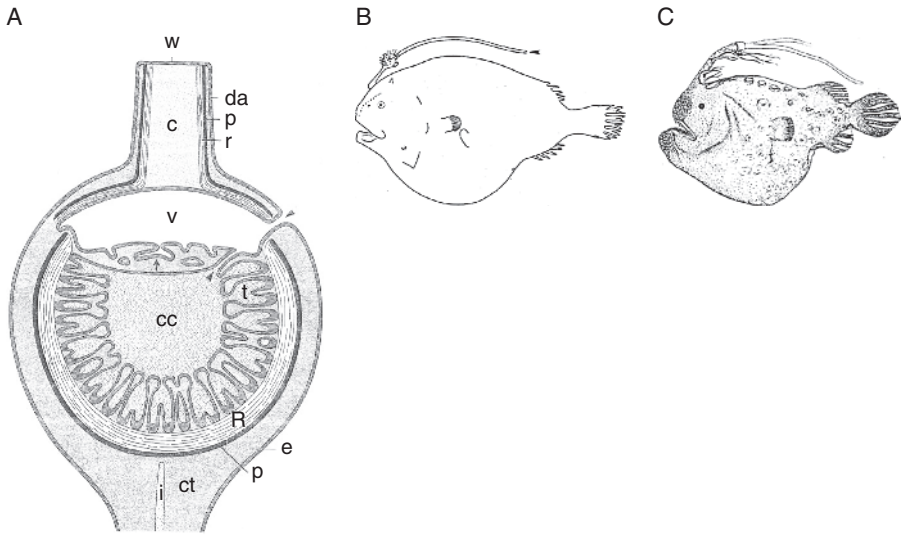


**Figure 1.18** The Ceratioid escal gland and light guides: A. Median section through a ceratioid esca; [w – window; c – light guide core; da – distal appendage; p – pigmented layer of light guide wall; r – reflecting layer of light guide wall; v – vestibule; t – branched globular tubule filled with bacteria; cc – central cavity of the escal filled with bacteria; R – reflecting layer of the escal cup; P – pigmented layer of the escal cup; e – epidermis; ct – connective tissue; i – illicium]. B. *Phyllorhinichthys micractis* with a single elongated light guide on top of the escal light organ (arrow marks point of light emission; C. *Himantolophus albinares* with two distal appendages of the escal which function as light guides (Munk 1999. Reproduced with permission of John Wiley & Sons).

core and the tubules contain pure cultures of gram negative bacteria which occupy these spaces extracellularly (Figure 1.18A-cc, -t). These rod-shaped bacteria lack capsules, spores and flagella and are 4–10 μm in length and 1 μm in diameter (Bassot, 1966; O'Day, 1974; Hansen and Herring, 1977; Nealson *et al*., 1981). The early reports of these bacteria as intracellular (Hulet and Musil, 1968) probably resulted from the confusing appearance of the tubule invaginations in relatively poorly fixed material (Herring, 1982).

In the light organs of four ceratioid species bacterial luciferase with fast decaying kinetics was found (Leisman *et al*., 1980). These findings corroborate the previous reports of presence of bacteria in the light organs, strengthening the notion that the light of anglerfishes is due to bacterial luminescence. According to Haygood *et al.* (1992) the number of bacteria in the esca of a juvenile *Melanocetus johnsoni* was estimated to be $4 \times 10^5$ cells, based on sample dilution and PCR of small subunits (16S) rRNA genes. According to O'Day (1974) the fine structure of the esca suggests that the bacteria obtain certain nutrients from the tissues of the host, with possible exceptions of some dissolved salts and trace elements that might be extracted from the water. Attempts to culture these bacteria in the laboratory have failed so far, probably due to metabolic changes occurring in the bacteria after establishment of the symbiosis (Haygood and Distel, 1993). Recent findings on the identity of the symbiotic bacteria of anglerfishes based on molecular techniques (Haygood *et al*., 1992; Haygood and Distel, 1993) are discussed in the section dealing with the evolution of the fish-luminescent bacteria symbiosis.

A usually slit-shaped epithelium lined space, the vestibulum (Figure 1.18A-v), lies above the distal part of the light gland. The vestibule is connected with the interior of the gland through one or several ducts and with the exterior by a single opening – the escal pore. According to Munk (1999) there are intraescal smooth muscles that differ among species in their spatial location. Ring-shaped smooth sphincter muscles may occur along the dorsal rim of the cup, enclosing the entire cup and in association with vascular plexuses. Radiating strands of smooth muscles were found in the

connective tissues between the glandular tubules. The main blood vessels include a single median artery and vein located in the connective tissue surrounding the illicium with branches supplying the light gland and its associated structures. The esca is poorly innervated with only thin nerves in the connective tissue around the light organ and the escal appendages. In at least one species, *Chaenophryne draco*, a single pair of nerves, one on each side of the vein, was observed along the illicium with small branches entering the pigmented outer layer of the cup. No nerves, however, have so far been observed in association with glandular tubules. An accessory escal gland of unknown function was described by Munk (1992) in anglerfishes of the families Ceratiidae and Oneirodidae. These exocrine glands are located in the connective tissue around the illicium and parts of the esca and were suggested to secrete substances that would attract prey.

According to Munk (1999) the escae of many anglerfishes possess species specific structural light guides (Figure 1.18B, 1.18C) as well as accessory filaments. Light guides, which are connected to the luminescent escal core, consist of an axial light transmitting core of loose connective tissues and a tubular light proof wall with one or two windows that may bear lens like structures. The tubular wall consist of a relative thick inner layer of reflecting cells containing guanine crystals and a thin outer layer of pigmented cells (Figure 1.18A-r,p). Light may emerge from a number of separate and widely spaced apertures thanks to the light guides. The escae and its attached filaments are the most important structure for identification of anglerfishes. In the case that the esca is lost, the females can often not be identified on the basis of their morphology (Uwate, 1979; Pietsch, 1986; Bertelsen and Pietsch, 1996).

According to Munk (1999) different stages in the ontogenetic development of the escal light gland have been studied in a few species. This light gland develops from an epidermal invagination of a bulb-shaped escal primordium. The gland lumen, the vestibule and the duct which opens to the exterior originate from the breakdown of solid masses of central cells originally within the epithelial cells. It is not known how the symbiotic bacteria infect the light organ and the role they play in its development. Special secretory goblet cells, which are common in the tubules of juveniles but not in adults, were suggested to secrete pheromones that may attract the right strain of bacteria around the time of metamorphosis (Herring and Munk, 1994; Munk *et al.*, 1998). Whereas larvae contain absolutely no bacteria, small juveniles may contain no bacteria or only few. Since larval development lasts for about two months in most species of anglerfishes, bacterial infection since hatching seems to be delayed for a considerable period (Munk and Herring, 1996). Munk and Herring (1996) suggested that the presence of bacteria does not seem to be needed for the initiation of gland formation; however, presence of the bacteria could enhance rapid gland development during metamorphosis. According to Munk (1999) the escal appendages, including the light guides, show considerable post-metamorphic growth. No bacteria are present inside the light organ prior to the formation of a tube interconnecting the escal interior core with the external world via the escal pore. It is most likely that the symbiotic bacteria invade the gland from the water column (Munk *et al.*, 1998). Haygood (1993) suggested that during spawning the symbiotic bacteria may adhere to the egg mucus membrane and remain there until they invade the gland after formation of the escal pore.

The production of light by deep sea anglerfishes has been recorded for less than twenty species (Munk, 1999). Light emission was usually observed in a dark room on board a ship or following anglerfish injection with various substances, such as adrenalin, and the application of an electric current. Only in a few cases was the emitted light monitored and analyzed (Hansen and Herring, 1977; Herring and Munk, 1994). Three patterns of light emission from escal glands have been recorded: a continuous glow (O'Day, 1974), repeated flashes (Herring and Munk, 1994) and the extrusion of a luminous fluid (Haneda, 1968). The emitted light appeared to the observer as blue-green with the peak of the emission spectra within the 470–490 nm range (Munk, 1999). Very little is known and it is mainly speculation how anglerfish modulate the emitted light. The two major suggested mechanisms include changes in the blood supply (Bertelsen, 1951) and contraction of the escal smooth muscles (Hansen and Herring, 1977).

Bertelsen (1951) suggested that light control was mediated via the blood stream, given the absence of major controlling nerve fibers inside the escal gland. Herring and Munk, (1994) argued that the control of light by altering oxygen supply to the gland assumes that oxygen levels are limiting light production. Cutting up a dark esca indeed induced light emission. However, light production for a considerable time despite removal of esca from an anglerfish indicates that not only blood supply is involved. Changes in the escal transparent window

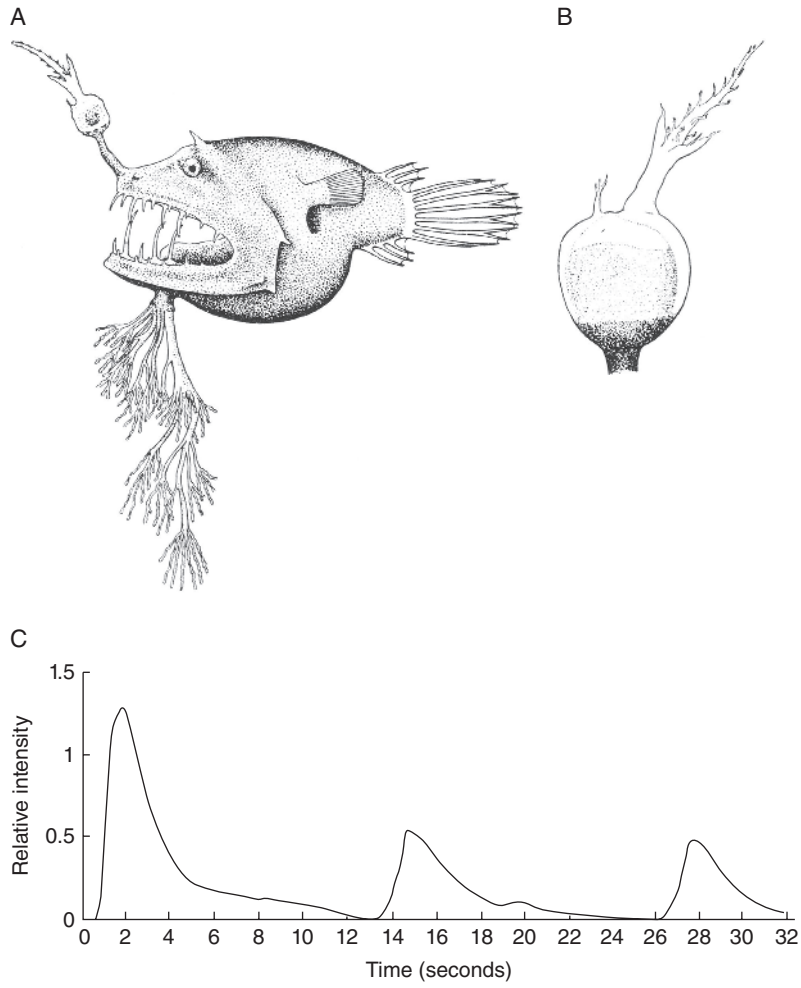


**Figure 1.19** Light from the escal gland of *Linophryne arborifera*: A. *Linophryne arborifera* (Regan 1926. Reproduced with permission of Carlsbergfondet.); B. The escal gland of *L. arborifera* (Bertelsen 1951. Reproduced with permission of Carlsbergfondet.); C. Continuous record of three bursts of light produced by a *L. arborifera* esca of a hand held fish (Hansen and Herring 1977. Reproduced with permission of John Wiley & Sons).

aperture concomitantly with changes in light intensity (Herring unpublished, cited in Munk, 1999) support the involvement of the escal sphincter muscles in regulating light intensity. However, repeated light pulses were generated in response to mechanical stimuli to anglerfishes, while the cup's aperture remained unchanged (Munk, 1999). According to Herring and Munk (1994) the kinetics of these light pulses, namely a rapid rise time and an exponential decay (Figure 1.19C), is typical of a triggered pulse rather than a shutter mechanism. The extrusion of a luminescent fluid in *Himantolophius groenlandicus* observed by Haneda (1968) is probably controlled by the escal sphincter muscles. Fluid extrusion has been rarely observed probably because captured fishes extruded these substances before arriving on the ship's deck, although contractions following irritation of the gland were witnessed (Munk, 1999).

The most straight forward mechanism for controlling light emission is that of the anglerfishes of the genus *Thaumatichthys*. These benthic deep sea fishes simply close their mouth in order to block the light of the esca (Bertelsen and Struhsaker, 1977).

In some species of anglerfishes, in addition to the escal light organs, light is also generated in other sites, such as the caruncles, the barbells and the skin. The caruncles are posterior dorsal fin rays of members of the family Ceratiidae modified into sessile luminous bulbs (Figure 1.16C) located just before the soft dorsal fin (Pietsch, 1986). From the caruncle's opening a granular luminous material can be extruded voluntarily by the fish (Young and Roper, 1977) or forcefully by pressing the gland (Bertelsen, 1951). These structures contain luminescent bacteria that are morphologically similar to those of the escal gland (Nealson *et al*., 1981) and have luciferase activity (Leisman *et al*., 1980). According to Haygood *et al. (*1992) bacteria from the caruncles had similar gene sequences to the bacteria of the escal gland; however, it was not possible to know whether they were derived from the same infection. The caruncles degenerate in adult *Ceratias* but persist in mature *Cryptopsaras* (Hansen and Herring, 1977).

Two anglerfish genera possess hyoid barbells. A rudimentary barbell is present in both sexes of the genus *Centrophryne*, whereas in the genus *Linophryne* only females possess a well developed barbell, which carries a number of luminous tubercules (Figure 1.19A). The luminescence is not bacterial but endogenic and the tubercules contain paracrystaline photogenic granules (Herring and Morin, 1978). According to Hansen and Herring (1977), the vast blood supply to the barbells and the absence of major nerve fibers suggest that the complex vascular network not only supplies oxygen but may also control luminescence. A steady blue glow with an emission maximum of 493 nm was emitted from the barbells of *Linophryne* sp. following immersion in dilute hydrogen peroxide. As different from the escal light gland which is of an ectodermal origin, the barbell light glands are probably of mesodermal origin, and thus these fishes bear light organs derived from two separate germinal layers. The function of light emission from the barbells is unknown and likewise it is not known whether it reinforces that of the escal luminescence or whether it serves some additional or entirely separate purpose. Luminesence has also been reported from the skins of the anglerfishes *Himantolophius azurlucens* (Beebe and Crane, 1947) and *Cryptopsaras couesi* with intensity changes in the laboratory matching in this species those of ambient downwelling light (Figure 1.20; Young and Roper, 1977).

