PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

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# Long-wave sensitivity in deep-sea stomiid dragonfish with far-red bioluminescence: evidence for a dietary origin of the chlorophyll-derived retinal photosensitizer of *Malacosteus niger*

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Both residual downwelling sunlight and bioluminescence, which are the two main sources of illumination available in the deep sea, have limited wavebands concentrated around 450–500 nm. Consequently, the wavelengths of maximum absorption ($\lambda_{max}$) of the vast majority of deep-sea fish visual pigments also cluster in this part of the spectrum. Three genera of deep-sea loose-jawed dragonfish (*Aristostomias*, *Pachystomias* and *Malacosteus*), however, in addition to the blue bioluminescence typical of most deep-sea animals, also produce far-red light (maximum emission >700 nm) from suborbital photophores. All three genera are sensitive in this part of the spectrum, to which all other animals of the deep sea are blind, potentially affording them a private waveband for illuminating prey and for interspecific communication that is immune from detection by predators and prey. *Aristostomias* and *Pachystomias* enhance their long-wave visual sensitivity by the possession of at least three visual pigments that are long-wave shifted ($\lambda_{max}$ values *ca.* 515, 550 and 590 nm) compared with those of other deep-sea fishes. *Malacosteus*, on the other hand, although it does possess two of these red-shifted pigments ($\lambda_{max}$ values *ca.* 520 and 540 nm), lacks the most long-wave-sensitive pigments found in the other two genera. However, it further enhances its long-wave sensitivity with a chlorophyll-derived photosensitizer within its outer segments. The fluorescence emission and excitation spectra of this pigment are very similar to spectra obtained from mesopelagic copepods, which are an important component of diet of *Malacosteus*, suggesting a dietary origin for this pigment.

**Keywords:** fish; visual pigment; bioluminescence; photosensitizer; chlorophyll; deep sea

The deep sea is by far the largest habitat on Earth, covering over 60% of its surface and having an average depth close to 4000 m. Despite this, less is known about it than about most other environments. However, it has long attracted the attention of vision scientists who have shown that the eyes of deep-sea fishes display an astonishing diversity of anatomical adaptations to their unique environment (Locket 1977; Wagner *et al.* 1998). The deep sea has also lured those with an interest in visual pigment ecology. One of the aims of such work is to relate the spectral absorption characteristics of an animal's visual pigments to its spectral environment. The deep sea holds perhaps a special attraction for such work as, unlike in shallow water or on land, its photic environment is in many aspects quite simple. Thus, some residual sunlight can penetrate the upper 1000 m of the ocean in ideal conditions, although in most waters visible sunlight is effectively extinguished at significantly shallower depths. Yet many animals, including most fishes, living well below the reach of sunlight, have well-developed visual systems. This allows them to view the second source of light in the deep sea: bioluminescence produced by over 80% of the species inhabiting this region. Both residual sunlight and bioluminescence are spectrally very restricted with most radiation being in the region 450–500 nm. Not surprisingly, therefore, the vast majority of fishes have visual pigments with peak absorbance ($\lambda_{max}$) in this region of the spectrum, providing a good match between environment and visual pigments, although detailed analysis shows that even here the relationship is not straightforward (Partridge *et al.* 1989; Douglas *et al.* 1998*a*).

Although the wavelength of maximum emission of most deep-sea bioluminescence occurs in the blue region of the spectrum, this is not always so. The most striking exceptions are three genera of deep-sea dragonfish (*Malacosteus*, *Aristostomias* and *Pachystomias*; order Stomiiformes, family Stomiidae), which, in addition to blue bioluminescence

