

A NOTE ON THE FIBRE-OPTIC LIGHT-GUIDES IN THE EYE PHOTOPHORES OF *WATASENIA SCINTILLANS*

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A brief account is given of the anatomy and fibre-optic-like light-guiding properties of rod-like elements in the eye photophores on the ventral surface of the eyeball of the Japanese firefly squid *Watasenia scintillans*. These light-guiding elements form a dominant proportion of the volume of the photophore (which is assumed to function in counter-illumination) and are aligned such that light from the bioluminescent core is directed in a cone downwards from the eye. A coplanar arrangement of lamellae in the light-guides strongly suggests that the light passing through will be narrowly restricted both in wavelength and polarization. These features are discussed with regard to other recent findings in this species.

In a series of studies some years ago, R. E. Young and his colleagues provided evidence that the mesopelagic enoploteuthid squid *Abralia trigonura*, *Abraliopsis* sp., *Enoploteuthis* sp., *Pterygioteuthis giardi*, *P. microlampas* and *Pyroteuthis addolux* produce downward-directed bioluminescence at intensities and wavelengths related to the intensity and spectrum of downwelling surface light (Young and Roper 1976, 1977, Young *et al.* 1979, 1980, Young and Mencher 1980, Young and Arnold 1982). This was interpreted as counter-illumination: a means of hiding the dark silhouette visible against downwelling light when an animal is viewed from below (a concept reviewed by Young and Mencher 1980; see, for example, Clarke 1963). It was also shown that differences in the wavelength of emitted light are induced in these species by changes in temperature: warming from 6–8°C through 23°C produced a shift in peak emission from around 485 to 535 nm, accompanied by a progressive increase in emission around 440 nm (Young and Mencher 1980, Young and Arnold 1982). This led to the suggestion that the observed temperature- and intensity-driven qualitative changes in the spectrum of bioluminescence are an ingenious way for these colour-blind animals to closely match the spectral characteristics of seawater at different depths during diurnal migrations and when coming to the surface to spawn (Young and Mencher 1980). However, there is evidence that the conclusions from the work of Young's group might not apply to *Watasenia scintillans* (Berry, 1911), the Japanese firefly squid (a mesopelagic enoploteuthid, sole member of the genus *Watasenia*). In experiments with this species, no progressive shifts have been observed in the wavelength of the emission maximum of any of the

photophores (J-M. Bassot, Centre Nationale de Recherche Scientifique, Paris, and IGG, unpublished experiments).

A survey of photophores in the Cephalopoda by Berry (1920a, b) noted that the underside of the eye is the commonest site in which photophores are to be found. Their high incidence in this position, and the dark pigmentation of the eyes (rendering them highly conspicuous when viewed from below against downwelling surface light), fulfils one of Young's criteria necessary to demonstrate that photophores function in counter-illumination (Young 1977). It should be noted that these photophores have rarely been observed to luminesce, leading Young and Arnold (1982) to suggest that they are not involved in counter-illumination. However, it is perhaps best to reserve judgement on this matter, because the animals have not yet been observed in the habitat in which they are likely to use counter-illumination (probably at a depth of 350 m during daytime; cf. Young 1977): such observation is another of Young's criteria to demonstrate that bioluminescence is used for counter-illumination. Also, in *Watasenia*, the peak wavelength of bioluminescent emission from the eye photophores is identical to that of most of the other photophores, 470 nm (J-M. Bassot and IGG, unpublished experiments). In the present paper, morphological and experimental evidence demonstrates the presence of fibre-optic light-guides in the eye photophores of *Watasenia*.

Herring (1985) has noted that, during their evolution, photophores have acquired additional optical structures which increase the efficiency of emission of light, restrict its angular direction, focus or collimate it, alter its spectral distribution or guide it from the source to a