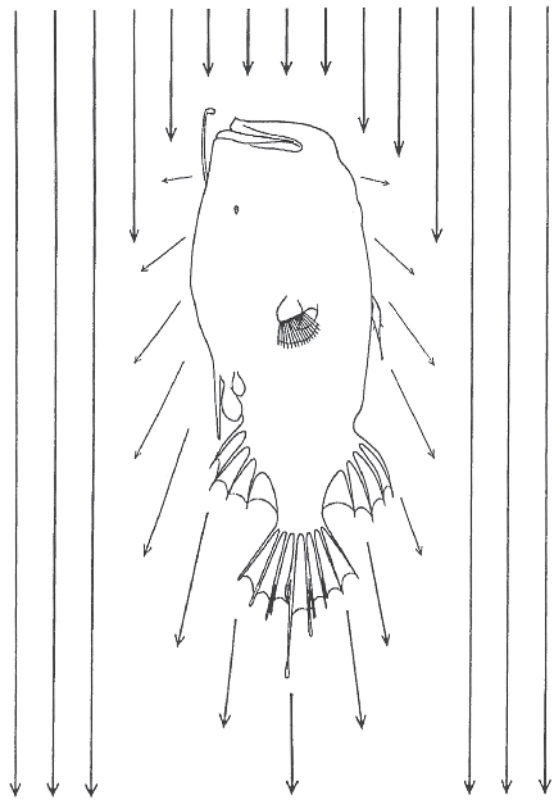

**Figure 1.20** Anglerfish with luminous skin: *Cryptopsaras couesii* in a head-up position; parallel vertical lines represent downwelling light; lines radiating from the fish represent relative luminescence (Young and Roper 1977. Reproduced with permission of National Marine Fisheries Service).

### *The Use of Lures by Anglerfishes*

So far, deep sea anglerfishes have not been witnessed using lights in their natural environment. Direct observations are limited to specimens that survived for a short time the ascent from deep water and were handled in various ways. Most of the knowledge concerning the behavior of these anglerfishes is based on inferences from behavioral, physiological and structural comparisons with their shallow water relatives. According to Luck and Pietsch (2008) observations carried out by deep sea submarines on live unrestrained animals using minimal invasive techniques are needed in order to verify the gained insights and to effectively study these animals.

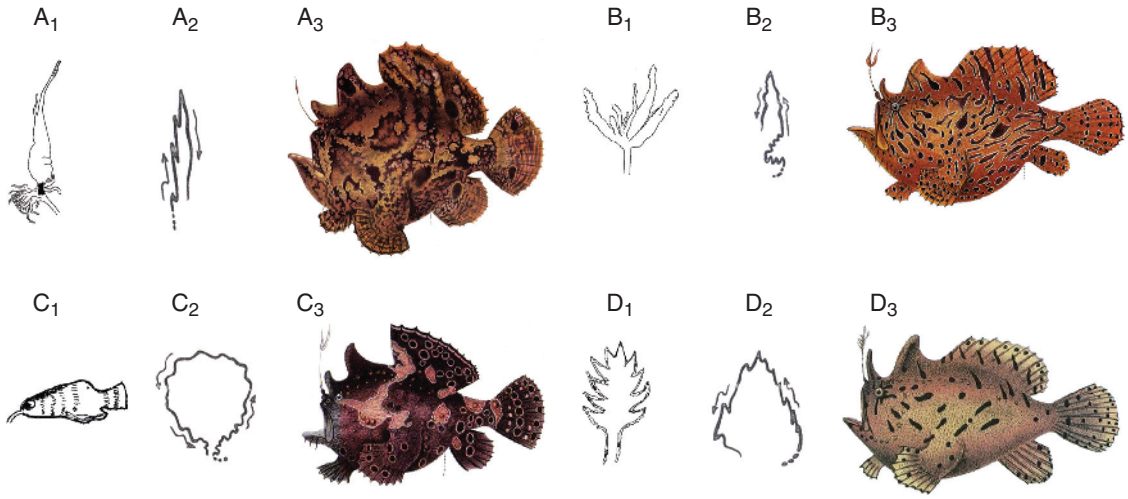


**Figure 1.21** Four species of antennarid frogfishes differing in lure structure and wiggling pattern: *Antennarius commersoni* ($A_3$) possess a lure that resembles a small shrimp ($A_1$) presented in an up and down motion ($A_2$); *Antennarius striatus* ($B_3$) possess a lure that resembles a polychaete (B1) presented in a rapid jerky motion ($B_2$); *Antennarius maculatus* ($C_3$) possess a lure that resembles a small fish ($C_1$) presented in a circular sweeping motion ($C_2$); *Antennarius hispidus* ($D_3$) possess a lure that resembles a tube-worm ($D_1$) presented in a roughly triangular motion ($D_2$) (Pietsch and Grobecker 1987, 1990). ($A_2$, $B_2$, $C_2$, $D_2$, $A_1$ & $B_1$ Pietsch and Grobecker 1987. Reproduced with permission of T.W. Pietsch, University of Washington; $C_1$ & $D_1$ drawn by Ilan Karplus from figures and photos $A_3$, $B_3$, $C_3$ & $D_3$ Bleeker 1865.)

Prey capture with a luring device was mainly studied in the relatively shallow occurring sea devils (suborder Lophioidei) and frogfishes (suborder Antennarioidei). Over 2000 years ago Aristotle in his "Historia Animalium" described the luring behavior of the sea devil *Lophius piscatorius* (cited in Gudger, 1947). Chadwick (1924) and Wilson (1937) described the luring behavior of this fish in captivity (both cited in Gudger, 1947), and Laurenson *et al.* (2004) monitored this behavior with a video camera at a depth of 350 m using a remotely operated vehicle (ROV). The sea devil concealed in the sand, erected its dorsal fins and reduced by half its exhalation rate to increase concealment when a potential prey approached within a distance of about 5 m. When the prey reached a distance of about 1–2 m and was positioned in front of the sea devil, the later used its luring device (i.e., the illicium with attached flashy flap – the esca) economically, in bouts of one to three casts each lasting only for several seconds. Most of our knowledge concerning deep sea anglerfish prey capture and feeding strategy is inferred from studies on shallow water frogfishes, which are small to medium sized, structurally diverse and easy to maintain in captivity. According to Pietsch and Grobecker (1987) frogfishes possess extremely diverse lures which are species specific and range in size from one-sixteenth of an inch to one inch or more. Lures were suggested to imitate morsels of food or small invertebrates and fishes (Figure 1.21$A_1$, 1.21$B_1$, 1.21$C_1$, 1.21$D_1$).

Frogfishes practice aggressive mimicry, they transmit false messages of the presence of a food item in order to attract potential prey to enter their deadly striking zone. Deception of the prey is increased by resembling an inanimate object such as a sponge or coral, adopting its coloration and remaining immobile and by wriggling the lure in a species specific pattern that resembles the mode of motion of the imitated prey (Pietsch and Grobecker, 1987; Figure 1.21$A_2$, 1.21$B_2$, 1.21$C_2$, 1.21$D_2$). The species specific structure of the lures was suggested to reduce interspecific competition among antennarids (Wickler, 1967). However, in a field study, Pietsch and Grobecker (1987) found that several sympatric Hawaiian frogfishes had a broad overlapping food diet. According to Pietsch and Grobecker (1987) lack of prey specificity may be due to the unpredictable

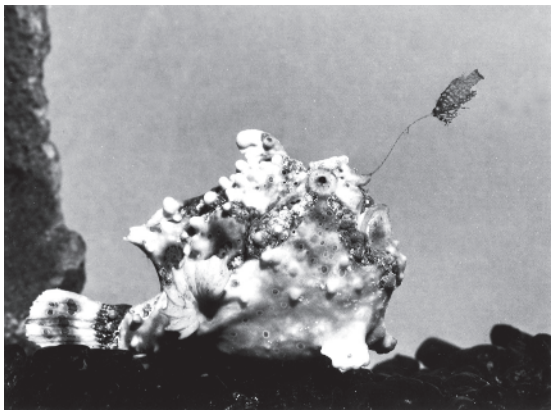

**Figure 1.22** *Antennarius maculatus* and its fish shaped lure (Reproduced with permission of T.W. Pietsch, University of Washington).

and complex food acquisition in the marine environment or due to frogfish being feeding generalists. Prey may have been taken when approaching the frogfish without being attracted to the lure, or while reacting to the lure as a potential competitor (e.g., *Dascyllus aruanus* interacting with the fish lure of *Antennarius maculatus*). Pietsch and Grobecker (1987) cautioned that their Hawaiian study was based on relative few fishes. Of special interest are the findings of Kuiter, from the Museum of Victoria, Melbourne (cited by Pietsch and Grobecker 1987) regarding the specialized feeding habits of *Phyllephryne scortea*. This frogfish, which possess a lure that resembles a pontogeneid amphipod, feeds mainly on gobies of the genus *Nesogobius* that feed primarily on amphipods.

Suction feeding is the method by which most deep sea anglerfishes (Bertelsen, 1951) and frogfishes (Pietsch and Grobecker, 1978, 1979, 1987, 1990) capture and swallow their prey, which may be very large and exceed that of the predator. According to Pietsch and Grobecker (1987) this feeding method is based on the sudden increase in the volume of the oral and gill cavity by lowering of the lower jaw and expanding the upper jaw forming a negative pressure which is filled with water that transports the prey into the predator mouth. In frogfishes there is about a 12-fold increase in the volume of the open bucal cavity versus the closed one, revealed by the injection of liquid parafin, which subsequently hardens. In other piscivores that use the same feeding method, the increase in the volume of the bucal cavity is much smaller (e.g., *Perca fluviatilis* displays only a sixfold increase in its buccal cavity). Analysis of suction feeding in several species of frogfishes with the aid of high speed cinematography (Pietsch and Grobecker, 1979) revealed swallowing of prey within 6 ms, a speed which exceeds by far the reported speeds of other piscivores practicing suction feeding (e.g., prey engulfment time of *Synancea verucosa* and *Perca fluviatilis* being 15 and 40 ms, respectively). The enormous increase in the volume of the buccal cavity allows frogfishes to effectively capture their prey from larger distances (i.e., strike distances) than other piscivores practicing suction feeding. Moreover, fish which practice this feeding strategy are able to remove a single prey without alerting other individuals in its vicinity.

Ramaiah and Chandramohan (1992) reported collecting several specimens of *Antennarius hispidus* from a depth of 50 m off Mumbai, which emitted a dim light from their lures. Luminescent bacteria isolated from these lures in the laboratory were identified as *Photobacterium leiognathi* on the basis of their biochemical reactions and growth on different types of culture media; these were identical to those of a reference strain of *Photobacterium leiognathi*. Regretfully, the structure of the light organ was not studied and neither was it compared to the structure of nonluminous lures of *Antennarius hispidus*. This finding is exciting in many ways. A luminescent esca was discovered in frogfishes for the first time, and the fish harboring these bacteria may be a different and unknown species. Moreover, the use of the luminescent lure for prey capture could greatly contribute to our understanding of deep sea anglerfishes.

The use of the luminescent lures by freshly captured deep sea anglerfishes has been reported for only three species of the genus *Himantolophus* (Beebe and Crane, 1947; Bertelsen and Krefft, 1988). According to Munk (1999) in all these species the lure could be held in a backward position resting in a median grove on top of the head or in a forward position with the esca overhanging the mouth. In a particularly active *H. groenlandicus* the lure was swept forward and backwards several times per minute. The *in situ* use of the lure by deep sea anglerfishes was so far only twice recorded with the aid of unmanned submersibles (Luck and Pietsch, 2008; Moore, 2002). Moore (2002) monitored three whipnose anglerfish of the genus *Gigantactis* drifting at a depth of 5000 m upside down with the lure extended just above the bottom. Luck and Pietsch (2008) analyzed 24 minutes of

video recording of a female anglerfish of the genus *Oneirodes* taken at a depth of about 1500 m. Most of the time the female was passively drifting and it deployed the lure for only 4% of the time. "It took about 7 s to rotate the lure forward and slightly longer, 10–12 s, to bring it back to the nonluring position. Each time the illicium was deployed it was held in a fully extended position for about 30 s without any discernible wriggling or vibration".

Attraction of prey to deep sea anglerfish lures is probably based on the positive phototactic response of many planktonic invertebrates and fishes. Young (1983) suggested that the continuously glowing lure may mimic fecal pellets that contain luminescent bacteria and constitute an important source of food ingested by mesopelagic and bathypelagic fishes and crustaceans. Prey was suggested to be chemically attracted to the lure by pheromones secreted in some species from the escal accessory glands (Munk, 1992). Here again, some insight may be gained from a shallow water frogfish *Antennarius striatus*. According to Pitsch and Grobecker (1987) this species possess unicellular secretory cells in its lure and was suggested to chemically attract its prey. In a controlled experiment, the response of individual *Dascyllus aruanus* to a rubber worm-shaped lure was contrasted while being presented either with fluid collected close to a luring *A. striatus* or with fluid collected from the same species that had its lure removed. Significantly more fish were attracted to the lure presented with fluid removed from an intact fish. Possibly also, in deep sea anglerfishes the accessory escal glands may fulfill a similar function.

Deep sea anglerfishes live in an environment with a sparse food supply due to the logarithmic decrease with depth of all particles (Sheldon *et al*., 1972). Under these circumstances it should be advantageous for bathypelagic fishes not to be choosey with regard to the size of food they ingest (Ebeling and Cailliett, 1974). According to Bertelsen (1951) adult and adolescent female anglerfish were indeed found to have a broad food intake, which consisted mainly of large and small crustaceans and fishes but also of cephalopods and chaetognaths. The crustaceans included copepods, ostracods, euphausids, amphipods and various decapods, especially peneids and hoplophorids. The ingested fishes could be very large, exceeding the anglerfishes in size. Despite the different structures of the lures no distinct differences were found in the food intake of the different species. It is not known how the deep sea anglerfishes are alerted for the presence of prey near their lures. Bertelsen (1951) suggested that vision usually is not involved, based on position of the female eyes and their development; however, the lateral line and the accessory filaments may be involved, responding to water vibrations and tactile stimulation by the prey. The very large mouth, the distensible stomach and suction feeding allow anglerfishes the intake of very large prey, whereas the tiny gill openings were suggested by Ebeling and Cailliett (1974) to prevent the escape of small items sucked in through their cavernous mouth.

An antipredatory function was ascribed to the emission of short flashes of light from the esca of handled fishes. These rapid bursts of light were suggested to startle and intimidate piscivores (Hansen and Herring, 1977). A similar function, particularly of confusing predators, was ascribed to the extrusion of a luminescent fluid (Haneda, 1968; Bertelsen and Krefft, 1988) that may be more commonly practiced than actually observed (Munk, 1999).

Bertelsen (1951) suggested the possible role played by the female luminescent escal in attracting males: "The esca with its light organ and specific attachments may presumably function as a distinguishing mark which males can recognize when they come sufficiently near". Some deep sea anglerfish males have extremely large and well developed eyes, whereas others have small degenerated eyes. The involvement of vision in mate location very likely differs among species. In view of the difficulty in pursuing this topic, little has been added since Bertelsen's (1951) corner stone monograph on deep sea anglerfishes. Finally, anglerfishes may actually have much greater communicative repertoires than initially anticipated, given the variety of patterns of light (i.e., continuous glow, pulses of light and extruded luminescent fluids) transmitted from the esca (Herring and Munk, 1994). However, decoding and understanding the signals of these fascinating creatures remain a challenge for future research.

## Ponyfishes

### *Structure, Distribution and Taxonomy*

Leiognathids are small fishes, ranging in size between 50 and 250 mm SL. They are laterally compressed, covered with small scales and possess a silvery ventrum and a darker mottled dorsum. The fish typically possess a single dorsal fin with a fin spine locking mechanism and a circum-esophageal light organ (LO), which usually contains a pure culture of the luminescent bacterium *Photobacterium*

*leiognathi*. Leiognathids are commonly known also as ponyfishes, referring to their extremely protractile mouth parts which give the head a horse like appearance. Ponyfishes are facultative schoolers that often form large schools of similarly looking multiple species assemblages. Despite their small size, ponyfishes are of economic importance in Southeast Asian countries, where they are used for human consumption, fish meal, manure and livestock feed. They are usually taken in demersal trawls, beach seines and lift nets (McFall-Ngai and Dunlap, 1984; Jones, 1985; Soars and Leis, 2010).

