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CAMOUFLAGE BY DISRUPTIVE ILLUMINATION IN LEIOGNATHIDS, A FAMILY OF SHALLOW-WATER, BIOLUMINESCENT FISHES

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Summary

Leiognathids are shallow-water, Indo-West Pacific fishes that have a circumesophageal, bacterial light organ. Visual observations of living fishes revealed a mottled ventral luminescence pattern, which was analyzed both behaviorally and morphologically. In behavioral experiments, these fishes responded to increases in intensity of downwelling light with increases in the intensity of ventral luminescence. However, while the absolute luminescence levels tracked the ambient light levels, they did not increase in direct proportion to those of increasing downwelling light; luminescence levels were closer to the intensity of downwelling light at low light levels.

The tissues that intervene between the internal light organ and the external environment are responsible for the observed mottled pattern of the ventral luminescence. Furthermore, these tissues, which have been incorporated into the light organ system, are involved in the control of the intensity, spectral quality and angular distribution of the fish's luminescence. The spectral peak of the bacterial luminescence from whole fish (500 nm) was shifted about 10 nm towards the green relative to the spectral peak of cultured light organ symbionts (485–490 nm). The luminescence had the greatest intensity of outward expression at an angle of 20–25° from the ventral midline and was undetectable dorsally. The ventral illumination behavior of leiognathids, with their associated morphology, is compared and contrasted with the counterillumination systems that have been described in a number of mesopelagic fishes, shrimps and squids.

Introduction

Counterillumination, the production of luminescence to match downwelling light, has long been suggested as a primary function of the ventrally directed bioluminescence characteristic of many luminous fishes, squids and crustaceans (Dahlgren, 1916; Clarke, 1963). Because most of the organisms thought to

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counterilluminate are mesopelagic, theories surrounding the nature of counterillumination behavior were designed around the conditions of that environment. The quantity of ambient light in the mesopelagic zone is low and the quality is highly predictable in intensity, color and angular distribution (Jerlov, 1968). The counterillumination theories proposed that selection would result in highly stereotyped luminescence patterns that would reflect the physical qualities of the ambient light (for a review, see Young, 1983). Experiments designed to test these theories have shown that certain squids (Young, 1977; Young and Mencher, 1980) and crustaceans (Warner *et al.* 1978; Latz and Case, 1982) are capable of very precise matching of experimental simulations of the mesopelagic photic environment. The direct testing of mesopelagic bioluminescent fishes has been limited because these fishes are usually severely stressed when collected (Case *et al.* 1977). However, the serial ventral photophores of most of these fishes provide the morphological correlates for such behavior, and the experimental measurements of their ventral luminescence spectra, the angular distribution and the maximum intensity have indicated the *potential* for bioluminescent matching of downwelling light patterns (Denton *et al.* 1972, 1985). Thus, studies of mesopelagic animals have supported the hypothesis that ventrally directed luminescence compensates for the silhouette of the organism, effectively camouflaging it in its environment.

Counterillumination has also been suggested as a function for the ventrally directed luminescence observed in certain shallow-water organisms (e.g. Hastings, 1971; Pauly, 1977; Morin, 1983). However, for these organisms, not only is the environment more spatially and temporally heterogeneous (e.g. shadows, terrigenous influences, surface ripple effects) than the mesopelagic zone, but the majority of shallow-water species have single, internal bacterial light organs rather than the multiple, ventral photophores common in midwater species (Table 1). In contrast to that of mesopelagic animals, the ventral luminescence of shallow-water species may express itself as a varied pattern of 'disruptive illumination' (Morin, 1983), analogous to the disruptive coloration patterns observed in many nearshore non-luminous fishes that occur in heterogeneous habitats (Endler, 1978).

The present paper examines the behavior and morphology associated with

Table 1. *Comparison of light organ system type and habitat quality between ventrally luminescing mesopelagic fishes and leiognathids*

	Counterilluminating systems of most mesopelagic fishes*	Counterilluminating systems of leiognathids
Light organs	Autogenic; several, serially arranged along the ventrum	Bacterial; single, internal
Relative quality of ambient downwelling light	Predictable with respect to intensity, wavelength and angular distribution	Unpredictable

* All species of 93 of the 96 genera showing ventral luminescence have the system described.

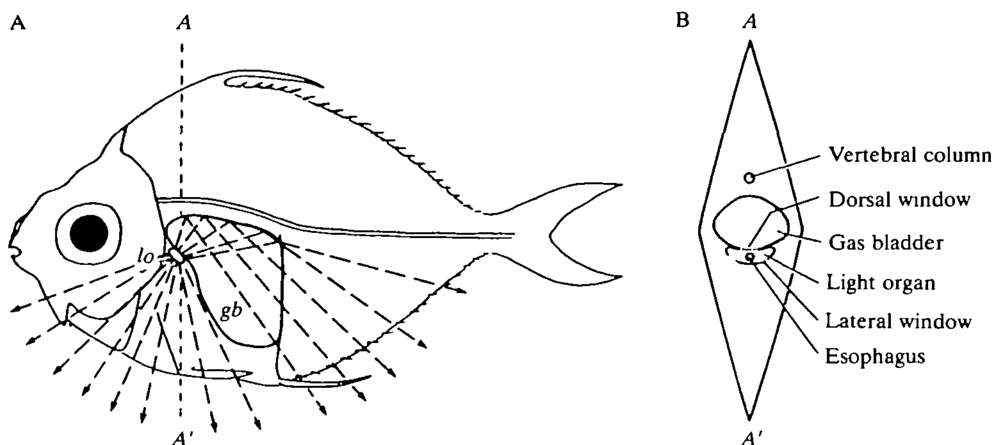

Fig. 1. The counterillumination pattern in a leiognathid fish. (A) Lateral view with the path of ventral luminescence indicated by the dashed lines and arrows. (B) Cross section, along line *A–A'* in A, illustrating the position of the light organ (*lo*) and the relationship of the light organ windows to the gas bladder (*gb*) and lateroventral musculature. In the majority of the analyzed systems, the light organ has three muscular shutters: a dorsal shutter, which permits light to exit into the gas bladder space, and a pair of lateral shutters, which open into translucent muscles anteroventrally. The light entering the gas bladder is reflected outwards through a posterior, transparent area on the anal fin pterygiophore into the translucent posteroventral musculature.

