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Pineal organs of deep-sea fish: photopigments and structure

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Summary

We have examined the morphology and photopigments of the pineal organs from a number of mesopelagic fish, including representatives of the hatchet fish (Sternoptychidae), scaly dragon-fish (Chauliodontidae) and bristlemouths (Gonostomidae). Although these fish were caught at depths of between 500 and 1000 m, the morphological organisation of their pineal organs is remarkably similar to that of surface-dwelling fish. Photoreceptor inner and outer segments protrude into the lumen of the pineal vesicle, and the outer segment is composed of a stack of up to 20 curved disks that form a cap-like cover over the inner segment. In all species, the pineal photopigment was spectrally distinct from the retinal rod pigment, with λ_{max} displaced to longer wavelengths, between ~485 and 503 nm. We also investigated the pineal organ of the deep demersal eel, *Synaphobranchus kaupi*, caught at depths below 2000 m, which possesses a rod visual pigment with λ_{max} at 478 nm, but the pineal pigment has λ_{max} at ~515 nm. In one species of hatchet fish, *Argyropelecus affinis*, two spectral classes of pinealocyte were identified, both spectrally distinct from the retinal rod photopigment.

Key words: photopigment, pineal organ, pinealocyte, pineal pigment, morphology, fish.

Introduction

The pineal organ in teleosts (and other lower vertebrates) is a functional, non-image-forming photodetector lying on the dorsal surface of the brain. In general, it consists of an end-vesicle attached to the brain *via* a pineal stalk and is located either directly below or within the cranium. This region of the cranium may lack melanophores, forming a depigmented 'pineal window'. The end-vesicle, which may be very large and cover the whole telencephalon, consists of a hollow sac with a central lumen, an extension of the third ventricle (Flight, 1979; Hafeez, 1971; Holmgren, 1965). The vesicle wall is surrounded by a dense plexus of capillaries. The pineal neuroepithelium is composed of several cell types, among them photoreceptors, neurons and supporting cells equivalent to radial glia. The outer segments of the pineal photoreceptor cells project into the lumen and, in general, they consist of a stack of 20–70 dome- or cup-shaped membrane lamellae that contain photopigment. Immunohistochemical studies have demonstrated that the photoreceptor cells contain elements of both the phototransduction cascade and the melatonin biosynthetic pathway (Ekström and Meissl, 1997).

Pineal photoreceptors function primarily as luminance detectors, since the lack of any focussing mechanism and the irregular organisation and convolutions of the pineal epithelium mean that only diffuse light reaches the pineal. In addition, the high convergence of photoreceptors to neurons and the slow time course of pineal photoreceptor responses (Marchiafava and Kusmic, 1993; Meissl and Ekström, 1988) imply that the pineal cannot distinguish discrete, rapidly changing light stimuli. The pineal is thus designed to detect slowly changing ambient light levels, ideal for the photic control of circadian and seasonal behaviour (Ekström and Meissl, 1997).

There is limited information as to the photosensitive pigments of pineal organs and, in teleosts, this is restricted to a small number of species representing only a few of the major teleost families. In salmonids, electrophysiological data from various species of trout and salmon have identified at least two 'pigments' with λ_{max} close to 530 and 500 nm (Marchiafava and Kusmic, 1993; Meissl and Ekström, 1988), but microspectrophotometric measurements have identified pigments with λ_{max} at about 460 and 560 nm (Kusmic et al., 1993). Similarly, in the pike (*Esox lucius*; Esocidae), there are potentially three pigments with λ_{max} at ~380, 530 and 620 nm (Falcón and Meissl, 1981). In these species, there is evidence for a chromatic output from the pineal. By contrast, data from the cyprinids suggest that only a single photopigment with λ_{max} close to 530 nm is present (Meissl et al., 1986; Nakamura et al., 1986). Because of the indirect methods employed, the assumption has been that even though the λ_{max} of these pigments were somewhat different from those of the visual

pigments, the pineal was expressing either rod or cone pigments or both. These data are supported by studies using immunocytochemical labelling of pineal photoreceptors, employing a range of antibodies (e.g. COS and OS) raised against retinal opsins (Forsell et al., 2001; Garcia-Fernandez et al., 1997; Tamotsu et al., 1994; Vigh-Teichmann et al., 1990, 1992), which again indicate that both rod-like and cone-like opsins may be present.