Ponyfishes feed mainly on zooplankton and phytoplankton, with some species consuming substantial quantities of benthic organisms. The morphology of their protractible mouths and teeth were related to the type of food they ingest (Jones, 1985). Members of the genus *Secutor*, with an upwards protracting mouth and blunt coniform teeth visible only microscopically, feed mainly on pelagic organisms, whereas *Gazza* species, with a forward protracting mouth and large canine teeth, feed on fishes and benthic invertebrates. A molecular phylogeny of ponyfishes suggests that the planktivorous and piscivorous feeding habits evolved from a bentivorous ancestor (Ikejima *et al*., 2004). Ponyfishes were suggested in early studies to be diurnally active, feeding in schools during the day and resting solitary quiescent at night in shallow water near the bottom (McFall-Ngai,1983; Morin, 1981). However, more recently, some species were considered as also nocturnally active on the basis of observations of fish feeding at night (Woodland *et al*., 2002), temporal stomach fullness (McFall-Ngai and Dunlap, 1983), and emission of signals at night by interacting fish. Moreover, some species ascend the water column at night to feed following the circadian migration of the zooplankton (Woodland *et al*., 2002).

Ponyfishes have a wide distribution in the tropical and subtropical Indo-West-Pacific. They range from the Red Sea and the eastern coast of Africa across the Indian Ocean and westwards in the Pacific as far as Hawaii and Tahiti (Jones, 1985). A single species *Equulites klunzingeri* migrated from the Red Sea via the Suez Canal into the Mediterranean where it established itself very successfully (Golani *et al*., 2006). Ponyfishes often occur in shallow waters with sandy and muddy bottoms in mangroves, estuaries and shore lines with typical high turbidity and low visibility. However, these fishes are also captured by demersal trawls from depth of a few hundred meters and in areas of excellent visibility (Pauly, 1977; Jones, 1985; McFall-Ngai and Morin, 1991).

The taxonomy of ponyfishes was until relatively recently in a confusing and ambiguous state, consisting traditionally of three genera, *Gazza, Secutor* and *Leiognathus* and about forty species (Sparks and Dunlap, 2004). The taxonomy of this group is complicated due to the fact that many type specimens were either never deposited or have subsequently been lost, and many of the original species descriptions were incomplete and of a rudimentary nature (Chakrabarty and Sparks, 2007). Furthermore, ponyfishes are extremely conservative and constant in many of their anatomical traits, which in other groups of fishes could serve to discriminate among species. All ponyfishes, for example, possess the same number of vertebra, fin spines and ray counts and their gill raker number overlap to a large degree among species (Jones, 1985). Understanding of ponyfish taxonomy and phylogeny was greatly improved following the use of the structure of the light organ system (LOS) for differentiating among species, in combination with nucleotide sequencing. On the basis of this new approach two subfamilies, eight genera and many new species were described (Sparks *et al*., 2005; Chakrabarty *et al*., 2011a, 2011b).

### *The Light Organ System (LOS) and Diversity of the Generated Light Patterns*

The morphology of the ponyfish light organ was studied with aid of light and electron microscopy (Harms, 1928; Haneda, 1940, 1950; Ahrens, 1965; Bassot, 1975; Haneda and Tsuji, 1976). This ring-shaped gland surrounds completely or partially the esophagus close to the point where it joins the stomach (Figure 1.26A). The gland is composed of epithelial cells which form 150–200 radially positioned tubules that extracellularly harbor luminescent bacteria (Bassot, 1975). In six species belonging to two genera, ponyfish tubules had external diameters ranging between 25 and 75 µm, with species differing also in wall thickness and tubule lumen diameters (Dunlap, 1984a). The glandular cells lining the tubules are rich in endoplasmatic reticulum, mitochondria and golgi. These cells have a microvillous border and show signs of secretory activity. Blood supply to the gland is little developed; nerve endings were not detected in this structure (Bassot, 1975). The lumina of the tubules empty into primary ducts leading to a collecting reservoir, which opens into the esophagus via two short ducts. A guanine

reflector between the esophagus and the internal layer of the gland directs the generated light outwards. An opaque white layer surrounds the external wall of the gland allowing exit of light only through a single small dorsal window and either a single ventral or two lateral windows. There are usually three muscular shutters embedded with chromatophores, which may block light passage through the windows. Closing the shutter may control the emission of light in two complimentary ways; namely, reducing the amount of generated light by a reduction of the supply of oxygen to the bacteria from the fish gas bladder jointly with the physical blockage of the generated light ((Dunlap and McFall-Ngai, 1987). According to McFall-Ngai and Dunlap (1983) it is not possible to directly observe the shuttering phenomena in living ponyfishes. However, both open and closed positions of the shutter can be seen in dissected fish. Moreover, the act of opening and closing the shutter can be witnessed through the "window" in the gas bladder in moribund dissected fishes. Both the light organ and virtually all the accessory structures and tissues involved in light transmission contain melanophores which were suggested to be involved in slow light intensity regulation through hormonal control (Herring and Morin, 1978).

99% of all the bacteria isolated from the light organ of seven different species of ponyfishes belonging to three genera were identified on the basis of their morphology and physiology as *Photobacterium leiognathi* with only 1% identified as chance contaminants or transients (Reichlet *et al*.,1977). Bacterial populations isolated from single light organs of the ponyfish *Nuchequula nuchalis* were found on the basis of nucleotide sequencing to belong to two or three genetically distinct strains (Dunlap *et al*., 2008). According to Dunlap (1984a) bacteria in the light organs of a variety of ponyfish species are gram negative, nonmotile, nonflagellated cocobaciloid to short rod shaped with shape distortion due to tight cell packing. These bacteria, averaging in size $1.6 \times 3.2\,\mu m$, are solidly packed within elongated thinly walled saccules inside the tubules of the light organ. Each tubule contains between one and twenty saccules. Within the saccules the bacteria are held at extremely high densities of about $1 \times 10^{11}$ cells/ml, which is about 15 times greater than the density estimated from total light organ volume. Maintenance of the bacteria within saccules was suggested to maximize induced luminescence, limit influx of nutrients and retain bacteria from escaping the light organ tubules. Luminescence of light organs of six species from three genera averaged at $2.4 \times 10^4$ quanta/s/cell, more than 10 times the maximum luminescence of *P. leiognathi* grown in culture. According to McFall-Ngai and Morin (1991) bacterial peak wave length, which is between 485 and 490 nm in culture, is shifted to the green (500 nm) in the intact light organ or the whole fish.

The location of the light organ deep inside the body of ponyfishes necessitates the involvement of additional structures, such as the gas bladder, and tissues, such as muscle, bone and skin, in the transmission of light from the inside to the outer surface. To be effective in this task these structures are modified in different ways, such as changes in the spatial orientation of the gas bladder, differential purine concentrations in the reflective layers and transparency of the various tissues which transmit light (McFall-Ngai, 1983). Haneda (1940) stated very properly in one of the earliest studies on ponyfish luminescence that these fishes resemble opaque milky bulbs in their diffuse glow, their light organs not seen similarly to incandescent filaments of the lamps. The gas bladder is of crucial importance for the transmission of light in ponyfishes in addition to the roles it fulfills in the supply of oxygen to bacteria in the light organ and regulation of fish buoyancy (McFall-Ngai, 1983). The light organ is positioned within the focus of the parabular shaped reflector of the bladder (Figure 1.23)

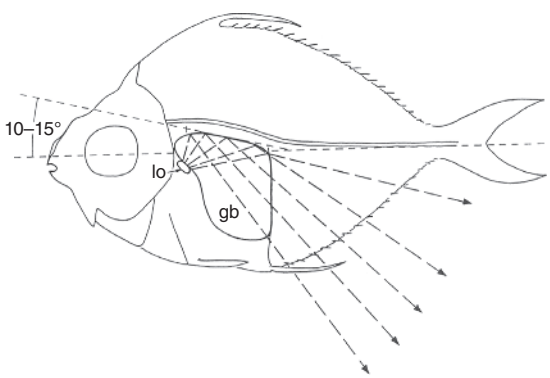


**Figure 1.23** The path of reflected bioluminescent light in *Leiognathus equulus* (indicated by the dashed lines and arrows) in the downward tilted gas bladder. lo – light organ; gb – gas bladder (McFall-Ngai 1983. Reproduced with permission of John Wiley & Sons).

with its dorsal window separated from the later by only a thin permeable membrane. Light is thus shining directly through this small window (i.e., 0.5 mm$^2$ in a 120 mm SL *Leiognathus equulus*) onto the reflecting surfaces of the bladder. The bladder is slightly tilted downward at the posterior end, so that its dorsal surface is at an angle of about 10-15° from the body axis. This spatial orientation assures the reflectance of light from the dorsal and dorsolateral surfaces downward through the posterior transparent parts of the bladder (Figure 1.23).

According to McFall-Ngai (1983) overall levels of purines in the reflectors of ponyfishes were much higher than those of other shallow water fishes, resembling the levels of deep sea fishes in which heavy purine deposition in the gas bladder is correlated with high pressure and high concentrations of oxygen. The correlation of purine concentrations in the bladder of ponyfishes with the path of light indicates that this structure is extremely well adapted for light transmission. The dorsal lining of the bladder of *Leiognathus equulus*, the primary site of incident luminescence, has the highest levels of purines (2.80 mg/cm$^2$), whereas the secondary reflecting surfaces, the lateral and ventral, contained significantly lower levels of purines (1.81 and 1.22 mg/cm$^2$, respectively). The levels of purines were greatly reduced in the bladder membrane adjacent to the dorsal window (0.09 mg/cm$^2$) and in the posterior region of the bladder (0.19 mg/cm$^2$) which both transmit light. The enigma of the oxygen supply to the luminescent bacteria in view of the poor blood supply to the light organ was solved by McFall-Ngai (Dunlap and McFall-Ngai, 1987; McFall-Ngai, 1991). About 20–30% of the volume of the gas bladder consists of oxygen, which diffuses through the oxygen permeable membrane adjacent to the dorsal window of the light organ and reaches the bacteria. Consumption of oxygen by the bacteria creates a steep diffusion gradient, which further facilitates the transfer of oxygen across the membrane. Oxygen is continuously supplied to the gas bladder from the circulatory system through a counter current exchange system (i.e., the red gland). Light emission from the light organ could be manipulated by exchanging the gas in the bladder with that of pure oxygen and nitrogen. Pure oxygen increased luminescence output 5–10 times over that of air whereas pure nitrogen yielded no detectable light from the light organ.

Luminescent signals of ponyfishes are more diverse, numerous and versatile than the light signals reported for any other living organism (Figure 1.24). The internal location of the light organ that allows the fish to control light emission at different locations with the aid of different structures probably underlies this extreme diversity (McFall-Ngai and Dunlap, 1983, 1984). Six ponyfish light signals are discussed in the following sections, with emphasis given to signal structure, the context and mechanism of light emission and the signal possible functions.

## *Disruptive Illumination*

Among the earliest reported patterns of light emission by ponyfishes was a ventral dim diffuse light that was mainly perceived when watching these fishes from below (Harms, 1928; Haneda, 1940, 1950). Subsequently, Hastings (1971) demonstrated that in *Leiognathus equulus* ventral light emission was detected by a photomultiplier only after the fish were stimulated from above by a flash light. This

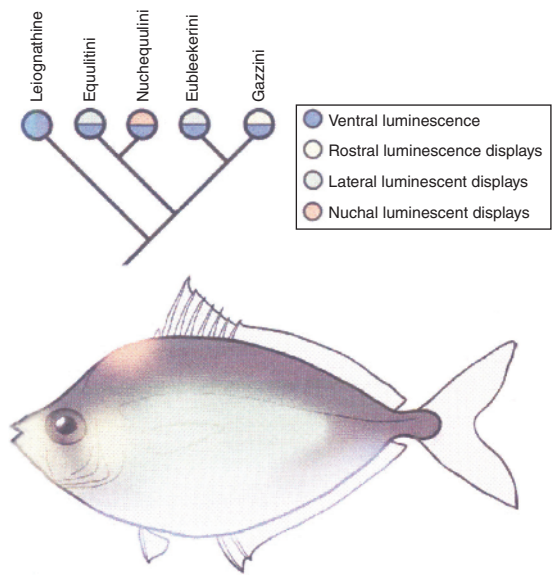


**Figure 1.24** Summary of the luminescent displays of leiognathid fishes incorporated into a simplified phylogeny of this family. Note that ventral luminescence is shared by all group members and that nuchal luminescent displays have not been observed but were suggested on the basis of a transparent nuchal patch (Chakrabarty *et al.* 2011a. Reproduced with permission of John Wiley & Sons).

observation lead Hastings (1971) to suggest that ponyfishes emit a ventral light in order to disrupt their silhouette by practicing counter illumination similarly to many mid-pelagic fishes, squids and crustaceans. These organisms emit light from ventral photophores as an antipredatory/predatory strategy to match the ambient downwelling light in intensity, spectral composition and angular distribution. Herring and Morin (1978) suggested that counter-illumination would be selectively advantageous in ponyfishes, since the fishes most commonly caught along with them were visually oriented piscivores, such as synodontids that sit on the bottom and ambush. Moreover, the amount of light produced by several species of leiognathids seemed to be inversely correlated with the depth they usually occupy (Pauly, 1977).

McFall-Ngai and Morin (1991) demonstrated in the laboratory that *Gazza minuta* responded to an increase in intensity of downwelling light with an increase in the intensity of ventral luminescence, with the greatest intensity displayed downwards at an angle of 20–35° from the body midline. *Gazza minuta* and several additional leiognathids displayed a ventral mottled pattern of luminescence when exposed to a downwelling light. This response was suggested to better match the heterogenous shallow water environment (e.g., shadows, terrigenous influences and surface ripple effects). Thus, counter-illumination, the uniform diffuse light displayed by mid-pelagic organisms which occupy a highly uniform and predictable environment, was replaced in the shallow water ponyfishes by disruptive illumination. In ponyfishes luminescence levels were closer to the intensity of downwelling light at low light levels, with a decrease in the proportion of light compensated by bioluminescence from 18.1 to 1.4% at low and high ambient light levels, respectively. This finding indicates a transition from disruptive illumination at low light levels (i.e., dawn and dusk, moonlight and /or turbid water) to contra-shading (i.e., ponyfish dark dorsum and silvery ventrum) based on reflected light at high light intensities. Disruptive illumination is probably most effective at dawn and dusk when crepuscular predatory fishes known to prey on leiognathids are most active (McFall-Ngai and Morin, 1991).