ventral luminescence in an abundant group of shallow-water fishes, the leiognathids (ponyfishes). There are approximately 30 species in three genera (*Leiognathus*, *Gazza* and *Secutor*) in the family Leiognathidae (Pauly and Wade-Pauly, 1981). These fishes occur in large schools in mangroves, estuaries and shorelines of the Indo-West Pacific, in areas where the water clarity, by Secchi disk readings, varies from turbid (<1 m) to very clear (>15 m) (Pauly, 1977; Pauly and Wade-Pauly, 1981). All representatives of the family are relatively small (averaging 40–250 mm in adult standard length), usually laterally compressed, and have highly silvered bodies. Surrounding their esophagus, they have a light organ that houses a pure culture of the facultatively symbiotic, marine luminous bacterium *Photobacterium leiognathi* (Fig. 1; Dunlap, 1984, and references therein). The general organization of their light organ system has been well described (Hastings, 1971; Haneda and Tsuji, 1976; McFall-Ngai, 1983; McFall-Ngai and Dunlap, 1983, 1984; Dunlap and McFall-Ngai, 1987). The various uses of the tissues of the light organ system (in particular, selective use of the different muscular shutters, as well as variations in the use of chromatophores in each portion of the system) permit a wide variety of luminescent behaviors in members of this family (McFall-Ngai and Dunlap, 1983; Dunlap and McFall-Ngai, 1987). Further, although the light organs of females of all species are approximately the same size, the male light organ shows varying degrees of hypertrophy among different species (McFall-Ngai and

Dunlap, 1984). In females, the light organ always appears as a symmetrical 'donut' of tissue around the esophagus.

We analyzed aspects of the ventral luminescence behavior, which is but one type of luminescent behavior, in these shallow-water fishes. Responses to changes in the intensity of ambient light, as well as the influence of tissues involved in transmission and diffusion of the light, were examined. The data presented for leiognathids support the hypothesis of 'disruptive illumination' as a function for the ventral luminescence of these nearshore fishes.

Materials and methods

Animals

Fishes used in this study were obtained from coastal bays and estuaries of the Orient. We used a variety of species because no one species was readily available at all times. *Gazza minuta* (Bloch) and *Leiognathus splendens* (Cuvier) were captured in cast nets in 1–3 m of water from South Bais Bay, Negros Oriental, the Philippines (9.36N, 123.07E), *Leiognathus equulus* (Forskål) were obtained from the fish corrals of Manila Bay, the Philippines (14.29N, 120.54E), and *Leiognathus nuchalis* (Temminck et Schlegel) were caught on barbless hooks on the shores off Misaki, Japan (35.08N, 139.37E). The specimens captured from South Bais Bay were maintained at Silliman University Marine Laboratory in Dumaguete in 15 000-l concrete tanks with aerated, recirculating sea water. Specimens from Manila Bay and Japan were transported to the University of California, Los Angeles, and maintained in 170-l glass aquaria for periods of up to 1 year. Female specimens were used for all experiments because the light organs are structurally similar for all species.

Equipment

The apparatus for producing overhead illumination was of simple design (see Fig. 2 for details) so that it could be easily transported and maintained under field conditions. A peak wavelength of 512 nm, with a half bandwidth of 98 nm, was produced by placing a Kodak Wratten filter between the light source and the specimen. The spectra of downwelling light were measured by a monochromator (Bausch & Lomb, Rochester, New York), with an exit slit width of 1 mm, coupled to a photomultiplier (PMT). The PMT used in all experiments was an RCA 1P21 in a light-tight housing. Although the spectral quality of the water from which the leiognathids were captured was not determined, the light provided in these experiments overlapped a significant portion of the average spectral quality of bay water (Jerlov, 1968). Furthermore, the ambient light spectrum was near the visual pigment sensitivity measured for one of the species, *Leiognathus nuchalis* (507 nm, F. Crescitelli and M. J. McFall-Ngai, unpublished data). All equipment except the variable power supply for the light source, the high-voltage source to the PMT, the amplifier and the voltmeter were enclosed in a light-proof cage (Fig. 2). Light intensity was measured as quanta $cm^{-2} s^{-1}$.

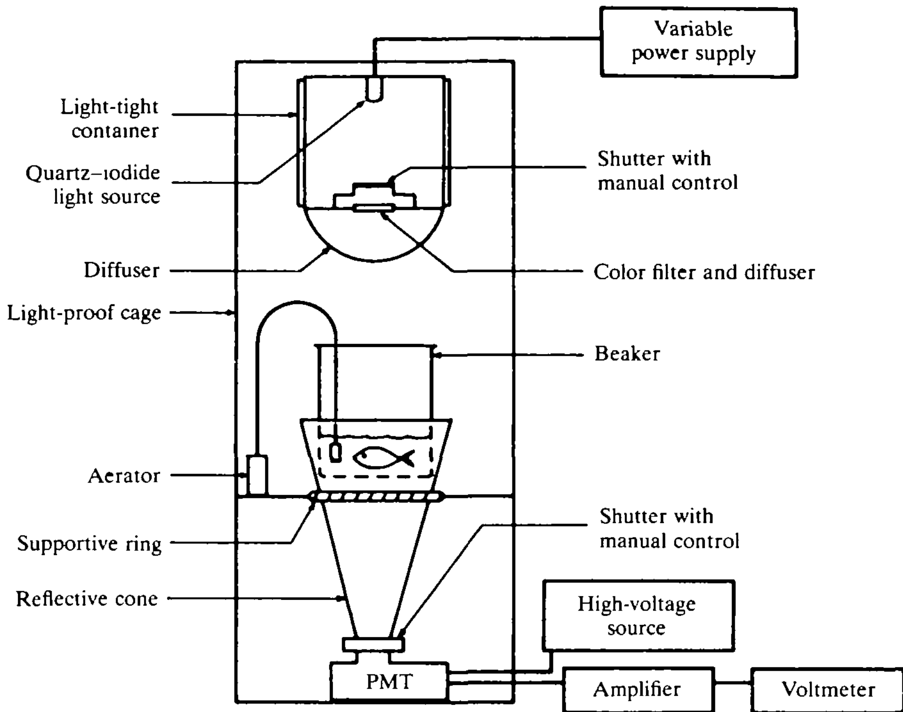

Fig. 2. Experimental apparatus for measurement of leiognathid counterillumination behavior. Light emitted from the lamp was permitted to shine through the base of the container *via* a camera shutter with a 5 cm aperture. Beneath this aperture were mountings for filters and a diffuser. A milk-white hemisphere was attached to the base of the box to further diffuse the light. The set-up thus provided uniform, diffuse downwelling light. The light-tight container and filters, mounted on a frame, were 36 cm from the experimental animal, which was contained in 1 l of aerated sea water in a 4-l beaker. A reflective foil-lined cone supported the beaker. The base of the cone surrounded a camera shutter, also with a 5 cm aperture, mounted above the aperture of the photomultiplier (PMT). Both camera shutters were operated by pneumatic shutter releases. Amplified light signals from the PMT were recorded from an analog voltmeter.