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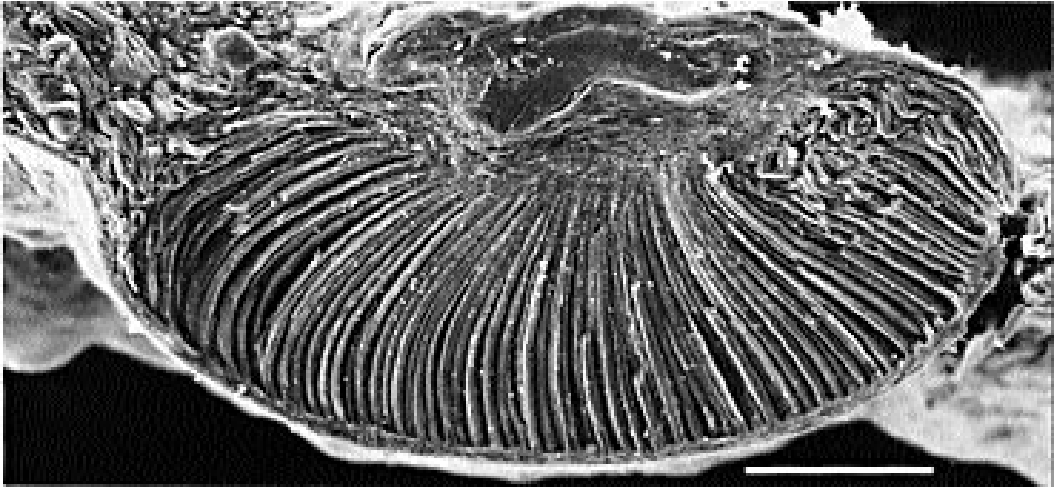


Fig. 1: Scanning electron micrograph of a freeze-fractured eye photophore of *Watasenia scintillans*. This species possesses five such large photophores on the ventral surface of each eye. Note the rod-like elements radiating out from the central core, directed predominantly ventrally, or downwards in the living animal. Scale bar 100 μm

distant point of emission. Most light organs are backed by a pigment cup containing a reflector. In some species of fish, these structures may be present also in a single tubular extension guiding light in a direction very close to the vertical. Arnold and Young (1974) and Arnold *et al.* (1974) described an eye photophore of *Pterygioteuthis microlampas*, but none of the structures mentioned resemble the light-guides of *Watasenia* described here. Young and Arnold (1982) described a rather irregular bunch of what they termed “ribbons” in photophores on the ventral surface of the head, arms, funnel and mantle of *A. trigonura*. Also, in the median abdominal photophores of *Pyroteuthis margaritifera*, Butcher *et al.* (1982) noted the presence of bundles of collagen fibres. Herring (1985) interprets all of these structures as light-guides, but these direct light by reflection among the outer surfaces of several ribbons or fibres running in a similar direction. None have been reported to conduct light inside them, as apparently occurs in *Watasenia* eye photophore light-guides: structures in a position analogous to those mentioned above. However, in the large photophore of the eye of *Sandalops melancholicus*, Young (1977) illustrated light-guides formed from rod-like iridophores, composed of long concentric ribbons of iridosomal platelets occupying much of the volume of the photophore. They are similar to structures assumed to be light-guides in *Bathothauma lyromma* (Dilly and Herring 1974), but much more loosely organized than those reported here in *Watasenia*.

Figure 1 shows the arrangement of rod-like elements which occupy much of the volume of the eye photophore. They appear arranged together in a highly ordered fashion to distribute light in a cone away from the central core, directed predominantly downwards from the base of the eye. Figure 2 shows individual elements in more detail in a transverse section viewed with a transmission electron microscope, revealing that each is an independent unit made up of a system of lamellae producing bands of alternating strong and weak electron density. The biochemical composition of these lamellae is unknown. Figure 3 demonstrates that the rod-like structures in the photophore are light-guides. In Figure 3a, a disc of light (from a fluorescence microscope ultraviolet source) is placed at the edge of the photophore, whereas in Figure 3b it is close to the centre. Note that in both micrographs the light reflected into the microscope produces a similar pattern of illumination: the numerous spots of light are apparently the points of exit of light from the rod-like elements (cf. Figs 1, 2). This strongly suggests that, although the amount and direction of light entering the photophore differs between Figures 3a and 3b, the structure of the photophore is such that light can reach the microscope only through certain of the “rods” which happen to be directed towards the microscope lens. It is therefore concluded that light reflected from or fluorescing from the central region of the photophore is conducted along the “rods”, each of which acts as a light-guiding fibre. In Figure 3c, the disc of light is on the opposite side of