### *Discrete Projected Luminescence (DPL)*

McFall-Ngai and Dunlap (1983) described in *Gazza minuta* a unique discrete projected luminescence (DPL) that consisted of two beams of light each emanating from a clear patch of skin lying at the posterior margin of the opercular cavity. These light beams differ from all other ponyfish light signals in having a point source character being directed from the skin patch antero-ventrally with a maximum intensity at an angle of about 30–45° from the midline (Figure 1.25A). The light controlling mechanisms include the lateral shutters of the light organ, the opercular flap, which is slid forward during light emission, and the chromatophores in the skin peripheral to the patch. The light passes only a distance of about 3 mm through translucent tissue from the light organ to the transparent skin due to the extremely compressed body of *G. minuta*. A similar DPL was probably also mentioned by Harms (1928) in the first description of luminescence in ponyfishes. According to McFall-Ngai and Dunlap (1983) DPL was not displayed during the day or at night under bright illumination, but only at dim light or darkness. Usually, groups of *G. minuta* in captivity started to display 20–60 minutes after dusk. The number of displaying individuals increased with time, shifting from infrequent and short displays to frequent and long ones. The pattern of light display is varied and includes both short and long periods of on and off (Figure 1.25B). Little is known about the function of this display, which was only observed in captivity, except that the light is emitted similarly in males and females. DPL has been suggested to be involved in avoiding nocturnally active predators, helping the fish to orient to its surrounding at night and possibly to be involved in intraspecific communication, such as spacing foraging individuals and reproductive activities. Only *G. minuta* was observed to produce discrete light beams, whereas several additional leiognathid species maintained and observed under similar aquarium conditions failed to do so. Possibly, this type of display is limited to other species of the genus *Gazza* and *Secutor* which possess a similar transparent patch on the posterior margin of the opercular cavity.

### *Ventral Body Flash*

A ventral flash (i.e., maximal uniform luminescence over two thirds of the fish) was emitted by *G. minuta* in the darkness while it was laterally facing the approach of a large object and just before

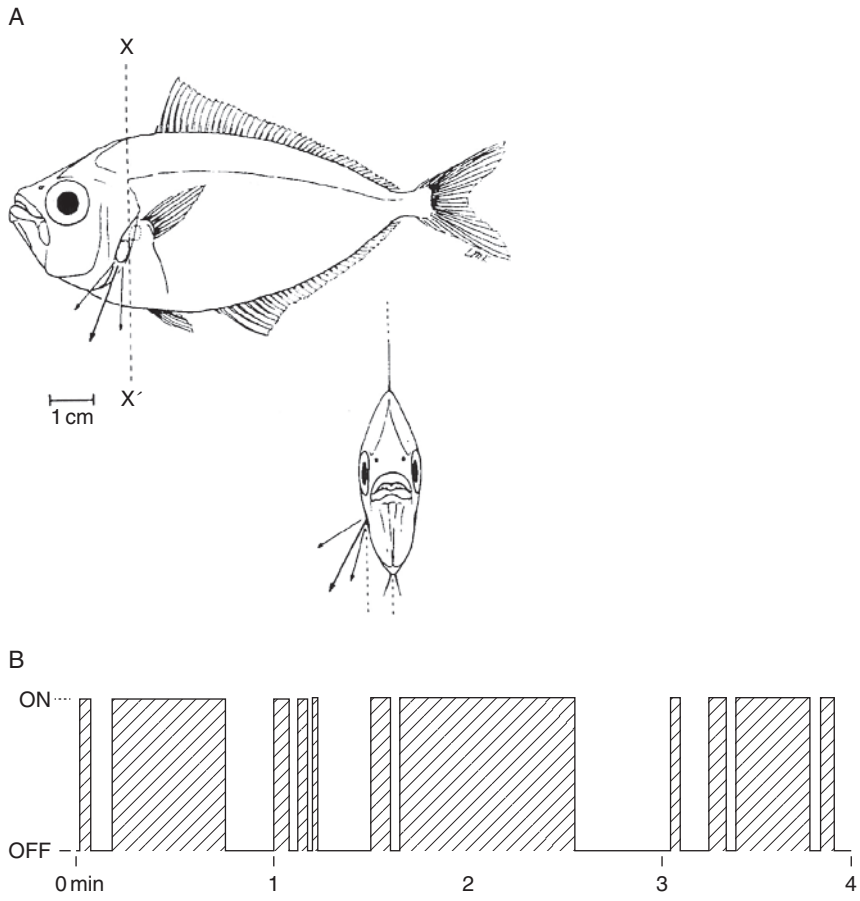


**Figure 1.25** A discrete projected luminescence (DPL) display generated by *Gazza minuta*: A. *Gazza minuta* with a clear patch area (solid line) from which a light beam emanates with maximum intensity at an angle of 30–45° to the body midline (dotted line indicates light organ position); B. Temporal profile of a typical DPL display (McFall-Ngai and Dunlap 1983. Reproduced with permission of Springer Science + Business Media).

darting away (McFall-Ngai and Dunlap, 1983). According to McFall-Ngai and Dunlap (1983) all three shutters of the light organ were briefly opened resulting in bright light, possibly startling a predator or briefly jamming its visual system.

### *Opercular Flash*

According to McFall-Ngai and Dunlap (1983) this display was elicited when *G. minuta* were netted and handled, with light being emitted at variable intensity from the entire area of the operculum. The lateral shutters and the chromatophores of the light organ were suggested to be involved in this display, which possibly serves to startle and intimidate a piscivore. This display had been described before by Haneda (1940, 1950) and Herring and Morin (1978).

### *Buccal Luminescence*

This type of luminescence was only observed in moribund *G. minuta* when being handled. According to McFall-Ngai and Dunlap (1983) light shone from the light organ directly into the buccal cavity by passing through a clear membrane at the back of the throat. Buccal luminescence was suggested to be involved in feeding, luring visually attracted prey.

### *Sex-Specific Signaling*

This mode of communication is discussed in detail in the last section, dealing with ponyfish luminescence.

## *Inception of the Association between Luminescent Bacteria and Ponyfishes*

Bacteria from the water surrounding ponyfishes ingested while feeding or breathing were suggested by Harms (1928) to infest the fish light organ from the digestive tract via the gland's ducts that open into the esophagus. About fifty years later, Hastings and Mitchell (1971) stated that the time and mode of ponyfish infection is unknown, while Reichlet *et al.* (1977) added that there is no suggested mechanism for regulating symbiotic bacteria specificity. The little we know about the inception of the partnership between bacteria and ponyfishes is based on the rare capture of larvae of these fishes and their examination with light and electron microscopy (Dunlap and McFall-Ngai, 1987). According to McFall-Ngai (1991), it has been assumed that infection begins anew with each generation because ponyfish eggs have not been shown to carry symbiotic bacteria that can be cultured from water where leiognathids are found (Reichlet *et al.*, 1977). Moreover, the formation of the light organ from the gut by out pocketing of the esophagus tissue assures that the fish are exposed to the bacteria with the first feeding.

Dunlap and McFall-Ngai (1987) stated that study of the initiation of the association would be crucial for our understanding of specificity as well as the involvement of the bacteria in the development of the light organ. However, to achieve these goals large numbers of small larvae had to be captured or these fishes had to be reared from the eggs in captivity. Progress was made following the closing of the leiognathid life cycle, the capture of large numbers of ponyfish larvae and the application of nucleotide sequencing to the bacteria contained in the larval light organs. Wada *et al.* (1999) demonstrated the transfer of symbiotic luminescent bacteria from adult *Nuchequula nuchalis* to larvae and juveniles. Aposymbiotic larvae (i.e., larvae without symbiotic bacteria), were reared several times from eggs for about 45–50 days and were subsequently exposed inside perforated plastic cages to water that either contained adults or no fish in the control groups. 33–100 % of the larvae exposed to adult ponyfish became luminescent in less than 48 hours and only few individuals in one of the control groups, possibly due to the presence of symbiotic bacteria in the nonsterilized sea water used in these experiments. Groups of aposymbiotic juveniles, 60 days old, were exposed for seven hours to either sea water that contained a light organ homogenate or plain sea water in order to study the dynamics of the acquired luminescence. Seven of the eight juveniles exposed to the light organ homogenate developed luminescence within 10 hours of inoculation with an increase in light intensity with time, whereas all control juveniles remained nonluminescent. This study conclusively demonstrated that ponyfish are free from symbiotic bacteria at hatching and produce luminescence only after infection with symbiotic bacteria from surrounding sea water. Moreover, *N. nuchalis* as young as four weeks post-hatch may already be able to establish the luminous symbiosis in the presence of symbiotic bacteria due to the possession of a light organ in these larvae.

Inception of bioluminescent symbiosis in *Nuchequula nuchalis* was studied by examination of the development of the light organ and its microbiological status in a large number of flexion and post-flexion larvae, juveniles and adults captured in the field (Dunlap *et al.*, 2008). Flexion larvae 6.0–6.5 mm NL (i.e., notochord length) either contained luminescent bacteria or not, whereas specimens larger than 6.5 mm NL invariably contained symbionts. All symbionts were identified as *Photobacterium leiognathi* by nucleotide sequencing of the *lux* A region of the bacterium. In flexion larvae between 6.0 and 6.5 mm NL the light organ contained tubules varying widely in diameter and was covered dorsally by a layer of pigment, possibly to prevent light from passing dorsally through the larva transparent epiaxial musculature. The stomach had not yet differentiated and the gas bladder was small and had not yet established the interface with the light organ that is characteristic of juvenile and adult ponyfishes. The presence of the light organ in specimens not yet colonized by bacteria indicates that development of the light organ precedes to host acquisition of symbiotic bacteria. Bacteria identified as *Vibrio harveyi*, which have never before been reported in partnership with fishes, were present in 15% of the colonies originating from the light organ of one larval specimen. According to Dunlap *et al. (*2008) that occurrence may be rare, since it may lead to the death of the fish due to the pathogenicity of this bacterium. Alternatively, *V. harveyi* may gain a temporary

foothold in the light organ but is later outcompeted by *Photobacterium leiognathi*. Bacterial populations of the light organ of larvae were similarly diverse as those harbored by adult fish, being typically composed of two to three genetically distinct strain types of *P. leiognathi*. Symbiont strain "sharing" was rare. The bacterial populations of the light organ of each specimen were composed of completely different strains, indicating that colonization of the light organ appeared to be random with regard to symbiont strain type.

Finally, because the Dunlap *et al. (*2008) study was based on wild caught specimens, their results also provided insight into life history traits that are critical for the continuity of the association. Adult *N. nuchalis* in Suruga Bay, Honshu, Japan, are demersal, occurring in relative shallow water. The adults spawn in this habitat and the small planktonic eggs are dispersed by currents into open waters of the bay up to 2 km offshore where the larvae hatch. The preflexion aposymbiotic larvae migrate inshore for the next 15–20 days. They acquire the symbiotic bacteria as flexion larvae in the shallow wave zone where these bacteria are readily found in contrast to their scarcity in the off shore waters.

### *Sexual Dimorphism of the LOS, Sex-Specific Signaling and the Role of Sexual Selection in the Evolution of Leiognathid Fishes*

The occurrence of sexual dimorphism in small schooling fishes is unusual and not expected, given the selection pressure by predation for increased similarity of shape, size and coloration of school members. However, the external sexual dimorphism of ponyfishes remains hidden and is only revealed through light flashes when the fish are not threatened, not jeopardizing concealment afforded by counter shading and/or disruptive illumination (McFall-Ngai and Dunlap, 1984). A similar concealed sexual dimorphism is displayed by schooling males of the acanthurid *Naso tapenosoma*, which are usually similar to the females in coloration but are able in an instant to display a flamboyant courtship coloration (Eibel-Eibesfeldt, 1962). The incidents of sexual dimorphism of the light organ and the structures associated with light transmission (LOS) are high among ponyfishes involving about two thirds of the so far described species (Chakrabarty *et al*., 2011a). In early studies (Haneda and Tsuji, 1976), few dimorphic species were found, probably because transparent patches may have been ascribed to abrasion during trawl collection and the geographical differences in the dimensions of the light organ may have confounded the gender effect (McFall-Ngai, and Dunlap, 1984). Sexual dimorphism in the light organ includes differences in volume and structure of this gland. In the extreme case of *Equulites elongates* the male light organ may be 100 times larger than that of the female (Dunlap and McFall-Ngai, 1984). Hypertrophy of the male light organ consists mainly of an increase in size of the dorsolateral and ventrolateral lobes (Figure 1.26).

In a recent study, MRI (Magnetic Resonance Imaging) technology was applied to examine light organs of ponyfishes *in situ* without damaging this delicate organ by dissection (Chakrabarty *et al*., 2011a). A light organ index (LOI) was calculated by dividing the light organ volume by whole body volume and multiplying by $10^3$. Among different ponyfish species, male LOI ranged between 0.3 in *Leiognathus equulus*, a species lacking sexual dimorphism, and 17.7 in *Equulus rivulatus*, a highly dimorphic species. According to Ikejima *et al. (*2008) stronger bioluminesence from larger LOs in males might have created displays more attractive to females and/or could have deterred male competitors more effectively from intruding into their private territories, leading to greater reproductive success. Many of the species with sexually dimorphic LOs also possess sexually dimorphic structures involved in light reflectance, transmission and diffusion, such as light guides and transparent patches.

Five distinct and highly specialized morphologies for male specific lateral signaling have evolved (Sparks *et al*., 2005). For example, in *Equulites* sp. the dorsolateral lobes of the light organ are hypertrophied (Figure 1.26B-a, 1.26C-a) extending posteriorly into the gas bladder, with a clearing of its silvery lining adjacent to a transparent flank patch (Figure 1.27A, 1.27B). Very differently in *Gazza* sp. the ventrolateral lobes are hypertrophied (Figure 1.26B-c, 1.26C-c) extending into an anterior silvery guanine lined reflective chamber allowing for light transmission and reflection to the opercular margin patch (Figure 1.27F). According to Sparks *et al.* (2005), within each clade of externally sexually dimorphic ponyfishes, the size, shape or orientation of the transparent external patches vary interspecifically (Figure 1.27A, 1.27B). Moreover, Chakrabarty *et al.* (2011b) added that ponyfish species may also differ in light intensity, the wave

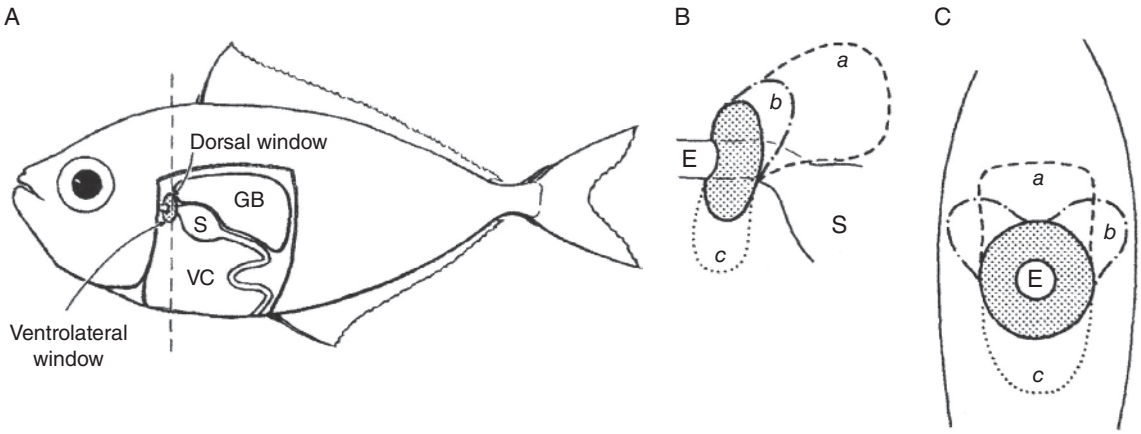


**Figure 1.26** The leiognathid light organ structure and position: A. position of the light organ relative to the fish internal organs [E – esophagus; GB – gas bladder; S – stomach; VC – visceral cavity]; B. lateral view of the hypertrophy of the light organ dorsolateral and ventrolateral lobes in males of sexually dimorphic species; C. cross-sectional view of the above [a. – *Equulites leuciscus* and E. *elongates*, b. – *Photopectoralis bindus* and *P. aureus*, c. – *Gazza* and *Secutor* species]. Stippled area represents the light organ in females and in males of nondimorphic species (McFall-Ngai and Dunlap 1984. Reproduced with permission of John Wiley & Sons).

length of the emitted light and the signaling rate. Ancestral character states were reconstructed using likelihood methodology (Chakrabarty *et al*., 2011a). Internal and external dimorphism in males were found to be statistically correlated. These features probably evolved once in the stem species of the Leiognathidae, with their subsequent loss in the subfamily Leiognathinae. Two additional losses of externally sexually dimorphic structures associated with light transmission have possibly occurred in the genera *Eubleekeria* and *Karalla*. Reconstruction of the transparent patch shape and its location on the body of the common ancestor was not possible due to extreme variability in these features.