Analyses of counterillumination behavior

Visual observations of counterillumination were made on *Leiognathus nuchalis*, *L. splendens* and *Gazza minuta* in this experimental apparatus from which the PMT had been removed. After dark-adapting for approximately 20 min, we watched the fishes from below, with and without downwelling light.

To document the external pattern of ventral bioluminescence, time exposure photographs were taken of the fish's ventrum. Fishes were injected in the epaxial musculature with 0.5 ml of a 0.1 mmol l^{-1} solution of epinephrine (Barnes and Case, 1974), then anesthetized in tricaine methanesulfonate (MS 222) at a concentration of 100 p.p.m. The fishes were mounted in air with their anterior ends upward and their ventral surfaces aimed toward the camera. Sea water

containing MS 222 was perfused over the gills *via* a tube inserted into the mouth. The fishes were kept moist and, even after 1 h of this treatment, they fully recovered within 5 min when placed back in normal sea water. The ventral surfaces of the fishes were photographed using Kodak Tri-X film with an exposure time of 30 s to 10 min.

A series of experiments was performed to assess the ability of *Gazza minuta* to respond to changes in ambient light intensities. The highest ambient light intensity used was similar to intensities at the sea surface at about 5 min after sunset (Jerlov, 1968; McFarland, 1986). The lowest ambient light intensity used was approximately the average light intensity at the sea surface 20 min after sunset, or the intensity of light produced by a quarter moon high in the night sky; but this lowest light level is above that of starlight (McFarland, 1986). Lower light levels were not used because the bioluminescence response of *G. minuta* could not be measured reliably below this ambient light intensity. These experiments were carried out at the Silliman University Marine Station on Negros Island in the Philippines with *G. minuta* that had been recently captured (within 1 week). Fish were dark-adapted for 1 h and then placed in 1 l of sea water in a 4-l beaker. Each fish was then exposed to one of six light intensities (Table 2) for 30 min. Preliminary experiments showed that this period was the minimum necessary to reach full expression at the highest light intensities. After adaptation to a given light intensity, the shutter to the overhead light was closed and the shutter above the PMT opened. The fish bioluminescence level was recorded immediately and then at 5 s intervals for 1 min. Each of the six light intensities was tested on six individuals [average standard length=68±3 mm (S.E.M.)]. After each light level, the water in the beaker was changed, and the fish adapted to another light level. The light levels were presented in an unordered sequence. The decay curves obtained for each fish for the six light intensities were normalized by taking the highest light level of a given specimen as 100 %, and then the six values for any given interval at each light level were averaged.

The intensity of bioluminescence that would be required to match downwelling light was estimated in two ways: (1) using freshly killed, nonluminescing fish of various sizes; (2) using black cardboard fish silhouettes of appropriate sizes, determined from measurements of the fish. The black cardboard cutouts were used to determine the influence of body size alone without the potentially compounding influence of the silvery sides of the fish. The intact fish control was suspended in the experimental apparatus, just above the bottom of the beaker, while the silhouette mimics were placed in the bottom of the beaker. In both cases, the baseline levels were compared between ambient light impinging on the PMT with and without the fish or silhouette mimic. This procedure gave the percentage of light intercepted in each case, the average values of which were found to be the same for both circumstances. The percentage of the light intercepted was plotted against the size of the sham. Because this gave a straight line, the percentage of the ambient light intercepted by fish of any size could be extrapolated from the regression line. For the 60–70 mm fishes used in these experiments, the amount of

ambient light intercepted was determined to be approximately 5.5 % of the total downwelling light. This percentage could then be compared with the actual maximum measured bioluminescence response of each of the six fish (see Table 2).

Angular distribution

Leiognathus equulus was used to determine the angular distribution of ventral luminescence. Freshly killed specimens were dissected to expose the back of the throat of the fish. The esophagus and light organ were pulled down from the gas bladder 'window', and a 3 mm diameter light guide from a tungsten light source was placed against the window surface to mimic the luminescent organ. Light from this source could then be measured as it reflected off the gas bladder and passed through the ventral tissues of the fish. The fish was mounted with its ventral side up. A PMT was mounted on a rotating platform above the fish, so that the angle of the PMT could be changed with respect to the fish. The quantity of light detected by the photometer from the fish's ventral surface was recorded at 5° increments over the 180° subtended by the midlateral level of the right side to the midlateral level of the left side. This procedure was repeated with the ventral surface of the fish (1) intact, (2) with scales removed and (3) with skin removed.

Spectral analyses

Leiognathus splendens from Bais Bay were used in the spectral studies. A monochromator (Bausch & Lomb, Rochester, New York), in series with the PMT, was used to determine the relative spectral characteristics of luminescence. The exit slit width was set at 1 mm. Because we were interested in the relative spectral characteristics and correction would not appreciably alter the relative differences between peaks, the spectra were not corrected for the differential excitability of the PMT at different wavelengths.