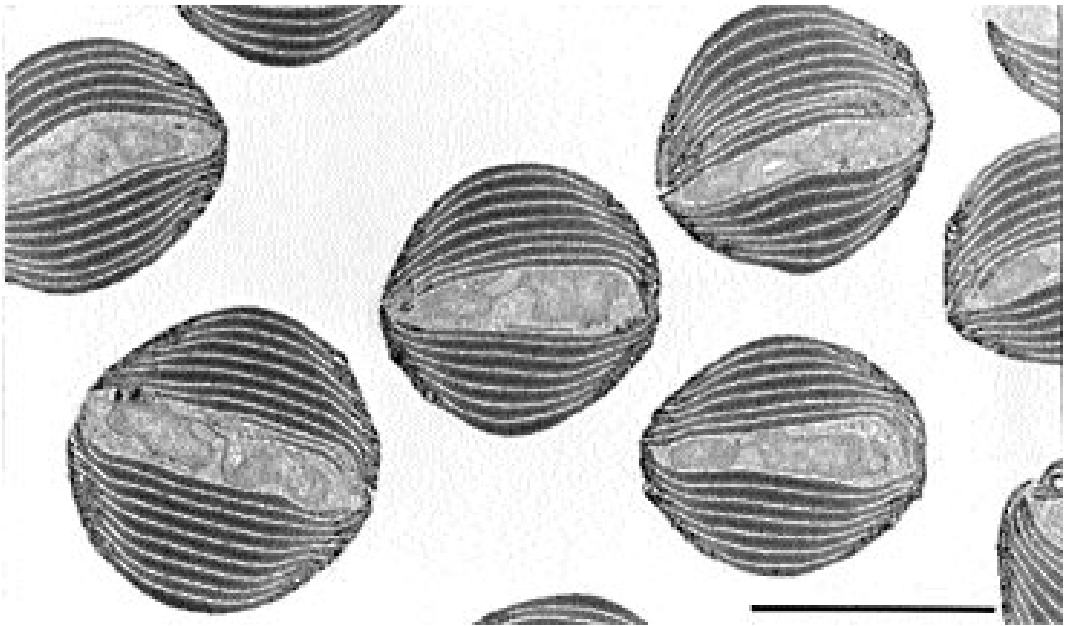


Fig. 2: Transmission electron micrograph of a thin tangential section through the peripheral region of the eye photophore, showing the rod-like elements in cross-section. Scale bar 5 μm

the photophore from Figure 3a and very little light reaches the microscope. This suggests that, rather than being reflected from within the photophore, the ultraviolet light stimulates part of the central core to fluoresce, such that only fluorescence generated near the base of the light-guides directed at the microscope can be seen in this series of light micrographs.

The morphology of the light-guides is in agreement with observations that light in the visible range passing through will be guided along the lamellae: Denton and Land (1971) have measured the reflectance characteristics of a number of layered structures in both cephalopods and fish, noting in particular that reflectance of a given wavelength (λ) is related to optical density and the thickness of the layer(s) as a function of $\lambda/4$. The small size of the light-guides (4–6 μm diameter), as shown in Figure 2, renders direct measurements of light passing through the lamellae very difficult, so as present the efficiency of the light-guiding effect and changes in the spectral characteristics of light passing through are unknown. (It is noted in passing that it might be possible to vary the thickness of the layers, and therefore the spectral characteristics, by physiological changes in fluid and solute content of the lamellae.) Also of interest is the arrangement of the lamellae predomi-

nantly in one plane, such that the light emerging from the light-guides is likely to be polarized: recent studies have demonstrated that wavelength as well as polarization characteristics of light may be important in the life of this species.

Watasenia lives in a twilight environment in which blue light predominates because it is a characteristic of water to absorb shorter and longer wavelengths with increasing depth (e.g. Kampa 1970, Dartnall 1975). In addition, light is polarized at the air-water interface (e.g. Waterman 1984). It has been demonstrated experimentally that polarization sensitivity is an important parameter of visual contrast in the eyes of cephalopods (Shashar and Cronin 1996, Shashar *et al.* 1996, Hayasaki *et al.* in prep.). It is also known that *Watasenia* has a specialized region of its retina which contains cells sensitive both to different wavelengths and to four different directions of polarization of light (Michinomae *et al.* 1994). The ventrally directed bioluminescence projected by these squid closely matches the downwelling ambient light both in wavelength (around 470 nm) and intensity, but they also simultaneously emit light downwards in the green (around 530 nm) from green photophores in the skin (Kito *et al.* 1992, J-M. Bassot and IGG, unpublished experiments). The ventral region of *Watasenia*'s retina receives mostly