There is some indirect (Azuma *et al*., 2005; Ikejima *et al*., 2008) and direct evidence (Sasaki *et al*., 2003; Woodland *et al*., 2002) for sex-specific signaling in ponyfishes. The development of the LOs and the gonads over the yearly cycle was monitored in *Equulites rivulatus* by examination of specimens collected at depths of 20–60 m, Kanagawa prefecture, Japan (Ikejima *et al*., 2008). In males there was a significant positive correlation between the GSI (gonado somatic index) and the PLW (percentage light organ weight of total body weight), whereas in females there was no change in the PLW, irrespective of the increase during the breeding season (i.e., June through August) in the GSI. The onset of sexual maturity in males (55 mm SL) coincided with that of the light organ enlargement. These findings, as stated by Ikejima *et al.* (2008), clearly indicate a functional coupling between reproduction and bioluminescence in this ponyfish species. The involvement of bioluminescence in the reproductive activity of *Nuchequula nuchalis* was demonstrated in captivity (Azuma *et al*., 2005). Luminescence was monitored with a photometer during day and night, in and outside the breeding season in a group of fish maintained in constant darkness in a circular tank. Light was detected only during the breeding season. The frequency of light signals increased during the night and particularly during spawning hours (20.00–24.00).

The best evidence for sex-specific signaling in ponyfishes was provided by Sasaki *et al.* (2003) from their early night, field observations of groups of interacting *Equulites elongates*. These schools, which contained luminescent and nonluminescent individuals, were repeatedly observed from mid-May to early September at depths of about 15 m over open sand or a soft bottom. Luminous individuals were displaying on and off at about one

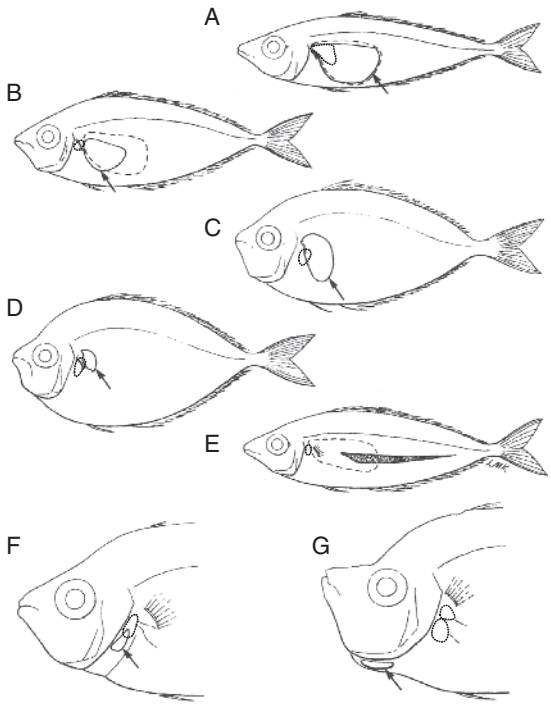


**Figure 1.27** Position of the light organs and transparent patches in several sexually-dimorphic leiognathid species: Dotted line indicates location of light organ, dashed line indicates location of gas bladder; solid line and arrow indicate position of clear skin patch. A. *Equulites elongates*; B. *Equulites rivulatus* C. *Photopectoralis aureus*; D. *Photopectoralis bindus*; E. *Equulites stercorarius*; F. *Gazza* sp.; G. *Secutor* sp. (McFall-Ngai and Dunlap 1984. Reproduced with permission of John Wiley & Sons).

second intervals a distinct rectangular luminescent patch on their flanks. The luminescent individuals seemed to chase the nonluminous ones. Luminescent fish were very likely males, since in this species only males possess transparent light transmitting patches. Occasionally synchronized flashing was observed in more than 80% of the luminescent individuals.

The synchronized nature of the ponyfish light display was also reported from Ambon, Indonesia for *Eubleekeria splendens* (Woodland *et al.*, 2002). Schools composed of several hundred individuals were observed on three different nights with little moonlight synchronously displaying. Flashes of light from the entire latero-ventral surface of their bodies were emitted on and off at, respectively, 0.2 and 0.4 second intervals. The fish were continuously on the move while flashing their lights, swimming rapidly, while the whole school, which had a diameter of about 10 m, maintained its position relative to the bottom. The fish responded to the sudden turning on of a dive light by stopping light emission and diving to the bottom. However, one minute after the dive light was extinguished flashing recommenced. At first only a few fish but gradually most, if not all, resumed flashing. About 30 seconds after the school was reformed in mid-water all flashing became synchronized. Woodland *et al.* (2002) suggested four possible roles for the synchronized light display – reproduction, feeding, predator avoidance, and school cohesion. According to these authors, if the signals were not synchronized the structure of the signal would be lost against a background of randomly flashing lights. Although, *E. splendens* lack external sexual dimorphism, males possess larger light organs than females. The on and off pattern could be species specific and the displaying fish may all be males flashing in synchrony to attract females.

Sexual selection has been hypothesized by Sparks *et al.* (2005) as driving diversification of ponyfishes. According to these authors, species-specific signals coupled with female choice frequently function to create pre-zygotic reproductive barriers among close sympatric species [e.g., courtship signaling in fire flies (Lloyd, 1966)]. Luminescent courtship signals by ponyfishes may similarly function to attract females, induce spawning and segregate species for reproduction. In the absence of sexual selection it is difficult to envision another mechanism underlying the extreme sexual dimorphism of these fishes. Natural selection is unlikely, since the male signals render the males more vulnerable. Moreover, if the system had evolved under selection pressures to avoid predators or to facilitate prey capture, both genders would be expected to exhibit similar morphologies. Finally, species-specific male signals in ponyfishes permit the coexistence of morphological similar species in a habitat with often reduced visibility, with the luminescent signal being instrumental in diversification of this group.

The role played by sexual selection in the tempo and mode of ponyfish evolution could be tested by Chakrabarty *et al.* (2011b) thanks to the fact that this group of fishes included both sexually

dimorphic and nondimorphic clades. According to these authors, there is no conclusive evidence that sexual selection mechanisms have influenced any significant increase or decrease in the rates of diversification in this group. In ponyfishes sexual selection may be acting only as an isolating mechanism that has allowed ponyfish to diversify continuously over time regardless of habitat or niche constraints not slowing down following density dependent speciation. However, sexual selection could also supplement other mechanisms of diversification through genetic isolation (Chakrabarty *et al*., 2011b).

In contrast to the impressive progress in recent years in the study of ponyfish LOS, phylogeny and taxonomy with the description of numerous new species, there is still very little evidence for sex-specific signaling. There is need for both field and controlled laboratory studies in order to describe the light signals given by males of different species and to analyze intraspecific sexual communication particularly in the context of mate choice and reproductive isolation.

## Specificity of the Partnerships between Luminescent Bacteria and Fishes

Luminescent bacteria contained in fish light organs have been identified on the basis of multiple traits, including structure, nutritional versatility on minimal media, production of extracellular enzymes, type of decay kinetics of *in vitro* luciferase assays and, recently, nucleotide sequencing (Reichlet and Baumann, 1973; Ruby and Nealson, 1976; Kaeding *et al*., 2007; Dunlap *et al*., 2008). Until recently, strict specificity of the partnership at the level of host family–bacterial species was demonstrated. For example, fishes of the family Monocentridae were associated only with *Aliivbrio fischeri* (Ruby and Nealson, 1976; Fitzgerald, 1977), whereas members of the family Leiognathidae were only associated with *Photobacterium leiognathi* (Reichlett *et al*., 1977). The mechanism regulating specificity is still unknown, although this issue was frequently addressed (Reichlet *et al*., 1977; Hastings *et al*., 1987; Nealson and Hastings, 1990). In Herring's own words "The task faced by a newly hatched larva (recognizing and capturing the right symbiont) makes finding a needle in a haystack seem a wholly trivial problem" (Herring, 1993).

There is considerable evidence that the appropriate bacterial species is newly acquired every generation from the environment and not transferred vertically from parents to offspring. This is mainly based on lack of bacteria in early developmental phases of larvae of monocentrids, leiognathids and apogonids that subsequently associate with luminescent bacteria (Yamada *et al*., 1979; Leis and Bullock, 1986; Wada *et al*., 1999; Dunlap *et al*., 2008, 2009). Moreover, no luminous bacteria were found in the light organs of deep sea anglerfishes prior to the formation of a functional canal connecting the surrounding sea water with the lumen of the tubules in the light organs (Munk *et al*., 1998).

There have been several suggestions concerning the mechanism of specificity regulation. Haneda and Tsuji (1976) suggested that the ducts connecting the tubules with the exterior may be involved in the specificity of the initial infection. According to Herring and Munk (1994) the secretion from special goblet cells contained in the escal light organ of small individuals of deep sea anglerfishes but not in adults may play an important role in the initial establishment of the right strain of symbiotic bacteria. Hastings *et al.* (1987) suggested several mechanisms for regulating specificity involving fish lectins, antibodies and inhibitatory substances. Fish lectins could bind receptors on the symbiont cell surface, thereby enhancing nutrient exchange only with these bacteria. Fish antibodies could react with all bacteria but the symbionts. Finally, inhibitatory substances (e.g., antibiotics) to which the symbionts are resistant could be produced by the fish or by the bacteria themselves. Indeed, isolated symbiotic strains of *Photobacterium leiognathi* excreted substances inhibiting the growth of other bacteria (Hastings *et al*., 1987).

More recent studies that involved DNA sequencing for species and strain identification which are more accurate than methods based exclusively on comparisons of phenotypic traits (Ast and Dunlap, 2004), revealed lack of strict specificity in fish–luminescent bacteria partnerships. In a single light organ two species of *Photobacterium* or one species of *Photobacterium* and one species of *Aliivebrio* co-occurred, a situation termed cosymbiosis (Dunlap *et al*., 2007; Kaeding *et al*., 2007). According to Dunlap *et al*. (2007) the genetic and physiological differences between two species of bacteria occupying the same light organ may be unimportant to the symbiosis or the important attributes may be shared by the two species. However, the predominance of one species over the other may reflect competitive

interactions leading to changes with time in the bacterial populations. Within a given host family different fish species harbored different *Photobacterium* species or *Photobacterium* and *Aliivibrio* (Dunlap *et al*., 2007). In a single instance, a larval ponyfish harbored both *P. leiognathi* and a large population of *Vibrio harveyi*, a bacterium which usually does not occupy light organs (Dunlap *et al*., 2008). Several genotypically distinct strains are typically found within the light organs of larval and adult fishes indicating that within species variation seems to be the norm (Dunlap *et al*., 2004, 2008) and that light organs are colonized more than once. The complete absence of nonluminous bacteria from the light organs of fishes indicates that some mechanism of selection, possibly based on bacterial activity shared by all luminescent Vibrionaceae, namely luminescence or activity associated with luminescence is involved (Urbanczyk *et al*., 2011). Some support of this suggestion was provided from studies on squids harboring luminescent bacteria in their light organs. The light organs of *Euprymna scolopes* were found to have the molecular machinery (i.e., the genes that encode part of the visual transduction cascade) and the physiological potential to respond to light as revealed by electro retinograms (Tong *et al*., 2009). Possibly, the extra ocular photoreceptors of *E. scolops* are involved in the exclusion of dark mutants of *Allivibrio fischeri* from the squid light organs (Visick and McFall-Ngai, 2000). Despite the recent evidence that specificity of fish–luminescent bacteria is not strict, it is, however, high and some unknown mechanisms of selection of the fish host and/or symbiotic luminescent bacterium species are definitely involved in the formation of these partnerships and maintenance of their specificity.

## Optimization of the Benefits to Fishes from their Association with Bacteria

Theoretically, in order to maximize the fish benefit from the partnership with luminescent bacteria, light emission must be intense, as high or higher per cell than is achieved in cell culture whereas bacterial growth must be constrained to save the host resources (Hastings *et al*., 1987). Indeed that seems to happen; however, the mechanism underlying this phenomenon is still largely unknown. There are currently two lines of evidence that symbiotic bacteria growth is reduced in fish light organs compared to their growth rate outside these organs. Growth rate of *Photobacterium leiognathi* in the light organ was estimated to be twenty times slower than in culture media based on the percentage of bacteria in a duplication state (Dunlap, 1984a). The time required for duplication of the bacterial population inside the light organ can be estimated in the laboratory by monitoring the rate of bacteria expulsion into the surrounding water and estimating the number of bacteria in the organ. These values were subsequently compared to bacterial duplication time in a culture medium, revealing for several symbiotic species reduced growth rates inside the light organs. Duplication time of *Aliivebrio fischeri* inside the Japanese pine cone fish (*Monocentrus japonicus*) light organ was variable and ranged from 7.5 to 135 h, whereas in a culture medium it was only 45 minutes. Duplication time of bacteria contained in the light organs of the anomalopid fishes *Photoblepharon palpebratus* and *Kryptophanaron alfredi* were 23 and 8 h, respectively (Haygood *et al*., 1984). These values cannot be contrasted to duplication time in a culture medium, since these bacteria have so far not been cultured and seem to be obligately symbionts. However, duplication time in anomalopids seems to be slow, consistent with the single Ribosomal RNA operon copy detected in bacteria removed from *Kryptophanaron alfredi*. The reduction in the number of operon copies was suggested as typical for continuous slow growth in a single type of environment suiting the permanent adaptation to the light organ conditions (Wolf and Haygood, 1993).

Levels of produced light and bacterial growth rate were manipulated in the laboratory in several species of luminous bacteria in order to find factors which would have contradictory effects on light and growth. Several such factors, which were often species specific, were found, including low levels of oxygen, iron limitations and osmolarity (Hastings *et al*., 1987). In some strains of symbiotic *Photobacterium kishitani* and *Aliivebrio fischeri* luminescence and luciferase synthesis continue intensively under low oxygen levels with higher luciferase content than in cells grown in air whereas growth was limited. However, in another symbiont, *P. leiognathi*, luciferase synthesis was not stimulated by low oxygen (Nealson and Hastings, 1977; Hastings *et al*., 1987). In several species of symbiotic bacteria, distinctly different

effects of osmotic conditions on growth and luminescence were found (Dunlap, 1984b). In *P. leiognathi* optimal luminescence occurred at about 30% sea water whereas optimum salt concentrations for growth were found to be near that of sea water. In contrast, in *P. kishitani* the situation is reversed, luminescence is optimal slightly above sea water osmolarity while growth is favored at about 50% sea water. Dunlap (1984b) proposed that the physiological differences between bacterial species with regard to the effect of osmolarity may be related to the osmotic conditions maintained in the light organs, where a particular symbiotic species occurs such that growth is restricted and luminescence is favored. In leiognathid's light organs which are internal, osmolarity is low, favoring in *P. leiognathi* luminescence and inhibiting growth, whereas in macrurid's light organs, which lead to the hindgut where surplus salts are discharged, osmolarity is high, favoring in *P. kishitani* luminescence and inhibiting growth. Hastings *et al. (*1987) suggested that luminescence and growth of symbiotic bacteria may be controlled by a multiplicity of factors.