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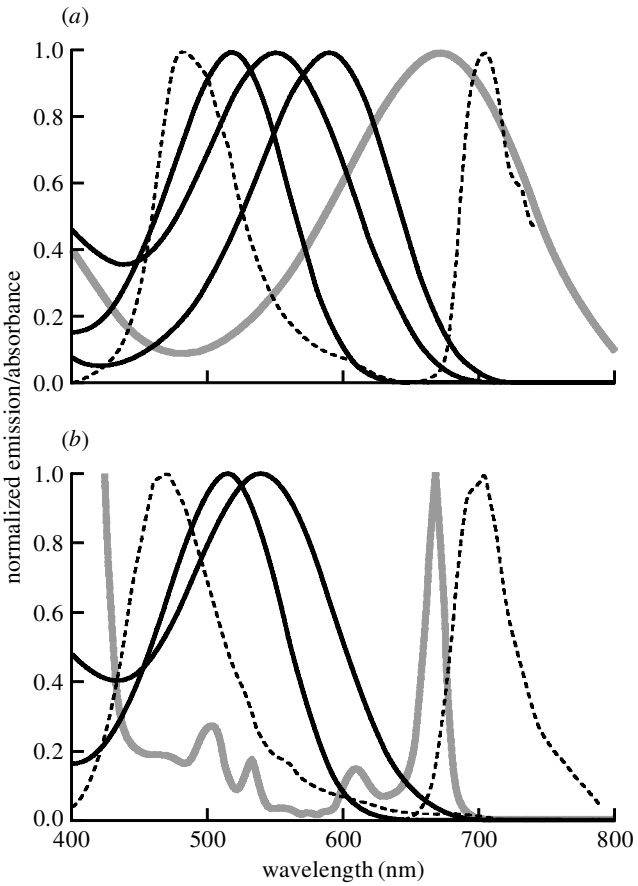


Figure 1. (*a*) Bioluminescence of *Aristostomias tittmanni* (dashed line; Widder *et al*. 1984), and the best-fit templates (solid lines) of the three visual pigments so far identified in its retina (a rhodopsin–porphyropsin pigment pair with $\lambda_{max}$ values 520 and 551 nm and a rhodopsin with a $\lambda_{max}$ at 588 nm) (Partridge & Douglas 1995). The lighter line represents a theoretical porphyropsin with $\lambda_{max}$ at 669 nm, which is the 'partner' of the long-wave-sensitive rhodopsin (calculated using a formula based on empirical data relating $\lambda_{max}$ values of pigments in a rhodopsin–porphyropsin pigment pair) (after Douglas *et al*. 1998*a*). (*b*) Bioluminescence of *M. niger* (dashed line; Widder *et al*. 1984), and best-fit templates (solid lines) of the two identified visual pigments (a rhodopsin with $\lambda_{max}$ 515 nm and a porphyropsin with $\lambda_{max}$ 540 nm). The absorption spectrum of the putative photosensitizer (lighter line) is represented by a purified diethyl ether extract of a retinal suspension (after Douglas *et al*. 1999).

similar to that of other deep-sea animals, also have suborbital photophores producing far-red bioluminescence with spectral emissions peaking sharply at wavelengths beyond 700 nm (Denton *et al*. 1970, 1985; Widder *et al*. 1984).

We, and others, have shown, using conventional retinal extract spectrophotometry, that to facilitate perception of their own long-wave bioluminescence, members of all three genera have at least two visual pigments that are long-wave shifted compared with those of other deep-sea animals ($\lambda_{max}$ *ca*. 515 and 550 nm) (e.g. Partridge *et al*. 1989; Bowmaker *et al*. 1988; Partridge & Douglas 1995; Douglas *et al*. 1998*b*, 1999). Such pigments form a 'rhodopsin–porphyropsin pigment pair' based on the same opsin, which in some photoreceptors is bound to retinal and in others to 3,4-dehydroretinal.

Although such pigments are a better match to the far-red bioluminescence of these animals than the visual pigments of most deep-sea animals that have $\lambda_{max}$ values around 480–490 nm, the match between pigments absorbing optimally at 515–550 nm, and bioluminescent emissions peaking above 700 nm, is still far from perfect. Using a retinal whole-mount technique, however, which isolates pigments that do not survive retinal extraction or any form of preservation (Douglas *et al*. 1995), we have been able to show that both *Aristostomias tittmanni* (Partridge & Douglas 1995) and *Pachystomias microdon* (Douglas *et al*. 1998*a*) possess a third pigment with $\lambda_{max}$ around 588–595 nm (figure 1*a*). These pigments, which are by some margin the most long-wave-sensitive rod pigments ever described, appear, based on the shape of their absorption spectrum, to use retinal as their chromophore bound to a second long-wave opsin. It would therefore not be unreasonable to suppose that these retinas might actually contain a fourth visual pigment composed of 3,4-dehydroretinal bound to this long-wave opsin. Such a pigment would give a virtually perfect match to these animals' bioluminescence (figure 1*a*). Unfortunately we were unable to isolate such a pigment, probably because tissue preparations were made under dim red illumination, which would cause such long-wave-sensitive pigments to bleach.