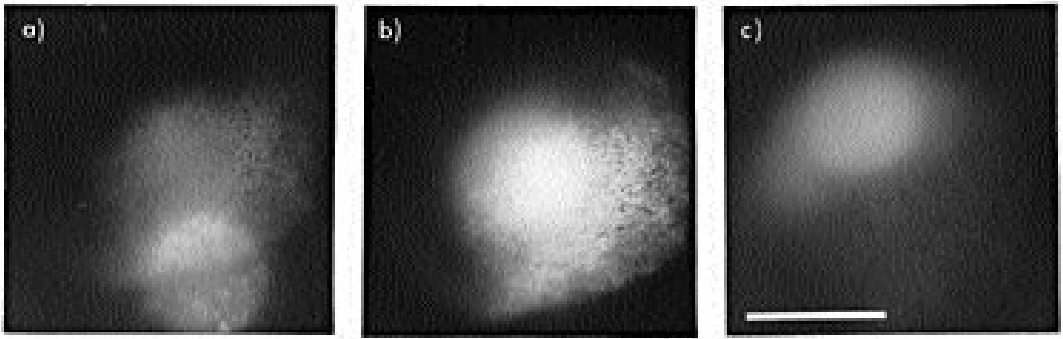


Fig. 3: A series of light micrographs demonstrating the light-guiding properties of the eye photophore. An intact photophore was dissected from a freshly dead firefly squid and placed in seawater with its downward-directed surface uppermost in a deep cavity glass slide. Using a fluorescence microscope with a UV barrier filter, UV light was directed onto different regions of the photophore as follows: (a) a peripheral region; (b) centrally; (c) a different part of the periphery. Note that the pattern of dots of emitted light is the same, where visible (very faint in (c)). The irregularity of the pattern is presumably a product of distortion during dissection of the photophore from the surface of the eye. See text for further interpretation. Scale bar 200 μ m

downwelling light, but it is sensitive to both blue and green, utilizing visual pigments based on two different chromophores: retinal and 4-hydroxyretinal (Seidou *et al.* 1990). However, the dorsal photosensitive vesicles (which measure the intensity of downwelling light; Young *et al.* 1979) of *Watasenia* contain only one visual pigment, based on the chromophore retinal (Seidou *et al.* 1990). These findings have led to the suggestion that, rather than true colour vision, the green photophores operate as part of a “wavelength-specific behaviour” (cf. Marshall *et al.* 1991): “secret” communication among schools of *Watasenia* that is at a wavelength and intensity that other mesopelagic animals cannot detect (Seidou *et al.* 1990, Gleadall 1994).

As pointed out by Rossel (1989), the basic quantitative response of photoreceptors to photon stimulation means that simultaneous sensitivity of a photoreceptor to both wavelength and polarization would preclude the passage of useful information about either parameter to the central nervous system. It has therefore been argued that the three different parameters of contrast detection (brightness, hue and polarization) that *Watasenia* probably uses in its light-poor environment, permit more efficient discrimination of prey, predators and its congeners (Gleadall 1994). If this interpretation is correct, the eye of the firefly squid is an example of a further step in the evolution of the basic molluscan eye from relatively simple brightness-based pattern vision, building upon the fundamental property of lateral inhibition between visual cells by utilizing both wavelength and angle/degree of polarization to enhance contrast qualitatively in a twilight environment quantita-

tively deficient in information.

A great deal more research is needed, however, for full understanding of the roles of colour and polarization of light emitted by the eye photophore light-guides, and of colour and polarization in general in the life of *Watasenia*.

ACKNOWLEDGEMENTS

We thank Mr T. Yano of Shinminato, Toyama, for donating live squid in good condition. IGG is supported by a grant from the Fujiwara Natural History Foundation.

LITERATURE CITED

- ARNOLD, J. M., and R. E. YOUNG 1974 — Ultrastructure of a cephalopod photophore. 1. Structure of the photogenic tissue. *Biol. Bull. mar. Biol. Lab., Woods Hole* **147**: 507–521.
- ARNOLD, J. M., YOUNG, R. E. and M. V. KING 1974 — Ultrastructure of a cephalopod photophore. 2. Iridophores as reflectors and transmitters. *Biol. Bull. mar. Biol. Lab., Woods Hole* **147**: 522–534.
- BERRY, S. S. 1920a — Light production in cephalopods. 1. An introductory survey. *Biol. Bull. mar. Biol. Lab., Woods Hole* **38**: 141–169.
- BERRY, S. S. 1920b — Light production in cephalopods. 2. An introductory survey. *Biol. Bull. mar. Biol. Lab., Woods Hole* **38**: 171–195.
- BUTCHER, S., DILLY, P. N. and P. J. HERRING 1982 — The comparative morphology of the photophores of the squid