No information is available concerning whether or not and how factors controlling light and growth in the laboratory operate in the fish light organs (Hastings *et al.*, 1987). Two models, the oxygen limitation model and the continuous culture model, were suggested to relate laboratory experiments and light organ ultrastructure to the control of bacterial light and growth in the light organs (Nealson, 1979). According to the oxygen limitation model, the Japanese pine cone fish provides the bacterium *Aliivibrio fischeri* with glucose that is metabolized by the bacterium into pyruvate. The epithelial cells lining the tubules are rich with mitochondria, which take up the pyruvate and oxidize it, reducing the oxygen levels available to the bacteria in the tubule lumen. The reduction in oxygen tension is such that bacterial growth is retarded and luminescence enhanced. A simple negative feedback loop operates to maintain slow growth and high light. Faster bacterial growth results in more pyruvate excretion, hence more mitochondrial metabolism and lower oxygen levels, which result in slower growth and enhanced luminescence. *Photobacterium leiognathi* the symbiotic bacteria contained in the circum esophageal light organs of members of the family Leiognathidae, require high levels of oxygen for luminescence, they do not excrete pyruvate and mitochondria rich cells are not present in their tubule linings. The continuous culture model was suggested for saving the host resources in these partnerships. The bacterial contents from the light organs empty directly into the fish stomachs where part of them are digested. This is similar to the situation in ruminants, where the microbial symbionts from the first stomach are passed to a later stomach where they are digested. Another suggestion by Nealson (1979) was that ponyfish light organs may operate like a carbon-limited chemostat with reduced growth of bacteria but with maximized light.

## The Evolution of the Partnerships between Fishes and Luminescent Bacteria

The structural similarity of the *lux* operon of all luminescent bacteria supports the notion that luminescence in bacteria evolved once (as discussed in the first section of this chapter). Generated light levels in the early stages of the evolution of luminescence in bacteria were probably too low to be noticed by higher organisms as being beneficial and selected for. It was suggested that other functions, such as removal of detoxifying deleterious oxygen derivatives (Rees *et al.*, 1998) and repair of damaged DNA sequences by the photoreactivation reaction, effective also at very low light levels (Czyz *et al.*, 2000), were driving the early stages of the evolution of luminescence. Only after the generated light levels were bright enough to be noticed, was luminescence per se selected for (Wegrzyn and Czyz 2002).

Quorum sensing fulfilled a central role in the evolution of bacterial luminescence, since it assured that light was produced in an energy conserving manner, not by a single cell but in large enough groups to produce light levels that are detectable (Widder, 2010). The benefits to bacteria from luminescence probably differed when they were free living, gut symbionts or light organ symbionts involving different selection pressures for light enhancement. Partnerships between fishes and luminescent bacteria may have arisen as gut symbionts, in conjunction with production of the enzyme chitinase by these bacteria, which aid in the digestion of crustaceans exoskeletons (Morin, 1981). Luminous gut symbionts of fishes are excreted with the fecal pellets, which they render luminescent. These pellets are ingested by a variety of fishes due to their nutritive value (Turner and

Ferrante, 1979). Luminescent fecal pellets were found in laboratory experiments to be more attractive than nonluminous ones (Morin, 1983). The generated light thus increases the chances of these luminescent bacteria returning to the nutrient rich gut environment (Herring, 1982; Nealson and Hastings, 1990). Luminous light organ symbionts obtain from their host a sheltered environment, oxygen and nutrients.

The fish–luminescent bacteria partnerships probably evolved numerous times, based on the diversity and distant taxonomic links among the host fishes which involve 21 families, members of seven different orders. Moreover, the light organs differ among groups in structure and location, including both internal and external light organs located below the eyes in anomalopids, in the lower jaw of monocentrids and in the escal lure of deep sea anglerfishes. The internal light organs which are connected to the digestive tract could have been easily invaded by the luminescent enteric bacteria. However, the external light organs have no obvious morphological precursors for their colonization. The fact that members of different families associate with different bacterial species further supports the suggestion of multiple independent events of evolution of these partnerships (Ruby and Morin, 1978; Herring, 1982; Haygood, 1993). According to McFall-Ngai (1991) the occurrence of partnerships between fishes and light organ symbionts is restricted to the more recently evolved teleost species. The taxa in which most associated fishes occur (e.g., Gadiformes, Perciformes and Ceratioidei) arose during the Cenozoic beginning approximately 65 million years ago. The Beryciformes (e.g., anomalopids and monocentrids) are probably among the oldest fishes with light organ symbionts, with a fossil record that dates from the Cretaceous. In all cases the presence of symbionts defines a taxon, characterizing all the species of a genus or family. There are several attributes of the fish–luminescent bacteria partnership which suggest coevolution. Among these attributes are the host dependence on bacterial light, with all fishes being obligately associated with luminescent bacteria. Further attributes include the special anatomical adaptations of the host for harboring and controlling light emission, the host–bacteria metabolic integration with part of the bacteria probably becoming obligately associated with the fish and, finally, the host family–bacterium species specificity, which may enhance coevolutionary processes between fishes and bacteria (Urbanczyk *et al*., 2011).

The symbiotic bacteria associated with light organs belong to two groups depending on their level of attachment to their host. The facultative bacteria, such as *Photobacterium leiognathi* and *Aliivibrio fischeri*, can be grown in the laboratory on culture media as well as extracellularly in the fish light organs. The facultative symbiont *P. kishitanii* removed from two hosts belonging to different orders possessed indistinguishable 16SrRNA genes (Haygood and Distel, 1993). The large number of Ribosomal RNA operons copies in facultative symbionts, ranging between 8 and 11,were suggested to allow effective shifts between a fast or feast environment, namely between the poor sea water environment and the nutrient rich light organs (Haygood, 1993; Urbanczyk *et al*., 2011). The so far unculturable bacteria contained in the light organs of anomalopid flashlight fishes and ceratioid deep sea anglerfishes were initially believed to be related to the facultative light organ bacteria (Herring, 1993). However, sequencing of 16SrRNA genes of these bacteria, contained as pure cultures inside the light organs, revealed that they belong to two different monophyletic groups, which differed from a third monophyletic group of facultative light organ symbionts (Figure 1.3; Haygood and Distel, 1993). According to Haygood (1993) it is reasonable to hypothesize that obligate light organ symbionts began as facultative ones, gradually becoming isolated from genetic exchange with free living populations. Obligate symbionts should cospeciate with their specific hosts, changing genetically and losing adaptations for living in a nonsymbiotic environment. Differences among luminescent Vibrionaceae are thus not due to a dramatic different origin but rather due to changes in the obligately associated bacteria along with their hosts. According to Hendry and Dunlap (2011) organization of the genes flanking the *lux* operon in bacteria removed from *Anomalops katoptron* differed from those of *Photobacterium* leading to the creation of a new genus and species *Photodesmus katoptron*. Whether the bacteria contained in the light organ of other flashlight fishes belong to the same new genus is still unknown. Increased nucleotide substitution rate in *Photodesmus katoptron* compared to other members of the Vibrionaceae is consistent with

population bottlenecks at their transfer to members of a new host generation and is suggestive of an obligate relationship.

Phylogenies based on DNA sequencing of several genes of host fishes belonging to seven teleost families and the facultative bacteria isolated from their light organs revealed no meaningful congruence (Dunlap *et al.*, 2007). The lack of codivergence was suggested to reflect independent evolutionary processes not likely to have arisen through coevolutionary interactions. The independent evolution of the bacteria and fish lineages was suggested to be related to the fact that the facultative symbionts recolonize the host every new generation while they occupy in addition to the light organs also a variety of other marine habitats, including intestinal tracts, skin, sediments and sea water. Colonization of the host occurs in a somewhat general manner, with luminescence being the most important contribution of the bacteria to the partnership. The fact that the host light organs develop independently and are not triggered by a specific bacterial species further supports the notion of an independent evolution of these associated organisms (Dunlap *et al.*, 2007).

Different from facultative partnerships, the partnerships between host fishes and luminescent bacteria which have not been cultured in the laboratory and may be obligatory symbionts, may have coevolved. Indeed the bacteria isolated from two different families of deep sea anglerfishes and several genera of flashlight fishes differed and may have coevolved with their hosts (Wolf and Haygood, 1991, Haygood *et al.*, 1992, 1993). Different from expected the topolgy of the anomalopid symbionts based on 16SrRNA genes is in conflict with the accepted phylogeny of the host fishes based on morphological characters (Figures 1.8 and 1.28). Haygood and Distel (1993) suggested that the divergence between the phylogenies may be due to an error in the host phylogeny. Alternatively, the symbiont of either *Anomalops* or *Photoblepharon* having displaced the other when they became sympatric and subsequently diverged along with their hosts, being closer to one another than to *Kryptophanaron*. Finally, the *Kryptophanaron* symbiont may have become earlier an obligate symbiont and was isolated from free living populations prior to *Photoblepharon* and *Anomalops*. Further research on the congruence of the phylogenies of deep sea anglerfishes and their bacterial symbionts and additional species of flashlight fishes and their symbionts will further refute or corroborate the involvement of coevolutionary processes.

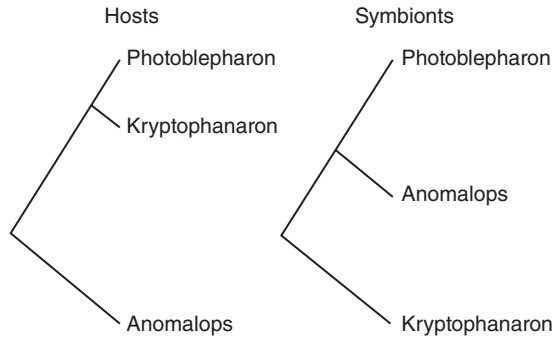


**Figure 1.28** Comparison of phylogenetic relationships of anomalopid hosts based on morphology and symbionts based on small subunit rRNA analysis (Haygood 1993. Reproduced with permission of Taylor & Francis).

Several researchers have stressed environmental congruence or host–symbiont habitat overlap as an explanation for host–symbiont affiliation (Ruby and Morin, 1978; Haygood, 1993; Urbanczyk *et al.*, 2011). It was suggested that the environmental distribution of the luminescent bacteria, where the species or its ancestral form were most abundant and metabolically active, and the host environmental distribution, particularly while being receptive to bacterial colonization, largely determine the identity of the bacteria–fish partnership. Psychotrophic *Photobacterium kishitanii*, which occur in relative cold water, occupy the light organs of the deep water chlorophthalmids and opisthoproctids, whereas the light organs of ponyfishes, which live in the warm coastal waters of the tropics and subtropics, are occupied by the more mesophilic *P. leiognathi*. However, host fishes harbor a specific species also in areas where several other symbiotic luminescent bacteria co-occur. Thus environmental congruence seems to have played an important role in the early evolution of these partnerships, but host and/or symbiont selection are probably currently involved in the establishment and maintenance of specific partnerships.

## References


Ahrens, G. 1965. Untersuchungen am Leuchtorgan von *Leiognathus klunzingeri* (Steindachner). Zeitschrift fuer Wissenschaftliche Zoologie173: 90–113.

Al Ali, B., Garel, M., Cuny, P., *et al*. 2010. Luminous bacteria in the deep sea waters near the ANTARES underwater neutrino telescope (Mediterranean Sea). Chemistry and Ecology 26: 57–72.

Ast, J.C. and Dunlap, P.V. 2004. Phylogenetic analysis of the *lux* operon distinguishes two evolutionary distinct clades of *Photobacterium leiognathi*. Archives of Microbiology 181: 352–361.

Azuma, N., Furubayashi, C., Shichiri, T., *et al*. 2005. Bioluminescence and mating behavior in ponyfish *Leiognathus nuchalis* In: *Bioluminescence and Chemiluminescence Progress and Perspectives* (eds Tsuji, A., Matsumoto, A., Maeda, M. *et al*.). World Scientific Publishing Co. Pte. Ltd., Singapore, pp. 3–6.

Baldwin, C.C. and Johnson, G.D. 1995. A larva of the Atlantic flashlight fish, *Kryptophanaron alfredi* (Beryciformes: Anomalopidae), with a comparison of Beryciform and Stephanoberyciform larvae. Bulletin of Marine Science 56: 1–24.

Baldwin, C.C., Johnson, G.D. and Paxton, J.R. 1997. *Protoblepharon rosenblatti*, a new genus and species of flashlight fish (Beryciformes: Anomalopidae) from the tropical South Pacific, with comments on anomalopid phylogeny. Proceedings of the Biological Society of Washington 110: 373–383.

Bassot, J-M. 1966. On the comparative morphology of some luminous organs. In: *Bioluminesence in Progress* (eds Johnson, F.H. and Haneda, Y.). Princeton University Press, Princeton, NJ, pp. 557–610.

Bassot, J-M. 1968. Les organs lumineux a bacteries symbiotiques du teleosteen *Anomalops*. Donnees histologiques. Bulletin de la Societe Zoologique de France 93: 569–579.

Bassot, J-M. 1975. Les organs lumineux a bacteries symbiotiques de quelques teleosteens leiognathides. Archives de Zoologie Experimentale and Generale 116: 359–373.

Beebe, W. and Crane, J. 1947. Eastern Pacific Expeditions of the New York Zoological Society XXXVII. Deep sea ceratioid fishes. Zoologica 31; 151–181.

Bertelsen, E. 1951. The ceratioid fishes. Ontogeny, taxonomy, distribution and biology. Dana Reptort 39: 1–276.

Bertelsen, E. 1986. Ceratioidei. In: *Fishes of the North-Eastern Atlantic and the Mediterranean*, vol. 3 (ed. P.J.P. Whithead *et al*.). UNESCO, Paris, pp. 1371–1414.

Bertelsen, E. and Krefft, G. 1988. The ceratioid family Himantolophidae (Pisces, Lophiiformes). Steenstrupia 14: 9–89.

Bertelsen, E. and Pietsch, T.W. 1984. Results of the research cruises of FRV "Walther Herwig" to South America LXIII. A resurrection of the ceratioid anglerfish *Ceratias tentaculatus* (Norman, 1930), with notes on the occurrence of the species of *Ceratias* in the Atlantic Ocean (Pisces: Lophiiformes). Archiv fuer Fischereiwissenschaft 35: 43–51.

Bertelsen, E. and Pietsch, T.W. 1996. Revision of the ceratioid anglerfish genus *Lasiognathus* (Lophiiformes: Thaumatichthyidae), with the description of a new species. Copeia 1996: 401–409.

Bertelsen, E. and Struhsaker, P.J. 1977. The ceratioid fishes of the genus *Thaumatichthys*: osteology, relationships, distribution, and biology. Galathea Report 14: 7–40.

Bleeker, P. 1865. Atlas ichthyologique des Indes Orientales Neerlandaises, publie sous les auspices du Gouvernement Colonial Neerlandais. V. Baudroies, Ostracions, Gymnodontes, Balistes. Frederic Muller, Amsterdam.