The uncorrected emission spectra were determined for: (1) fresh cultures of the symbiont *Photobacterium leiognathi* from *Leiognathus splendens*; (2) the intact light organ; (3) the anesthetized fish. To isolate *P. leiognathi*, the contents of the light organ were streaked on sea water complete agar (Nealson, 1978). Bright, isolated colonies were then streaked onto fresh plates (N=3). After 2 days of growth at room temperature (25 °C) the bacterial cultures were brightly luminescent. The uncorrected spectral emission of these cultured bacteria was determined by placing the opened plates directly over the monochromator slit. To measure intact light organ spectra, light organs were dissected out of freshly killed specimens, the light organ shutters were removed, and the light organ was placed directly over the slit of the monochromator. To determine the spectra emitted by the fish, it was necessary to inject the fish with epinephrine to bring about full light expression. Each specimen (N=6) was injected in the epaxial musculature with 0.5 ml of a 0.1 mmol l^{-1} solution of epinephrine (Barnes and Case, 1974). The fish were then anesthetized in MS 222 at a concentration of 100 p.p.m. and suspended directly above the monochromator. The intensities of the luminescence curves were normalized and averaged (N=6) to produce the final spectral curve.

Results

Although other luminescent behaviors have been reported for leiognathids in the dark (McFall-Ngai and Dunlap, 1983), no sustained ventral emission that could be called counterillumination was detected in the absence of ambient light. However, in response to downwelling light, ventral luminescence was seen in all three of the species visually observed from below (*Leiognathus nuchalis*, *L. splendens* and *Gazza minuta*). In all cases, the ventral luminescence appeared as a mottled pattern of light. The brightest areas were in the gill region, which appeared to match or nearly match the intensity of the background light, especially at the low ambient light levels. In other areas, less intense regions were broken by brighter regions. The total effect was an obscuring of the shape of the fish.

The long-exposure photograph of injected *Leiognathus splendens* illustrates mottled ventral luminescence patterns (Fig. 3) representative of that seen in visual observations of unanesthetized fish responding to downwelling light with ventral luminescence. Such patterns were also observed during preliminary analyses of *L. nuchalis* luminescence, made with the aid of a different counterillumination apparatus and image intensifier (M. McFall-Ngai, J. G. Morin, J. F. Case and J. Warner, unpublished data). In all three species, two large and diffuse bright areas were present just behind the gill opercula. Based on computer-digitized analysis of photographs of ventral luminescence (e.g. Fig. 3), these paired spots represent about 8–10% of the area of the ventral silhouette. The head and

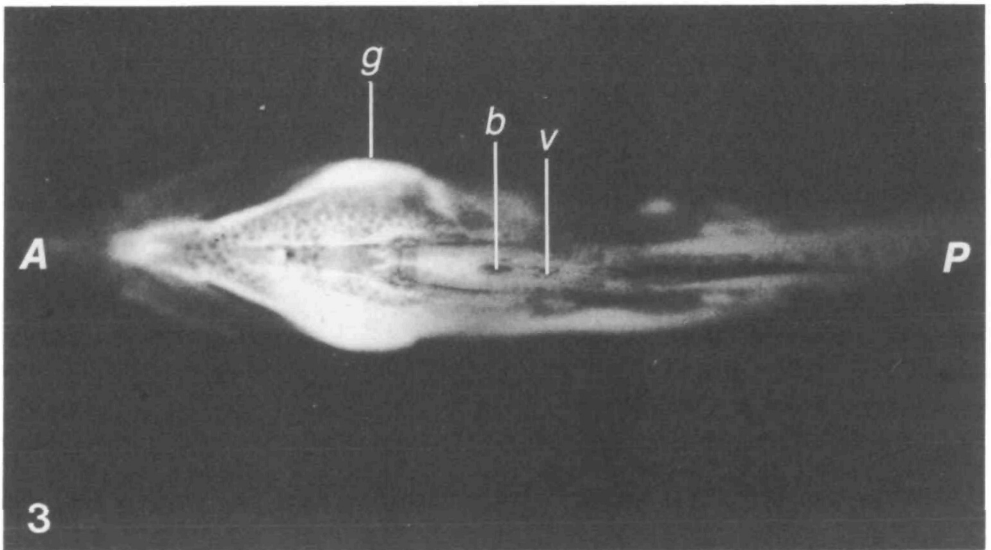

Fig. 3. Photograph of the ventral illumination pattern of a 65 mm *Leiognathus splendens*, a pattern representative of the visually observed counterillumination of all species studied. The photograph is a 5 min exposure taken with Kodak Tri-X film. *A*, anterior; *P*, posterior; *b*, basipterygiophore of the pelvic girdle; *g*, gill region; *v*, vent.

posterior portions were covered by a mottled, dim glow, the extent of which was largely dependent upon the degree of dermal chromatophore contraction.

Although all species showed a similar ventral pattern of luminescence, the decay of luminescence, when the downwelling light was turned off, was of two distinct types. In *L. nuchalis*, the half-decay time was very rapid (<0.1 s) and any ventral luminescence was undetectable after about 1 s. In *G. minuta* and *L. splendens*, however, when the overhead light was turned off, there was no dramatic drop in luminescence intensity within the first 0.1 s. Rather, the time required to drop to half-maximum intensity during decay was approximately 5 s for all light intensities (Fig. 4). In these two species, the ventral luminescence, as perceived by the human eye, dropped off steadily, particularly during the first 30 s (Fig. 4), but persisted for an average of about 3 min and, in somes case, for up to 10 min.

Measurements of counterillumination behavior

With increasing ambient light intensities, the bioluminescent decay curves of *Gazza minuta* (Figs 4 and 5; Table 2) showed concomitant increases in intensity. There was strong overlap in the decay curves obtained above a downwelling light intensity of 180×10^{11} quanta $cm^{-2}s^{-1}$, suggesting that the response capabilities of the fish had reached an asymptote at a light intensity between 36×10^{11} and 180×10^{11} quanta $cm^{-2}s^{-1}$. The average initial light levels of the fish ranged between 1.9×10^{9} quanta $cm^{-2}s^{-1}$ at an ambient light intensity of 1.8×10^{11} quanta $cm^{-2}s^{-1}$ and 27.0×10^{9} quanta $cm^{-2}s^{-1}$ at an ambient light intensity of 360×10^{11} quanta $cm^{-2}s^{-1}$ (Table 2). The fish's light output was much less than the amount of light intercepted by a freshly killed, nonluminescing fish or comparable silhouette mimic (5.5 % of the ambient). Moreover, as ambient light intensity increased, the proportion compensated by bioluminescence decreased from 18.9 to 1.4 % (Fig. 5; Table 2).