- Pyroteuthis margaritifera* (Cephalopoda: Eupoloteuthidae). *J. Zool., Lond.* **196**: 133–150.
- CLARKE, W. D. 1963 — Function of bioluminescence in mesopelagic organisms. *Nature, Lond.* **198**: 1244–1246.
- DARTNALL, H. J. A. 1975 — Assessing the fitness of visual pigments for their photic environments. In *Vision in Fishes. New Approaches in Research*. Ali, M. A. (Ed.). New York; Plenum: 543–563.
- DENTON, E. J. and M. F. LAND 1971 — Mechanism of reflexion in silvery layers of fish and cephalopods. *Proc. R. Soc. Lond.* **178A**: 43–61.
- DILLY, P. N. and P. J. HERRING 1974 — The ocular light organ of *Bathohtauma lyromma* (Mollusca: Cephalopoda). *J. Zool., Lond.* **172**: 81–100.
- GLEADALL, I. G. 1994 — A model for enhancing the visual information available under low-level light conditions: multiple contrast channels created by stepwise changes in detector parameters in the banked ventral retina of the firefly squid. *Interdiscipl. Inform. Sci.* **1**(1): 67–75.
- HAYASAKI, Y., GLEADALL, I. G., TASAKI, K. and Y. TSUKAHARA (in preparation) — Contrast enhancement in the octopus retina: lateral inhibition and polarization sensitivity.
- HERRING, P. J. 1985 — How to survive in the dark: bioluminescence in the deep sea. *Symp. Soc. expl Biol.* **39**: 323–350.
- KAMPA, E. M. 1970 — Underwater daylight and moonlight measurements in the eastern North Atlantic. *J. mar. biol. Ass. U.K.* **50**(2): 397–420.
- KITO, Y., SEIDOU, M., NARITA, K. and M. MICHINOMAE 1992 — Visual pigments and bioluminescence of the firefly squid. *Nikkei Sci.* **22**(1): 30–41 (in Japanese).
- MARSHALL, N. J., LAND, M. F., KING, C. A. and T. W. CRONIN 1991 — The compound eyes of mantis shrimps (Crustacea, Hoplocarida, Stomatopoda). 2. Colour pigments in the eyes of stomatopod crustaceans: polychromatic vision by serial and lateral filtering. *Phil. Trans. R. Soc. Lond.* **334B**: 57–84.
- MICHINOMAE, M., MASUDA, H., SEIDOU, M. and Y. KITO 1994 — Structural basis for wavelength discrimination in the banked ventral retina of the firefly squid *Watasenia scintillans*. *J. expl Biol.* **193**: 1–12.
- ROSSEL, S. 1989 — Polarization sensitivity in compound eyes. In *Facets of Vision*. Stavenga, D. G. and R. C. Hardie (Eds). Berlin; Springer: 298–316.
- SEIDOU, M., SUGAHARA, M., UCHIYAMA, H., HIRAKI, K., HAMANAKA, T., MICHINOMAE, M., YOSHIHARA, K. and Y. KITO 1990 — On the three visual pigments in the retina of the firefly squid, *Watasenia scintillans*. *J. comp. Physiol.* **166A**(3): 769–773.
- SHASHAR, N. and T. W. CRONIN 1996 — Polarization contrast vision in *Octopus*. *J. expl Biol.* **199**: 999–1004.
- SHASHAR, N., RUTLEDGE, P. S. and T. W. CRONIN 1996 — Polarization contrast vision in cuttlefish — a concealed communication channel? *J. expl Biol.* **199**: 2077–2084.
- WATERMAN, T. H. 1984 — Natural polarized light and vision. In *Photoreception and Vision in Invertebrates*. Ali, M. (Ed.). New York; Plenum: 63–114 (NATO Advanced Science Institutes, Series A **74**).
- YOUNG, R. E. 1977 — Ventral bioluminescent countershading in midwater cephalopods. *Symp. zool. Soc. Lond.* **38**: 161–190.
- YOUNG, R. E. and J. M. ARNOLD 1982 — The functional morphology of a ventral photophore from the mesopelagic squid, *Abralia trigonura*. *Malacologia* **23**(1): 135–163.
- YOUNG, R. E., KAMPA, E. M., MAYNARD, S. D., MENCHER, F. M. and C. F. E. ROPER 1980 — Counterillumination and the upper depth limits of midwater animals. *Deep-Sea Res.* **27**(9A): 671–691.
- YOUNG, R. E. and F. M. MENCHER 1980 — Bioluminescence in mesopelagic squid: diel color change during counterillumination. *Science* **208**(4449): 1286–1288.
- YOUNG, R. E. and C. F. E. ROPER 1976 — Bioluminescent countershading in midwater animals: evidence from living squid. *Science* **191**: 1046–1048.
- YOUNG, R. E. and C. F. E. ROPER 1977 — Intensity regulation of bioluminescence during countershading in living midwater animals. *Fishery Bull., Wash.* **75**(2): 239–252.
- YOUNG, R. E., ROPER, C. F. E. and J. F. WALTERS 1979 — Eye and extraocular photoreceptors in midwater cephalopods and fishes: their roles in detecting downwelling light for counterillumination. *Mar. Biol.* **51**(4): 371–380.