Carnevale, G. and Pietsch, T.W. 2009. The deep sea anglerfish genus *Acentrophryne* (Teleostei, Ceratioidei, Linophrynidae) in the Miocene of California. Journal of Vertebrate Paleontology 29: 372–378.

Chakrabarty, P. and Sparks, J.S. 2007. Phylogeny and taxonomic revision of *Nuchequula* Whitley 1932 (Teleostei: Leiognathidae), with the description of a new species. American Museum Novitates 3588: 1–25.

Chakrabarty, P., Davis, M.P., Smith, W.L., *et al*. 2011a. Evolution of the light organ system in ponyfishes (Teleostei: Leiognathidae). Journal of Morphology 272: 704–721.

Chakrabarty, P., Davis, M.P., Smith, W.L., *et al*. 2011b. Is sexual selection driving diversification of the bioluminescent ponyfishes (Teleostei: Leiognathidae)? Molecular Ecology 20: 2818–2834.

Colin, P.L. 1988. Anomalopidae-Lanterneyes, flashlightfishes. In: *The Larvae of Indo-Pacific Shorefishes*. (eds Leis, J.M. and Trinski, T.). University of Hawaii Press, Honolulu, pp. 83–85.

Colin, P.L., Arneson, D.W. and Smith-Vaniz, W.F. 1979. Rediscovery and redescription of the Caribbean anomalopid fish *Kryptophanron alfredi* Silvester and Fowler (Pisces: Anomalopidae). Bulletin of Marine Science 29: 312–319.

Czyz, A., Wrobel, B. and Wegrzyn, G. 2000. *Vibrio harveyi* bioluminescence plays a role in stimulation of DNA repair. Microbiology 146: 283–288.

Dahlgren, U. 1908. The luminous organ of a new species of *Anomalops*. Science 27: 454–455.

Dunlap, P.V. 1984a. Physiological and morphological state of the symbiotic bacteria from light organs of ponyfish. Biological Bulletin 167: 410–425.

Dunlap, P.V. 1984b. *The ecology and physiology of the light organ symbiosis between Photobacterium leiognathi and ponyfishes*. PhD Dissertation. University of California, Los Angeles, CA.

Dunlap, P.V. 1999. Quorum regulation of luminescence in *Vibrio fischeri*. Journal of Molecular Microbiological Biotechnology 1: 5–12.

Dunlap, P.V. and McFall-Ngai, M.J. 1984. *Leiognathus elongatus* (Perciformes: Leiognathidae): two distinct species based on morphological and light organ characters. Copeia 1984: 884–892.

Dunlap, P.V. and McFall-Ngai, M.J. 1987. Initiation and control of the bioluminescent symbiosis between *Photobacterium leiognathi* and leiognathid fish. In: *Endocytobiology III* (eds Lee, J.J. and Frederick, J.F.). The New York Academy of Sciences, New York, pp. 269–283.

Dunlap, P.V. and Ast, J.C. 2005. Genomic and phylogenetic characterization of luminous bacteria symbiotic with the deep sea fish *Chlorophthalmus albatrossis* (Aulopiformes: Chlorophthalmidae). Applied and Environmental Microbiology 71: 930–939.

Dunlap, P.V., Jiemjit, A., Ast, J.C., *et al*. 2004. Genomic polymorphism in symbiotic populations of *Photobacterium leiognathi*. Environmental Microbiology 6: 145–158.

Dunlap, P.V., Ast, J.C., Kimura, S., *et al*. 2007. Phylogenetic analysis of host-symbiont specificity and codivergence in bioluminescent symbioses. Cladistics 23: 507–532.

Dunlap, P.V., Davis, K.M., Tomiyama, S., *et al*. 2008. Developmental and microbiological analysis of the inception of bioluminescent symbiosis in the marine fish *Nuchequula nuchalis* (Perciformes: Leiognathidae). Applied and Environmental Microbiology 74: 7471–7481.

Dunlap, P.V., Kojima, Y., Nakamura, S. and Nakamura, M. 2009. Inception of formation and early morphogenesis of the bacterial light organ of the sea urchin cardinalfish, *Siphamia versicolor*. Marine Biology 156: 2011–2020.

Ebeling, A.W. and Cailliet, G.M. 1974. Mouth size and predator strategy of midwater fishes. Deep-sea Research 21: 959–968.

Eibel-Eibesfeldt, I. 1962. Freiwasserbeobachtungen zur Deutung des Schwarmverhaltens verschiedener Fische. Zeitschrift fur Tierpsychologie 19: 165–182.

Fishelson, L., Gon, O., Goren, M. and Ben-David-Zaslow, R. 2005. The oral cavity and bioluminescent organs of the cardinal fish species *Siphamia permutata* and *S. cephalotes* (Perciformes, Apogonidae). Marine Biology 147: 603–609.

Fitzgerald, J.M. 1977. Classification of luminous bacteria from the light ogan of the Australian pinecone fish, *Cleidopus gloriamaris*. Archives of Microbiology 112: 153–156.

Fridman, D. 1972. Notes on the ecology of a new species of *Photoblepharon* (Pisces, Anomalopidae) from the Gulf of Elat (Aqaba). Scientific Newsletter Heinz Steinitz Marine Biology Laboratory 2: 1–2.

Girsch, S. 1976. Interfacing of vision and bioluminescence in the flashlight fish *Photoblepharon palpebratus*. Abstract in the 4th Annual Meeting of the American Society of Photobiology, Boulder, CO.

Golani, D., Ozturk, B. and Basusta, N. 2006. *The Fishes of the Eastern Mediterranean*. Turkish Marine Research Foundation, Istanbul, Turkey.

Gudger, E.W. 1947. The angler-fishes, *Lophius piscatorius* et *americanus*, use the lure in fishing. The American Naturalist 79: 542–548.

Haneda, Y. 1940. On the luminescence of the fishes belonging to the family Leiognathidae of the tropical Pacific. Palau Tropical Biological Station Studies 2: 29–39.

Haneda, Y. 1950. Luminous organs of fish which emit light indirectly. Pacific Science 4: 214–227.

Haneda, Y. 1968. Observations on the luminescence of the deep sea luminous angler fish *Himantolophus groenlandicus*. Science Report of the Yokosuka City Museum 14: 1–6.

Haneda, Y. and Tsuji, F.I. 1971. Light production in the luminous fishes *Photoblepharon* and *Anomalops* from the Banda Islands. Science, 173: 143–145.

Haneda, Y. and Tsuji, F.I. 1976. The luminescent systems of ponyfishes. Journal of Morphology 150: 539–552.

Hansen, K. and Herring, P.J. 1977. Dual bioluminescent systems in the anglerfish genus *Linophryne* (Pisces: Ceratioidea). Journal of Zoology London 182: 103–124.

Harms, J.W. 1928. Bau und Entwicklung eines eigenartigen Leuchtorgans bei *Equula* spec. Zeitschrift fuer Wissenschaftliche Zoologie 131: 157–179.

Harvey, E.N. 1921. A fish, with a luminous organ, designed for the growth of luminous bacteria. Science 53: 314–315.

Harvey, E.N. 1922. The production of light by the fishes *Photoblepharon* and *Anomalops*. Publication 312, The Carnegie Institute, Washington, D.C., pp. 45–60.

Harvey, E.N. 1952. *Bioluminescence*. Academic Press, New York.

Hastings, J.W. 1971. Light to hide by: ventral luminescence to camouflage the silhouette. Science 173: 1016–1017.

Hastings, J.W. and Mitchell, G. 1971. Endosymbiotic bioluminescent bacteria from the light organ of ponyfish. Biological Bulletin 141: 261–268.

Hastings, J.W., Makemson, J. and Dunlap, P.V. 1987. How are growth and luminescence regulated independently in light organ symbionts? Symbiosis 4: 3–24.

Haygood, M.G. 1993. Light organ symbioses in fishes. Critical Reviews in Microbiology 19: 191–216.

Haygood, M.G. and Distel, D.L. 1993. Bioluminescent symbionts of flashlight fishes and deep sea anglerfishes form unique lineages related to the genus *Vibrio*. Nature 363: 154–156.

Haygood, M.G., Tebo, B.M. and Nealson, K.H. 1984. Luminous bacteria of a monocentrid fish (*Monocentris japonicus*) and two anomalopid fishes (*Photoblepharon palpebratus* and *Kryptophanaron alfredi*): population sizes and growth within the light organs, and rates of release into the seawater. Marine Biology 78: 249–254.

Haygood, M.G., Distel, D.L. and Herring, P.J. 1992. Polymerase chain reaction and 16S rRNA gene sequences from the luminous bacterial symbionts of two deep sea anglerfishes. Journal of the Marine Biological Association of the United Kingdom 72: 149–159.

Heemstra, P.C., Hissmann, K., Fricke, H. and Smale, M.J. 2006. Fishes of the deep demersal habitat at Ngazidja (Grand Comoro) Island, western Indian Ocean. South African Journal of Science 102: 444–460.

Hendry, T.A. and Dunlap, P.V. 2011. The uncultured luminous symbiont of *Anomalops katoptron* (Beryciformes: Anomalopidae) represents a new bacterial genus. Molecular Phylogenetics and Evolution 61: 834–843.

Herring, P.J. 1977. Bioluminescence of marine organisms. Nature 267: 788–793.

Herring, P.J. 1982. Aspects of the bioluminescence of fishes. Oceanography and Marine Biology: An Annual Review 20: 415–470.

Herring, P.J. 1990. Bioluminescent communication in the sea. In: *Light and life in the Sea*. (eds Herring, P.J., Campbell, A.K., Whitfield, M., and Maddock, L.). Cambridge University Press, pp. 245-264.

Herring, P.J. 1993. Light genes will out. Nature 363: 110p.

Herring, P.J. and Morin, J.G. 1978. Bioluminescence in fishes. In: *Bioluminescence in Action* (ed Herring, P.J.). Academic Press, New York, pp. 273–329.

Herring, P.J. and Munk, O. 1994. The escal light organ of the deep sea anglerfish *Haplophryne mollis* (Pisces: Ceratioidei) with observations on luminescence control. Journal of the Marine Biological Association of the United Kingdom 74: 747–763.

Hoff, M. 2009. Going with the glow: how bacteria integrate molecular signals to synchronize bioluminescence. PLOS Biology 7: 418.

Howland, H.C., Murphy, C.J. and McCosker, J.E. 1992. Detection of eyeshine by flashlight fishes of the family Anomalopidae. Vision Research 32: 765–769.

Hulet, W.H. and Musil, G. 1968. Intracellular bacteria in the light organ of the deep sea angler fish, *Melanocetus murrayi*. Copeia 1968: 506–512.

Ikejima, K., Ishiguro, N.B., Wada, M., *et al*. 2004. Molecular phylogeny and possible scenario of ponyfish (Perciformes: Leiognathidae) evolution. Molecular Phylogenetics and Evolution 31: 904–909.

Ikejima, K., Wada, M., Kita-Tsukamoto, K., *et al*. 2008. Synchronized development of gonad and bioluminescent light organ in a highly sexually dimorphic leiognathid fish, *Photoplagios rivulatus*. Marine Biology 153: 1009–1014.

Johnson, G.D and Rosenblatt, R.H. 1988. Mechanisms of light occlusion in flashlight fishes, family Anomalopidae (Teleostei: Beryciformes), and the evolution of the group. Zoological Journal of the Linnean Society 94: 65–98.

Johnson, G.D., Seeto, J. and Rosenblatt, R.H. 2001. *Parmops echinatus*, a new species of flashlight fish (Beryciformes: Anomalopidae) from Fiji. Proceedings of the Biological Society of Washington 114: 497–500.

Jones, G. 1985. Revision of the Australian species of the fish family Leiognathidae. Australian Journal of Freshwater Research 36: 559–613.

Kaeding, A.J., Ast, J.C., Pearce, M.M., *et al*. 2007. Phylogenetic diversity and cosymbiosis in the bioluminescent symbioses of *Photobacterium mandapamensis*. Applied Environmental Microbiology 73: 3173–3182.

Kessel, M. 1977. The ultrastructure of the relationship between the luminous organ of the teleost fish *Photoblepharon palpebratus* and its symbiotic bacteria. Cytobiologie 15: 145–158.

Kitchell, J.F. 1983. Energetics. In: *Fish Biomechanics* (eds Webb, P.W. and Weihs, D.). Praeger Publishers, New York, pp. 312–338.

Konishi, Y. and Okiyama, M. 1997. Morphological development of four trachichthyoid larvae (Pisces: Beryciformes), with comments on trachichthyoid relationships. Bulletin of Marine Science 60: 66–88.

Laurenson, C.H., Hudson, I.R., Jones, D.O.B. and Priede, I.G. 2004. Deep water observations of *Lophius piscatorius* in the north-eastern Atlantic Ocean by means of a remotely operated vehicle. Journal of Fish Biology 65: 947–960.

Leis, J.M. and Bullock, S. 1986. The luminous cardinalfish *Siphamia* (Pisces, Apogonidae): development of larvae and the luminous organ. In: *Indo-Pacific Fish Biology: Proceedings of the 2nd International Conference on Indo-Pacific Fish* (ed. Uyeno, T.). Ichthyological Society of Japan, Tokyo, pp. 703–714.

Leisman, G., Cohn,D.H. and Nealson, K.H. 1980. Bacterial origin of luminescence in marine animals. Science 208: 1271–1273.

Lloyd, J.E. 1966. Studies on the flash communication system in *Photinus* fireflies. Miscellaneous Publication No. 130, Museum of Zoology, University of Michigan, Ann Arbor, MI.

Luck, D.G. and Pietsch, T.W. 2008. *In situ* observations of a deep sea anglerfish of the genus *Oneirodes* (Lophiiformes: Oneirodidae). Copeia 2008: 446–451.

Marshall, N.B. 1967. The olfactory organs of bathypelagic fishes. Symposium of the Zoological Society of London 19: 57–70.

McCosker, J.E. 1977. Flashlight fishes. Scientific American 236: 106–114.

McCosker, J.E. 1982. Discovery of *Kryptophanaron alfredi* (Pisces: Anomalopidae) at San Salvador, Bahamas, with notes on anomalopid light organs. Revista de Biologia Tropical 30: 97–99.

McCosker, J.E. and Lagios, M.D. 1975. Les petits peugeots of Grande Comore. Pacific Discovery 28: 1–6.

McCosker, J.E. and Rosenblatt, R.H. 1987. Notes on the biology, taxonomy, and distribution of flashlight fishes (Beryciformes: Anomalopidae). Japanese Journal of Ichthyology 34: 157–164.

McFall-Ngai, M.J. 1983. Adaptations for reflection of bioluminescent light in the gas bladder of *Leiognathus equulus* (Perciformes: Leiognathidae). The Journal of Experimental Zoology 227: 23–33.

McFall-Ngai, M.J. 1991. Luminous bacterial symbiosis in fish evolution: adaptive radiation among leiognathid fishes. In: *Symbiosis as a Source of Evolutionary Innovation* (eds Margulis, L. and Fester, R.). MIT Press, Cambridge, MA, pp. 381–409.

McFall-Ngai, M.J. and Dunlap, P.V. 1983. Three new modes of luminescence in the leiognathid fish *Gazza minuta*: discrete projected luminescence, ventral body flash, and buccal luminescence. Marine Biology 73: 227–237.

McFall-Ngai, M.J. and Dunlap, P.V. 1984. External and internal sexual dimorphism in leiognathid fishes: morphological evidence for sex-specific bioluminescent signaling. Journal of Morphology 182: 71–83.