Thus both *Aristostomias* and *Pachystomias* contain three, and possibly four, very long-wave-shifted visual pigments allowing them to see their own bioluminescence, which would be invisible to all other species. Such a system potentially enables these species to illuminate prey and communicate with conspecifics immune from detection by potential predators and prey alike (Partridge & Douglas 1995).

The third genus of dragonfish that produces far-red light, *Malacosteus*, is also sensitive to long-wave bioluminescence, although the mechanism it employs to be so is very different from that used by the other two species. Thus, although *Malacosteus niger* possesses two long-wave-shifted visual pigments ($\lambda_{max}$ values *ca*. 520 and 540 nm) similar to those based on the shorter-wave opsin one can extract from *Aristostomias* and *Pachystomias* (figure 1*b*), it lacks the second opsin that enables the other species to have pigments with $\lambda_{max}$ beyond 550 nm (Douglas *et al*. 1998*b*, 1999). However, microspectrophotometry has shown that the outer segments of *M. niger*, in addition to their two visual pigments, also contain one or more additional pigments with absorption maxima around 670 nm that are not bleached significantly by light (figure 1*b*; Bowmaker *et al*. 1988; Partridge *et al*. 1989). We have shown that these pigments are, as suggested by Bowmaker *et al*. (1988), used by *M. niger* as a photosensitizer to enhance its sensitivity to long-wave radiation (Douglas *et al*. 1998*b*, 1999). Thus, wavelengths around the $\lambda_{max}$ of the photosensitizer (671 nm) are more effective at bleaching *Malacosteus* visual pigments than other wavelengths (i.e. 654 nm) nearer the absorption maximum of the visual pigments. Therefore light cannot be bleaching the visual pigments directly. The photosensitizer therefore must absorb light at its absorption peak in the far-red and indirectly bleach the shorter-wave-sensitive visual pigments.

Although we do not yet know the mechanism of photosensitization, we have been able to identify the pigments involved as a mixture of bacteriochlorophyll derivatives.