Angular distribution

Maximum luminescence was emitted at 20–25° from the ventral midline (Fig. 6). Removal of the small, clear deciduous scales of the posteroventral surface did not markedly influence the angle of maximum luminescence, although at all angles there was a uniform increase in the overall intensity of about 40 % (Fig. 6). However, the removal of the guanine-laden, chromatophore-rich skin greatly changed the angle of maximum luminescence, resulting in a nearly free diffusion of luminescence through the lateral surfaces of the fish (Fig. 6). Also, while the angle of maximum intensity shifted to approximately 35–40° from the ventral midline, the ventral-most emissions remained approximately constant in intensity and angular profile. Because it was necessary to perform these experiments with freshly killed, partially dissected fishes, it was not possible to determine if the living fish is able to change the angle by modifications in the guanine platelets or chromatophores of the skin.

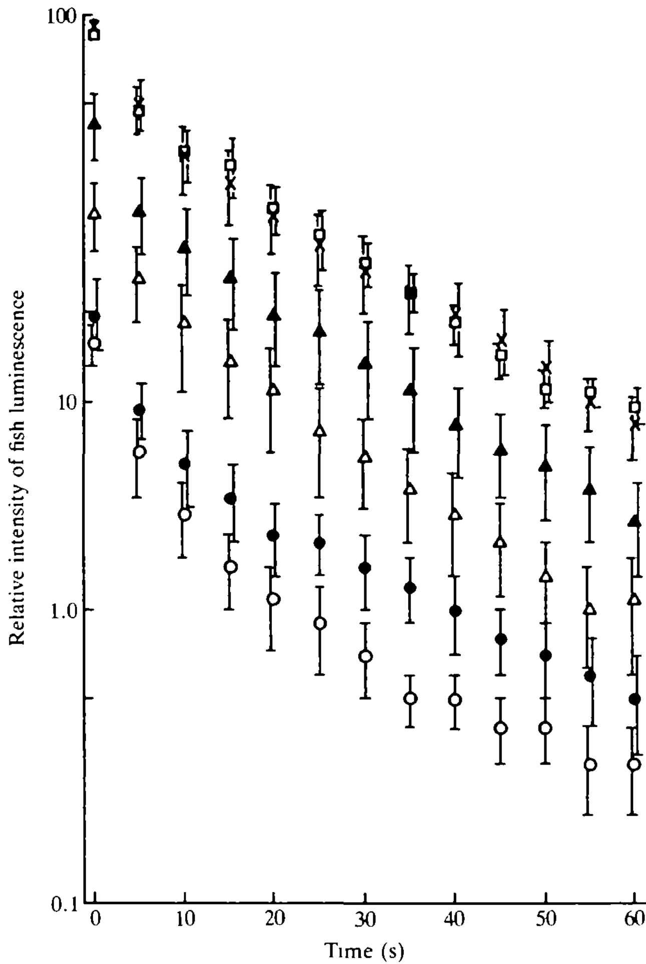

Fig. 4. Bioluminescence decay curves of *Gazza minuta* under varying downwelling light intensities. Symbols represent the average decay (±S.E.M.) of luminescence for six individuals adapted to the following ambient light levels: ○ 1.8×10¹¹; ● 3.6×10¹¹; △ 18.0×10¹¹; ▲ 36.0×10¹¹; □ 180.0×10¹¹; × 360.0×10¹¹ quanta $cm^{-2} s^{-1}$. Each individual was adapted to these light levels for 30 min. The ambient overhead light was then turned off and decay was recorded with a photomultiplier at 5 s intervals.

Spectral studies

The uncorrected spectra of freshly cultured bacterial symbionts (*Photobacterium leiognathi*) peaked at about 490 nm with a half-bandwidth of approximately 85 nm (Fig. 7). This peak wavelength is similar (491 nm) to that reported by Hastings and Mitchell (1971) for freshly cultured symbionts of *P. leiognathi*; the overall half-bandwidth was within 2 nm of their data. These values are all within the resolution of the equipment used in these experiments.

The average spectrum of the intact light organs showed a peak at about 500 nm, which is shifted 10 nm towards the green compared to the cultured bacteria; the

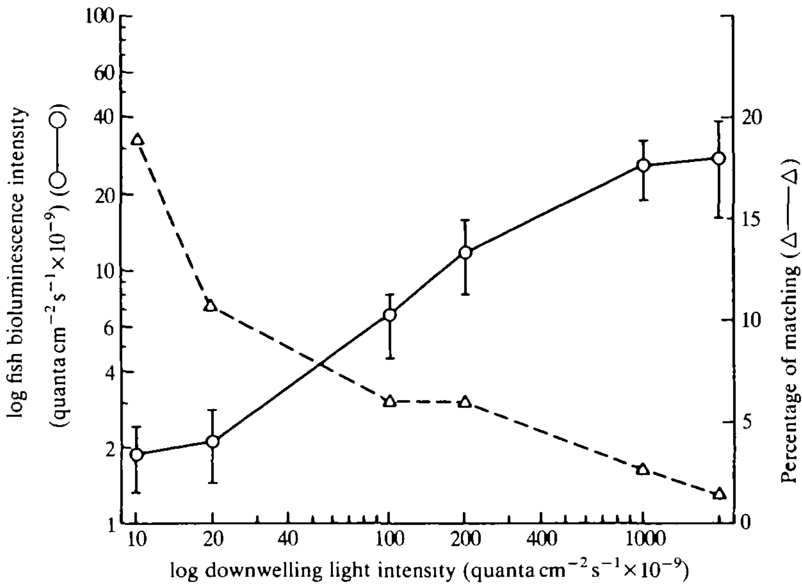

Fig. 5. Ventral luminescence in *Gazza minuta*. O, absolute luminescence intensity. Each point represents the maximum recorded luminescence at each of the six ambient light levels [mean values±S.E.M. (N=6)]. Δ, percentage matching or proportional matching of the fish's luminescence. These values represent the ratio of maximum bioluminescence intensity to the luminescence level that would be required for complete matching, as determined by the silhouette mimic controls.