McFall-Ngai, M.J. and Morin, J.G. 1991. Camouflage by disruptive illumination in leiognathids, a family of shallow-water, bioluminescent fishes. Journal of Experimental Biology 156: 119–137.

Mead, G.W., Bertelsen, E. and Cohen, D.M. 1964. Reproduction among deep sea fishes. Deep Sea Research 11: 569–596.

Meighen, E.A. 1991. Molecular biology of bacterial bioluminescence. Microbiological Reviews 55: 123–142.

Meyer-Rochow, V.B. 1976a. Loss of bioluminescence in *Anomalops katoptron* due to starvation. Experientia 32: 1175–1176.

Meyer-Rochow, V.B. 1976b. Some observations on spawning and fecundity in the luminescent fish *Photoblepharon palpebratus*. Marine Biology 37: 325–328.

Meyer-Rochow, V.B., Baburina, V. and Smirnov, S. 1982. Histological observations on the eyes of the two luminescent fishes *Photoblepharon palpebratus* (Boddaert) and *Anomalops katoptron* (Blkr.). Zoologischer Anzeiger, Jena 209: 65–72.

Miya, M., Satoh, T.P. and Nishida, M. 2005. The phylogenetic position of toadfishes (order Batrachoiformes) in the higher ray-finned fish as inferred from partitioned Bayesian analysis of 102 whole mitochondrial genome sequences. Biological Journal of the Linnean Society of London 85: 289–306.

Miya, M., Takeshima, H., Endo, H., *et al*. 2003. Major patterns of higher teleostean phylogenies: a new perspective based on 100 complete mitochondrial DNA sequences. Molecular Phylogenetics and Evolution 26: 121–138.

Moore, J.A. 2002. Upside-down swimming behavior in a whipnose anglerfish (Teleostei: Ceratioidei: Gigantactinidae). Copeia 2002: 1144–1146.

Morin, J.G. 1981. Bioluminescent patterns in shallow tropical marine fishes. In: *Proceedings of the 4th International Coral Reef Symposium*, Vol. 2 (eds Gomez, E.D. and Birkeland, C.E.). Marine Sciences Center, University of the Philippines, Manila, Philippines, pp. 569–574.

Morin, J.G. 1983. Coastal bioluminescence: patterns and functions. Bulletin of Marine Science 33: 787–817.

Morin, J.G., Harrington, A., Nealson, K., *et al*. 1975. Light for all reasons; versatility in the behavioral repertoire of the flashlightfish. Science 190: 74–76.

Munk, O. 1992. Accesory escal gland (AEG) in some deep sea anglerfishes. Acta Zoologica 73: 33–37.

Munk, O. 1999. The escal photophore of ceratioids (Pisces: Ceratioidei) – a review of structure and function. Acta Zoologica 80: 265–284.

Munk, O. 2000. Histology of the fusion area between the parasitic male and the female in the deep sea anglerfish *Neoceratias spinifer* Pappenheim, 1914 (Teleostei, Ceratioidei). Acta Zoologica 81: 315–324.

Munk, O. and Herring, P.J. 1996. An early stage in development of escae and caruncles in the deep sea anglerfish *Cryptopsaras couesi* (Pisces: Ceratioidei). Journal of the Marine Biological Association of the United Kingdom 76: 517–527.

Munk, O., Hansen, K. and Herring, P.J. 1998. On the development and structure of the escal light organ of some melanocetid deep sea anglerfishes (Pisces: Ceratioidei). Journal of the Marine Biological Association of the United Kingdom 78: 1321–1335.

Nealson, K.H. 1979. Alternative strategies of symbiosis of marine luminous fishes harboring light-emitting bacteria. Trends in Biochemical Sciences 4: 105–109.

Nealson, K.H. and Hastings, J.W. 1977. Low oxygen is optimal for luciferase synthesis in some bacteria. Ecological implications. Archives of Microbiology 112: 9–16.

Nealson, K.H. and Hastings, J.W. 1990. The luminous bacteria. In: *The Prokaryotes* (eds Balows, A., Truper, H.G., Dworkin, M., *et al*.). Springer-Verlag, pp. 625–639.

Nealson, K.H, Cohn, D., Leisman, G. and Tebo, B. 1981. Co-evolution of luminous bacteria and their eukaryotic hosts. Annals New York Academy of Sciences 361: 76–91.

Nealson, K.H., Haygood, M.G., Tebo, B.M., *et al*. 1984. Contribution by symbiotically luminous fishes to the occurrence and bioluminescence of luminous bacteria in seawater. Microbial Ecology 10: 69–77.

Neil, S.R.St.J. and Cullen, J.M. 1974. Experiments on whether schooling of prey affects hunting behaviour of cephalopods and fish predators. Journal of Zoology 172: 549–569.

Nicol, J.A.C. 1967. The luminescence of fishes. Symposium of the Zoological Society London 19: 27–55.

O'Day, W.T. 1974. Bacterial luminescence in the deep sea anglerfish *Oneirodes acanthias* (Gilbert, 1915). Contributions to Science 255: 1–12.

Olsson, R. 1974. Endocrine organs of a parasitic male deep sea angler-fish, *Edriolychnus schmidti*. Acta Zoologica 55: 225–232.

Parr, A.E. 1930. On the probable identity, life-history and anatomy of the free-living and attached males of the ceratioid fishes. Copeia 1930: 129–135.

Pauly, D. 1977. The Leiognathidae (Teleosts): an hypothesis relating their mean depth occurrence to the intensity of their countershading bioluminescence. Marine Research Indonesia 19: 137–146.

Peat, S.M. and Adams, B.J. 2008. Natural selection on the *lux*A gene of bioluminescent bacteria. Symbiosis 46: 101–108.

Penrith, M.J. 1967. Ceratioid angler-fishes from South Africa. Journal of Natural History 1: 185–188.

Pietsch, T.W. 1975. Precocious sexual parasitism in the deep sea ceratioid anglerfish, *Cryptopsaras couesi Gill*. Nature 256: 38–40.

Pietsch, T.W. 1976. Dimorphism, parasitism and sex: reproductive strategies among deep sea ceratioid anglerfishes. Copeia 1976: 781–793.

Pietsch, T.W. 1986. Systematics and distribution of bathypelagic anglerfishes of the family Ceratiidae (Order: lophiformes). Copeia 1986: 479–493.

Pietsch, T.W. 2005. Dimorphism, parasitism and sex revisited: modes of reproduction among deep sea ceratioid anglerfishes (Teleostei: Lophiiformes). Ichthyological Research 52: 207–236.

Pietsch, T.W. and Grobecker, D.B. 1978. The compleat angler: aggressive mimicry in an antennarid anglerfish. Science 201: 369–370.

Pietsch, T.W. and Grobecker, D.B. 1979. High speed cinematographic evidence for ultrafast feeding in antennarid anglerfishes. Science 205: 1161–1162.

Pietsch, T.W. and Grobecker, D.B. 1987. *Frogfishes of the World. Systematics, Zoogeography, and Behavioral Ecology*. Stanford University Press, Stanford, CA.

Pietsch, T.W. and Grobecker, D.B. 1990. Frogfishes. Scientific American 262: 96–103.

Pietsch, T.W. and Kenaley, C.P. 2007. Ceratioidei. Seadevils, deep sea anglerfishes. http://tolweb.org/Ceratioidei/22000/2007.10.02 (last accessed 19 June 2013).

Pietsch, T.W. and Orr, J.W. 2007. Phylogenetic relationships of deep sea anglerfishes of the suborder Ceratioidei (Teleostei: Lophiiformes) based on morphology. Copeia 2007: 1–34.

Ramaiah, N. and Chandramohan, D. 1992. Occurrence of *Photobacterium leiognathi*, as the bait organ symbiont in frogfish *Antennarius hispidus*. Indian Journal of Marine Sciences 21: 210–211.

Rees, J.P., De Wergifosse, B., Noiset, O., *et al*. 1998. The origins of marine bioluminescence: turning oxygen defense mechanisms into deep sea communication tools. Journal of Experimental Biology 201; 1211–1221.

Regan, C.T. 1926. The pediculate fishes of the suborder Ceratioidea. Dana Oceanographic Report 2: 1–45.

Regan, C.T. and Trewavas, E. 1932. Deep sea anglerfishes (Ceratioidea). Dana Reports 2: 1–113.

Reichlet, J.L. and Baumann,P. 1973. Taxonomie of the marine luminous bacteria. Archives of Microbiology 94: 283–330.

Reichlet, J.L., Nealson, K. and Hastings, J.W. 1977. The specificity of symbiosis: ponyfish and luminescent bacteria. Archives of Microbiology 112: 157–161.

Rosenblatt, R.H. and Johnson, G.D.1991. *Parmops coruscans*, a new genus and species of flashlight fish (Berciformes: Anomalopidae) from the South Pacific. Proceedings of the Biological Society of Washington 104: 328–334.

Rosenblatt, R.H. and Montgomery, W.L. 1976. *Kryptophaneron harveyi*, a new anomalopid fish from the eastern tropical Pacific, and the evolution of the Anomalopidae. Copeia 1976: 510–515.

Ruby, E.G. and Morin, J.G. 1978. Specificity of symbiosis between deep sea fishes and psychrotrophic luminous bacteria. Deep Sea Research 25: 161–167.

Ruby, E.G. and Nealson, K.H. 1976. Symbiotic association of *Photobacterium fischeri* with the marine luminous fish *Monocentrus japonica*: a model of symbiosis based on bacterial studies. Biological Bulletin 151: 574–586.

Sagi, G. 1978. *Communication by light signals and culture trials to characterize the luminescent bacteria from the light organ of the fish Photoblepharon palpebratus*. MSc Dissertation, The Hebrew University of Jerusalem.

Sasaki, A., Ikejima, K., Aoki, S., *et al*. 2003. Field evidence for bioluminescent signaling in the ponyfish, *Leiognathus elongates*. Environmental Biology of Fishes 66: 307–311.

Shedlock, A.M., Pietsch, T.W., Haygood, M.G., *et al*. 2004. Molecular systematics and life history evolution of anglerfishes (Teleostei: Lophiiformes): evidence from mithocondrial DNA. Steenstrupia 28: 129–144.

Sheldon, R.W., Prakash, A. and Sutcliff, W.H. 1972. The size distribution of particles in the ocean. Limnology and Oceanography 17: 327–340.

Shilo, M. and Yetinson, T. 1979. Physiological characteristics underlying the distribution patterns of luminous bacteria in the Mediterranean Sea and the Gulf of Elat. Applied and Environmental Microbiology 38: 577–584.

Soars, N.A. and Leis, J.M. 2010. Larval development of the common ponyfish, *Leiognathus equulus* (Teleostei: Leiognathidae). Ichthyological Research 57: 263–271.

Sparks, J.S. and Dunlap, P.V. 2004. A clade of non-sexually dimorphic ponyfishes (Teleostei: Perciformes: Leiognathidae): phylogeny, taxonomy, and description of a new species. American Museum Novitates 3459, 1–21.

Sparks, J.S., Dunlap, P.V. and Smith, W.L. 2005. Evolution and diversification of a sexually dimorphic luminescent system in ponyfishes (Teleostei: Leiognathidae), including diagnoses for two new genera. Cladistics 21: 305–327.

Steche, O. 1909. Die Leuchtorgane von *Anomalops katoptron* und *Photoblepharon palpebratus*, zwei Oberflachenfische aus dem Malaiischen Archipel. Zeitschrift fuer Wissenschftliche Zoologie 93: 349–409.

Studer, P. and Wirz, C. 1984. Experience in the keeping of flashlight fishes (*Photoblepharon palpebratus*, Anomalopidae) in the vivarium of the Basle Zoo. Vie Marine 6: 77–80.

Tett, P.B. and Kelly, M.G. 1973. Marine bioluminescence. Oceanography and Marine Biology Annual Review 11: 89–173.

Thacker, C.E. and Roje, D.M. 2009. Phylogeny of cardinalfishes (Teleostei: Gobiiformes: Apogonidae) and the evolution of visceral bioluminescence. Molecular Phylogenetics and Evolution 52: 735–745.

Tong, D., Rozas, N.S., Oakley, T.H., *et al*. 2009. Evidence for light perception in a bioluminescent organ. Proceedings of the National Academy of Sciences USA 106: 9836–9841.

Turner, J.T. and Ferrante, J.G. 1979. Zooplankton fecal pellets in aquatic ecosystems. Bioscience 29: 670–677.

Urbanczyk, H., Ast, J.C. and Dunlap, P.V. 2011. Phylogeny, genomics, and symbiosis of *Photobacterium*. FEMS Microbiological Review 35: 324–342.

Uwate, K.R. 1979. Revision of the anglerfish Diceratiidae with description of two new species. Copeia 1979: 129–144.

Visick, K.L. and McFall-Ngai, 2000. An exclusive contract: Specificity in the *Vibrio fischeri-Euprymna scolopes* partnership. Journal of Bacteriology 182: 1779–1787.

Wada, M., Azuma, N. Mizuno, N. and Kurokura, H. 1999. Transfer of symbiotic luminous bacteria from parental *Leiognathus nuchalis* to their offspring. Marine Biology 135: 683–687.

Watson, M., Thurston, E.L. and Nicol, J.A.C. 1978. Reflectors in the light organ of *Anomalops* (Anomalopidae, Teleostei). Proceedings of the Royal Society London B 1148: 339–351.

Wegrzyn, G. and Czyz, A. 2002. How do marine bacteria produce light, why are they luminescent, and can we employ bacterial bioluminescence in aquatic biotechnology? Oceanologia 44: 291–305.

Wickler, W. 1961. Uber die Paarbildung der Tiefsee-Angler. Natur und Volk 91: 381–390.

Wickler, W. 1967. Specialization of organs having a signal function in some marine fish. Studies of Tropical Oceanography 5, 539–548.

Widder, E.A. 2010. Bioluminescence in the ocean: origins of biological, chemical, and ecological diversity. Science 328: 704–708.

Wolf, C.J. and Haygood, M.G. 1991. Restriction fragment length polymorphism analysis reveals high levels of genetic divergence among the light organ symbionts of flashlight fish. Biological Bulletin 181: 135–143.

Wolf, C.J. and Haygood, M.G. 1993. Bioluminescent symbionts of the Caribbean flashlight fish (*Kryptophanaron alfredi*) have a single rRNA operon. Molecular Marine Biology and Biotechnology 2: 189–197.

Woodland, D.J., Cabanban, A.S., Taylor, V.M. and Taylor, R.J. 2002. A synchronized rhythmic flashing light display by schooling *Leiognathus splendens* (Leiognathidae: Perciformes). Marine and Freshwater Research 53: 159–162.

Yamada, K., Haygood, M. and Kabasawa, H. 1979. On fertilization and early development in the pine-cone fish, *Monocentris japonicus*. Annual Report of the Keikyu Aburatsubo Marine Park Aquarium 10: 31–38.

Yetinson, T. and Shilo, M. 1979. Seasonal and geographic distribution of luminous bacteria in the Eastern Mediterranean Sea and the Gulf of Elat. Applied and Environmental Microbiology 37: 1230–1238.

Young, R.E. 1983. Oceanic bioluminescence: an overview of general functions. Bulletin Marine Sciences 33: 829–845.

Young, R.E. and Roper, C.F.E. 1977. Intensity regulation of bioluminescence during countershading in living midwater animals. Fishery Bulletin 70: 239–252.