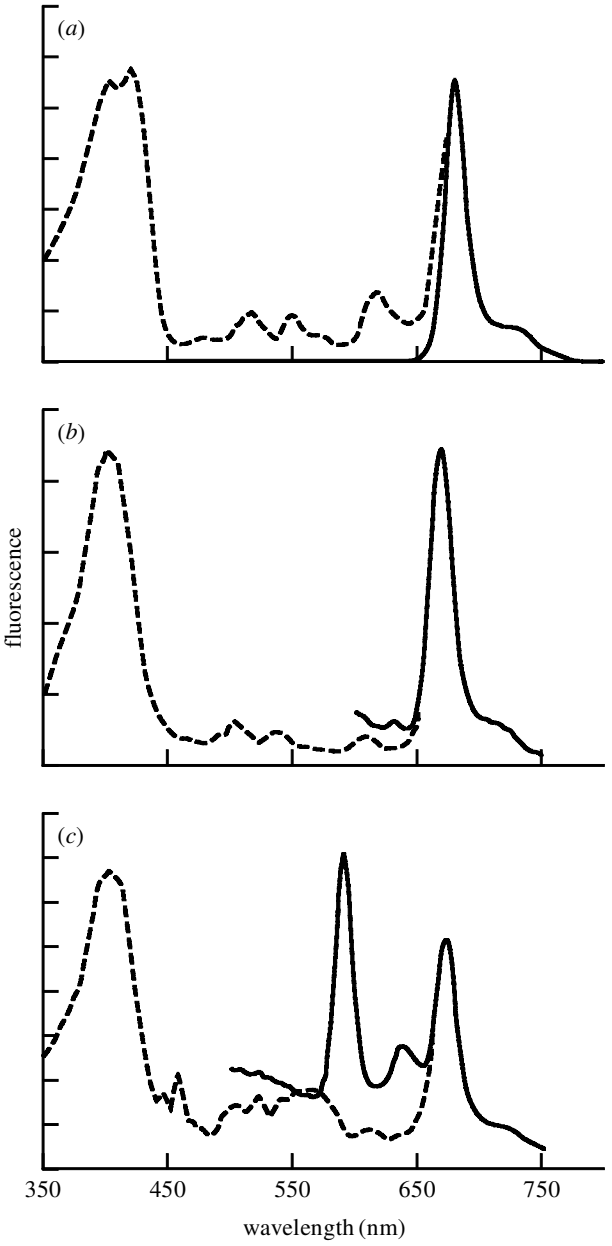


Figure 2. Fluorescent excitation (dashed lines) and emission (solid lines) spectra of (*a*) an unpurified *M. niger* retinal cell suspension in 20% sucrose in PIPES-buffered saline (excitation at 418 nm and emission at 670 nm) (after Douglas *et al*. 1999); (*b*) a methanol extract of whole copepods (*Euchaeta* sp.) (excitation at 400 nm and emission at 670 nm); (*c*) a methanol extract of *Malacosteus* stomach contents) (excitation at 420 nm and emission at 670 nm).

Specifically the photosensitizer is composed of several *Chlorobium* pheophorbides; that is a mixture of defarnesylated and demetallated derivatives of *Chlorobium* chlorophylls 660 (bacteriochlorophyll *c*) and *Chlorobium* chlorophylls 650 (bacteriochlorophyll *d*), with the latter predominating (Douglas *et al*. 1998*b*, 1999).

One of the outstanding questions is the source of this chlorophyll-derived photosensitizer. Two alternatives present themselves: the animal either synthesizes the material itself or obtains it from the diet. We know of no example of chlorophyll derivatives being synthesized in vertebrates. A dietary origin thus seems more likely, especially in view of the fact that the visual pigment chromophores, and the astaxanthin-based tapetum of this species (Denton & Herring 1971), are also derived from dietary sources. If they have access to chlorophyll, the two principal modifications required to produce the photosensitizing pigment, hydrolysis of the farnesyl group and demetallation, can easily take place in the alimentary tracts of organisms.

The *Malacosteus* photosensitizer is, however, derived from bacteriochlorophylls *c* and *d*, which are only known to occur in green photosynthetic bacteria (order Rhodospirillales, suborder Chlorobiineae, families: Chlorobiaceae, bacteriochlorophylls *c* and *d*; and Chloroflexaceae, bacteriochlorophyll *c*). Green bacteria have been identified in subtidal marine sediments but not in the open ocean. It is therefore unclear how they are incorporated into the open-ocean food-chain leading to *M. niger*. Nevertheless, *M. niger*, has a most unusual diet when compared with its close relatives. The stomiid dragonfish are a relatively large family of deep-sea fishes and most, including *Aristostomias* and *Pachystomias*, eat mainly myctophids (deep-sea lantern fish). In contrast, *M. niger* feeds primarily on euchaetid and aetideid copepods (Sutton & Hopkins 1996), which have direct trophic access to photosynthetic organisms.

We therefore compared the fluorescent excitation and emission spectra (recorded at room temperature with a Perkin-Elmer LS50 spectrofluorimeter) of a suspension of retinal cells in 20% sucrose (figure 2*a*), which highlights the photosensitizing pigment (Douglas *et al*. 1999), with similar spectra prepared from homogenates and methanol extracts of whole copepods (*Euchaeta* sp.) (figure 2*b*) collected in the Gulf of Main and the stomach contents of *Malacosteus* caught in the same region (figure 2*c*). The spectra derived from the retinal cell suspensions were typical of a magnesium-free chlorophyll derivative and were consistent with the identity of the photosensitizer as *Chlorobium* pheophorbides (Douglas *et al*. 1999). The spectra obtained from the copepods (figure 2*b*) were very similar to those of the retinal photosensitizer (figure 2*a*) consistent with a common origin. Nevertheless, copepods are thought to consume primarily phytoplankton, which contain derivatives of chlorophylls *a* and *b*, not bacteriochlorophylls *c* and *d*. Interestingly, however, anaerobic purple sulphur bacteria possessing bacteriochlorophyll *a* exist in open-ocean ecosystems and have recently been described in the guts of some pelagic copepods (Proctor 1997).