Table 2. *Ventral bioluminescence response of* Gazza minuta *to different downwelling ambient light intensities*

Ambient light intensity (quanta cm^{-2} s^{-1} × 10^{-11})	Light intercepted by fish silhouette mimic* (quanta cm^{-2} s^{-1} × 10^{-9})	Average (±S.E.M.) maximum fish luminescence (N=6) (quanta cm^{-2} s^{-1} × 10^{-9})	Percentage of complete matching
1.8	9.9	1.87±0.54	18.9
3.6	19.8	2.10±0.64	10.6
18.0	99.0	6.06±1.92	6.1
36.0	198.0	11.92±3.93	6.0
180.0	990.0	25.48±6.70	2.6
360.0	1980.0	27.03±10.98	1.4

*5.5% of ambient light intensities; i.e. amount of downwelling light to be matched for complete counterillumination (see text for details).

half-bandwidth was approximately the same, at 85 nm. The peak emission from the fish itself was the same as that of the intact light organ, but the half-bandwidth was much narrowed to 60 nm. About two-thirds of the 25 nm narrowing was eliminated from the shorter wavelengths.

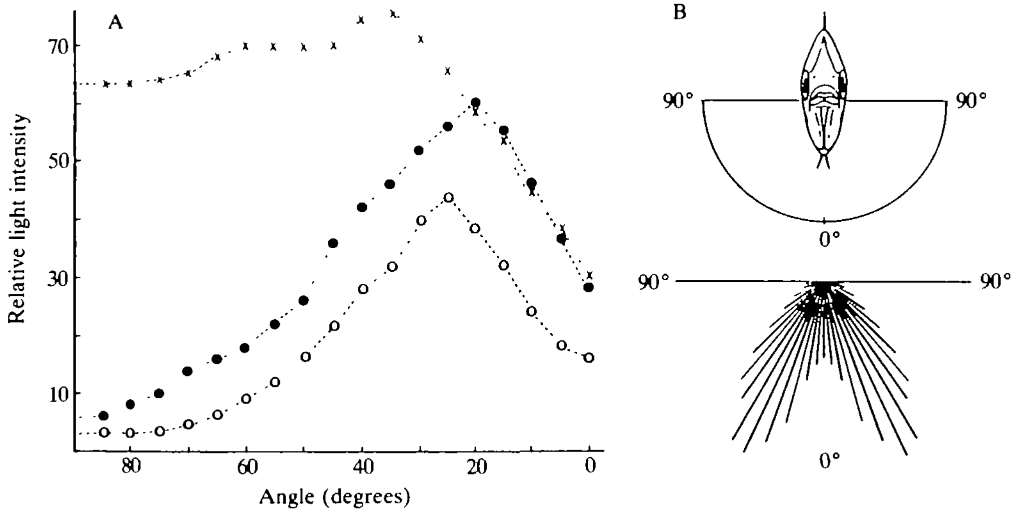

Fig. 6. Angular distribution pattern of *Leiognathus equulus* posteroventral luminescence. (A) The curves represent luminescence intensities at various angles over the fish's posteroventral surface. The maximum luminescence intensity, with all tissues present, was recorded at an angle of 25–30° from the ventral midline (○). Removal of the scales increased intensity by about 40% over the entire curve (●). Removal of guanine-rich skin changed the angular pattern such that light diffused freely from the lateral surfaces (×). (B) Schematic representation of the ventral counterillumination pattern, taken from A. The lengths of the lines emanating at 5° angular intervals represent the relative brightness of the luminescence at that angle.

Observations of leiognathid light organ morphology and anatomy related to ventral luminescent behavior

The morphological characteristics of the light organ systems of females of all leiognathid species (Fig. 8) are generally the same, although there are some minor differences. The bacteria in the light organs of all leiognathid species are contained within tubules (Fig. 8A) embryologically derived from the esophageal tissue (M. J. McFall-Ngai, A. Cabanban and J. G. Morin, unpublished data). Chromatophore abundance in the various tissues correlates well with the path of light progression through the total system. The shutters on the light organs of most species examined have both muscles and a large number of chromatophores in addition to the muscles (Fig. 8B). However, in one species, *Leiognathus nuchalis*, which showed only rapid decay kinetics of luminescence, the light organ shutters appear to be devoid of chromatophores. However, in all species the gas bladder is highly silvered (Fig. 8C) and contains extensive chromatophore coverage. This pigmentation is particularly apparent in the area adjacent to the tissue covering the anal fin pterygiophore, which is the transparent, unsilvered portion of the gas bladder. The dorsal musculature contains a dark pigmentation, which is largely absent from the ventral muscles through which the light is diffused (Fig. 8D). Finally, although the skin of the dorsum has species-specific mottling patterns,

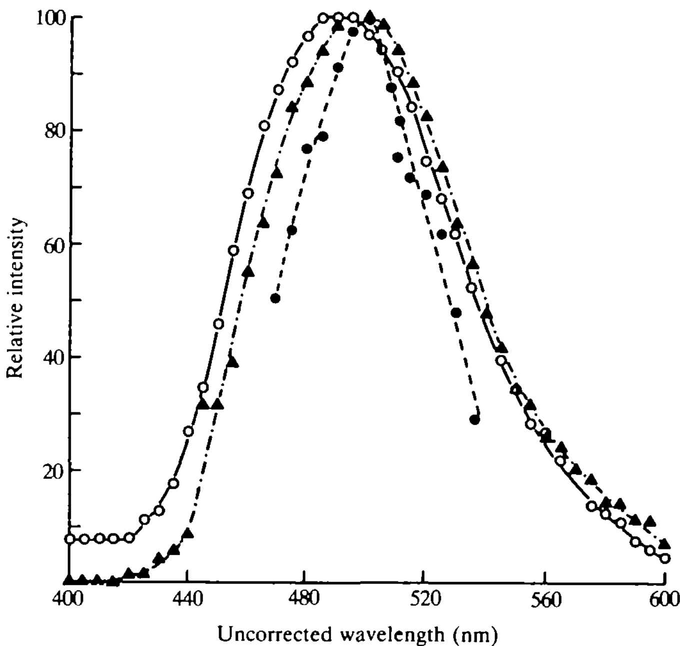

Fig. 7. Averages of spectra measured for the light organ system of *Leiognathus splendens*. ○, cultured symbionts (N=3); △, intact light organs (N=3); ●, whole, living specimens (N=6).

there are large concentrations of chromatophores in the skin of the ventrum that were observed to expand and contract under various experimental regimes, especially around the regions of maximal luminescence (Fig. 8E).

Discussion

The pattern of ventral luminescence in leiognathid fishes appears to be strikingly different from the counterillumination systems previously described in mesopelagic fishes. The dynamics of the luminescent behavior in leiognathids seems to be a direct result of the internal position of their light organ. This position allows the fish to control light emission not only proximal to the light organ, as in superficial photophores (Herring, 1982, 1985), but also in tissues at some distance from the light organ. This arrangement results in control not only of the intensity, color and angular distribution of luminescence in leiognathids, as is characteristic of midwater fishes, but also of the *pattern* of ventral luminescence. Among counterilluminating animals, only luminous squids have been demonstrated to show such control and versatility of luminescence expression (Young, 1977; Young and Mencher, 1980).

The complexity of leiognathid luminescence patterns is indicated by the results of our behavioral experiments. Under our experimental conditions, specimens of the leiognathid *Gazza minuta* increased their ventral luminescence intensity in

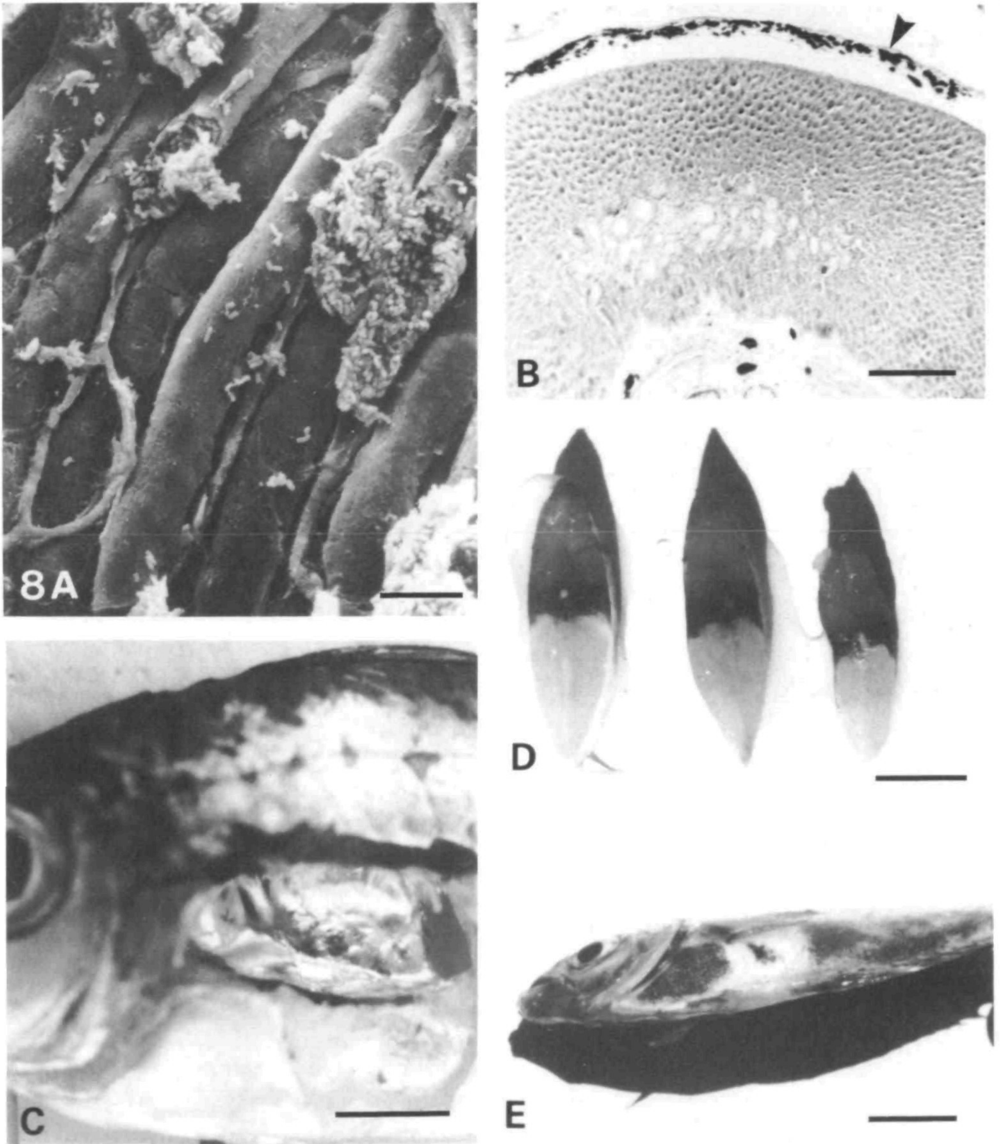

Fig. 8. Anatomy and morphology of the principal components forming the light organ system of a typical leiognathid, *Gazza minuta*. (A) Scanning electron micrograph (SEM) of the light organ tubules. Scale bar, 25 μm (for SEM methods, see Dunlap, 1984). (B) Light micrograph (5 μm section, stained with hematoxylin and eosin) of the dorsal portion of the light organ, showing chromatophore-rich window (arrowhead). Scale bar, 0.4 mm. (C) Cut-away of the lateral body wall to show silvery gas bladder surface. Scale bar, 10 mm. (D) Serial cross sections, 10 mm thick, of the posterior body showing chromatophore-rich dorsal musculature and translucent ventral musculature. Sections extend from just posterior to the gas bladder (left-hand section) to just anterior to the tail (right-hand section). Scale bar, 10 mm. (E) Ventral surface, showing mottling of the skin due to chromatophore expansion. Scale bar, 10 mm.

response to increases in downwelling light. However, their luminescence did not match the intensity of downwelling light over their entire ventral surface with an equal bioluminescent output. The maximum light output measured for this species, under high light conditions, was greater than the lowest ambient light intensities in which luminescence was detectable. These findings indicate that, although they do not match over their entire ventrum, they are *capable* of producing intensities that could match the low ambient light levels. Such a phenomenon was also observed for *Leiognathus nuchalis* in an independent experimental regime (M. McFall-Ngai, J. G. Morin, J. F. Case and J. Warner, unpublished data). It is possible that leiognathids do not behave in the same way under these experimental conditions as they would in nature. However, this is unlikely since we obtained identical results for leiognathids under two different types of experimental regimes, and, unlike mesopelagic fishes, leiognathids survive capture, maintenance and experimental manipulation well, and are thus more likely to show normal behavior in confined conditions.