The peak in the fluorescence excitation spectrum at 670 nm of the *Malacosteus* stomach contents (figure 2*c*) also suggests the presence of a chlorophyll derivative. However, the spectrum is more complex than those of pure chlorophyll derivatives or copepods due to the presence of other, non-chlorophyll-related, fluorescing compounds. Thus, while we cannot show beyond doubt that the origin of the *Malacosteus* photosensitizer is dietary, since we have not identified the chlorophyll-like substances in the diet of *Malacosteus* using definitive methods such as mass spectrometry, the fluorescence spectra are highly suggestive of it.

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## REFERENCES

Bowmaker, J. K., Dartnall, H. J. A. & Herring, P. J. 1988 Longwave-sensitive visual pigments in some deep sea fishes: segregation of 'paired' rhodopsins and porphyropsins. *J. Comp. Physiol.* A **163**, 685–698.

Denton, E. J. & Herring, P. 1971 Report to the council. *J. Mar. Biol. Ass. UK* **51**, 1035.

Denton, E. J., Gilpin-Brown, J. B. & Wright, P. G. 1970 On the 'filters' in the photophores of mesopelagic fish and on a fish emitting red light and especially sensitive to red light. *J. Physiol. Lond.* **208**, 72P–73P.

Denton, E. J., Herring, P. J., Widder, E. A., Latz, M. F. & Case, J. F. 1985 The roles of filters in the photophores of oceanic animals and their relation to vision in the oceanic environment. *Proc. R. Soc. Lond.* B **225**, 63–97.

Douglas, R. H., Partridge, J. C. & Hope, A. J. 1995 Visual and lenticular pigments in the eyes of demersal deep-sea fishes. *J. Comp. Physiol.* A **177**, 111–122.

Douglas, R. H., Partridge, J. C. & Marshall, N. J. 1998*a* The visual systems of deep-sea fish. I. Optics, tapeta, visual and lenticular pigmentation. *Prog. Ret. Eye Res.* **17**, 597–636.

Douglas, R. H., Partridge, J. C., Dulai, K., Hunt, D., Mullineaux, C. W., Tauber, A. & Hynninen, P. H. 1998*b* Dragon fish see using chlorophyll. *Nature* **393**, 423–424.

Douglas, R. H., Partridge, J. C., Dulai, K. S., Hunt, D. M., Mullineaux, C. W. & Hynninen, P. H. 1999 Enhanced retinal longwave sensitivity using a chlorophyll-derived photosensitiser in *Malacosteus niger*, a deep-sea dragon fish with far red bioluminescence. *Vision Res.* **39**, 2817–2832.

Locket, N. A. 1977 Adaptations to the deep-sea environment. In *Handbook of sensory physiology*, vol. VII/5 (ed. F. Crescitelli), pp. 67–192. Berlin: Springer.

Partridge, J. C. & Douglas, R. H. 1995 Far-red sensitivity of dragon fish. *Nature* **375**, 21–22.

Partridge, J. C., Shand, J., Archer, S. N., Lythgoe, J. N. & Van Groningen-Luyben, W. A. H. M. 1989 Interspecific variation in the visual pigments of deep-sea fishes. *J. Comp. Physiol.* A **164**, 513–529.

Proctor, L. M. 1997 Nitrogen-fixing, photosynthetic, anaerobic bacteria associated with pelagic copepods. *Aquat. Microb. Ecol.* **12**, 105–113.

Sutton, T. T. & Hopkins, T. L. 1996 Trophic ecology of the stomiid (Pisces: Stomiidae) fish assemblage of the eastern Gulf of Mexico: strategies, selectivity and impact of a top mesopelagic predator group. *Mar. Biol.* **127**, 179–192.

Wagner, H.-J., Fröhlich, E., Negishi, K. & Collin, S. P. 1998 The eyes of deep-sea fishes. II. Functional morphology of the retina. *Prog. Ret. Eye Res.* **17**, 637–685.

Widder, E. A., Latz, M. I., Herring, P. J. & Case, J. F. 1984 Far red bioluminescence from two deep-sea fishes. *Science* **225**, 512–514.





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