Although the overall luminescent output in *Gazza minuta* and *Leiognathus nuchalis* did not compensate for ambient light intensities, at low light levels certain portions of the body appeared camouflaged to the human eye. These locations were in the gill region, which represents about 8–10 % of the silhouette area of the ventrum. This bright luminescence occurred at the widest part of the body (Fig. 3), an area that would otherwise create the most prominent silhouette. If these areas alone were luminescing, then the portion that they obscure would account for 0.5 % rather than 5 % of the intercepted light. This difference would shift the matching curves (Table 1; Fig. 5) upwards by a factor of 10 to more than 100 % at low light levels and to over 10 % even at high light levels. However, because some of the light is also emitted throughout the remainder of the ventral silhouette, these values represent the upper limit of their contribution to matching at a given ambient light level.

The response of leiognathids to increases in downwelling light is further evidence that the ventral luminescence of these fishes is different from that of mesopelagic animals. Not only was the ventral luminescence of *Gazza minuta* less than the experimentally presented environmental light but, as light levels increased, their overall output compensated to a lesser degree at each increasing level of light intensity. This trend may indicate a gradual shift from dependence on luminescence for camouflage at low light levels to other mechanisms at higher light levels, such as use of reflected light. As is the case with many other species of shallow-water schooling fishes (Denton, 1970), leiognathids probably depend heavily on their silvery, reflective lateral surfaces when light levels are high. If this is so, the data from Fig. 5 on intensity responses to ambient light suggest that luminescence functions primarily at the low light levels (i.e. at dawn and dusk, under moonlight and/or in turbid water), while reflected light is used at higher (daytime) light levels. Based on our data, where ambient light levels were below 36×10^{11} quanta $cm^{-2} s^{-1}$, matching was significant. These ambient values represent illumination levels at the sea surface about 10 min after sunset and 10 min

before sunrise (Munz and McFarland, 1977; McFarland, 1986), but would shift to earlier or later, respectively, depending on the fish's depth in the water and the water clarity.

Crepuscularly active predatory fishes known to prey on leiognathids include lizard fishes (Synodontidae), barracudas (Sphyraenidae), snooks (Centropomidae), jacks (Carangidae) and snappers (Lutjanidae) (from unpublished data obtained in a study of the ecology of South Bais Bay by S. Alcazar of Silliman University, Dumaguete, Philippines; Salini *et al.* 1990). In general, these are fishes that attack from an ambush position obliquely below their prey. Thus, any disruption of the prey's silhouette would make the prey more difficult to detect. Presumably the disruptive illumination provides such a benefit at the low ambient light levels when predation is likely to be greatest. Twilight is apparently the time of day when predation by such piscivorous fishes is most intense (Hobson, 1979).

Effective counterillumination requires that the bioluminescent spectrum should be similar to that of the environment. The intrinsic photophores of most mesopelagic fishes luminesce in the blue wavelengths (around 475 nm), closely matching the blue color of the dimly lit habitat (Herring, 1983; Widder *et al.* 1983; Denton *et al.* 1985). However, coastal and estuarine water often appears green or blue-green (Jerlov, 1968). Visual pigment analyses of *Leiognathus nuchalis* indicated that spectral sensitivity of the visual system peaks around 507 nm (F. Crescitelli and M. McFall-Ngai, unpublished data), indicating adaptation to coastal waters. The bioluminescence of intact, living specimens of *L. splendens* has a peak at about 500 nm, the same peak wavelength as had been reported for another leiognathid, *Secutor insidiator* (Herring, 1983). *Photobacterium leiognathi* grown in culture has a peak wavelength between 485 and 490 nm (Fig. 7; Hastings and Mitchell, 1971). This difference in the peak wavelengths of emission between the intact fish and cultured bacteria could be due either to some change in the bacteria themselves, as a result of being under light organ 'culture' conditions, or to filtering of the light within the fish, distal to the culture chamber. Hastings and Mitchell (1971) showed that the peak wavelength of *Photobacterium leiognathi* cultured from the light organ (491 nm) was shifted towards the green by 6 nm compared to cultures of free-living isolates from the water column (485 nm). Thus, there is some evidence that this species of bacterium may have its peak wavelength of light emission altered by conditions within the light organ. Because our experiments showed that the spectrum from the intact light organ was shifted even farther to the green from that of laboratory cultures of the bacterial symbionts, anatomical or physiological features of the light organ itself may further affect the spectrum. The mechanisms by which this may occur are not known, although filters in light organ systems that shift the wavelength of emitted light are not uncommon (Herring and Morin, 1978; Denton *et al.* 1970, 1985).

Because a given species was not consistently available, various experiments of this study were carried out on four species of leiognathids. The morphology of the light organ systems of most leiognathid females is similar but, where we noted differences, there were concomitant differences in the behavior. These differences

indicate that there are interspecific variations in the control of luminescence in leiognathids. Particularly interesting were the long luminescence decays seen in *Gazza minuta* and *Leiognathus splendens*, compared to the short decay times observed in *L. nuchalis*. Consistent with these differences, our dissections of these species showed heavy chromatophore coverage on the light organ of *G. minuta* and *L. splendens*, the dynamics of which are potentially responsible for the slow decay, while *L. nuchalis* shutters had few, if any, chromatophores. As a consequence, the light organ type in *L. nuchalis* may not permit graded or slow decay of luminescence, but only rapid, shutter-controlled, on–off responses. Chromatophores in the skin of this species may be entirely responsible for modifying the intensity of light output.

In conclusion, our results provide evidence that a type of counterillumination behavior does occur in a shallow-water fish species. The mottled patterns of this ventral luminescence are reminiscent of the disruptive coloration patterns that are used as camouflage by many animals living in heterogeneous habitats. The bioluminescently produced mottling of leiognathids, which might be called disruptive illumination, differs from the more uniform ventral luminescence reported for mesopelagic animals, but the effect in camouflaging may be comparable. Further studies on leiognathids and other ventrally bioluminescing, neritic animals should provide insight into the variety of ventral luminescence patterns and the mechanisms for their control in this spatially and temporally heterogeneous environment.

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