Recently, a number of additional opsins have been identified in vertebrates that are not expressed in either retinal rods or cones and that belong to opsin families distinct from the rod and four classes of cone opsin. At least two of these, VA opsin (Foster and Hankins, 2002; Kojima et al., 2000; Moutsaki et al., 2000; Soni and Foster, 1997) and parapinopsin (Blackshaw and Snyder, 1997), have been located in areas associated with the teleost pineal. However, a more specific teleost pineal opsin, 'exo-rhodopsin', has been identified in zebrafish, *Danio rerio* (Mano et al., 1999), or 'extra-retinal rod-like opsin' (ERrod-like opsin) in Atlantic salmon (*Salmo salar*) and puffer-fish, *Fugu rubripes* (*Takifugu rubripes*) (Philp et al., 2000). This opsin, assumed to be ubiquitous in teleosts, clearly belongs to the rod opsin family but is not expressed in the retina (Bellingham et al., 2003). The term 'ERrod-like opsin', though cumbersome, is preferable, since it avoids the confusion that 'exo-rhodopsin' can introduce, given that pineal pigments may be either rhodopsins or porphyropsins, i.e. based on vitamin A_1 or vitamin A_2.

Photopigments, presumed to be ERrod-like opsins, have been measured by microspectrophotometry in a cyprinid, the goldfish (*Carassius auratus*; Peirson and Bowmaker, 1999), and a characid, the cavefish (*Astyanax fasciatus*; Parry et al., 2003). In the goldfish, where the retinal pigments are all porphyropsins, the rods have λ_{max} at 522 nm, whereas the pinealocytes appear to have a mixed pigment pair based on retinal and 3-dehydroretinal with a λ_{max} close to 512 nm (Peirson and Bowmaker, 1999). *Astyanax*, which also has retinal pigments that are A_1/A_2 mixtures, similarly has pinealocytes with λ_{max} at shorter wavelengths than the rods (Parry et al., 2003). In both species, no additional photopigments were identified in the pineal organ.

In mesopelagic fish from depths just within the reach of sunlight, the pineal organ is ideally situated to monitor the intensity of the down-welling daylight. Typically, the pineal end-vesicle is located underneath a conspicuous 'window' in the skull where the skin lacks melanophores and the skull shows a distinct thinning (McNulty, 1976; McNulty and Nafpaktitis, 1977). This is accompanied by an increase in size of the photoreceptor outer segments, including the number of discs, presumably to increase sensitivity to the very dim light at depth.

We have examined the photopigments and morphology of the pineal organs from a number of mesopelagic fish including representatives of the hatchet fish (Sternoptychidae), scaly dragon-fish (Chauliodontidae) and bristlemouths (Gonostomidae). We also investigated the pineal of the deep demersal eel, *Synaphobranchus kaupi* (Wagner and Mattheus, 2002). In all cases, a pineal photopigment was detected that was spectrally distinct from the retinal rod pigment and has a λ_{max} displaced to longer wavelengths. In one species of hatchet fish, *Argyropelecus affinis*, two spectral classes of pinealocyte were identified, both spectrally distinct from the retinal rod photopigment.

Materials and methods

Mesopelagic fish were caught at depths of between 500 and 1000 m off the west coast of Africa and the Cape Verde Islands during cruise 243 aboard *RRS Discovery*. The list of species is given in Table 1. The deep demersal eel, *Synaphobranchus kaupi*, was collected at a depth of ~2000 m on a subsequent cruise aboard *RRS Discovery* (255) to the Porcupine Seabight and abyssal plain in the Eastern North Atlantic. Fish were collected at night and kept in the dark.

Table 1. *λ_{max} of pinealocytes*

	Rod[a] λ_{max} (nm)	Number of fish	Pinealocyte λ_{max} (nm)
Stomiiformes			
Sternoptychidae			
Sternoptyx diaphana	478	5	485.6±1.5 (0.012)[b] 485.4±2.8 (50)[c]
Sternoptyx pseudobscura	479	4	486.5±1.8 (0.011) 486.1±3.4 (26)
Argyropelecus affinis	478	5	486.5±1.6 (0.011) 485.0±2.5 (13) 497.8±1.6 (0.014) 496.6±3.2 (47)
Argyropelecus gigas	477	2	497.2±1.0 (0.020) 496.1±2.6 (20)
Polyipnus polli	483	1	500.5±1.8 (0.010) 499.1±5.2 (7)
Gonostomatidae			
Gonostoma elongatum	483	1	501.9±1.3 (0.016) 501.1±2.7 (14)
Stomiidae			
Chauliodus sloani	485	3	503.4±1.6 (0.013) 503.9±2.3 (33)
Anguilliformes			
Synaphobranchidae:			
Synaphobranchus kaupi	478	3	515.3±0.6 (0.024) 515.3±2.3 (48)

[a]λ_{max} of rod pigments taken from Douglas and Partridge (1997) and Partridge et al. (1988, 1989, 1992). The data are from extracts and/or microspectrophotometry and may vary by 1–2 nm.

[b]λ_{max} of mean difference spectrum, with mean absorbance in parentheses.

[c]Mean of the λ_{max} of individual pinealocytes, with the number of cells in parentheses.

Histology

Pineal glands were isolated, preferably with parts of the cranium attached, and fixed in a mixture of 4% paraformaldehyde, 2% glutaraldehyde in 0.1 mol l^{-1} cacodylate buffer (Karnovsky, 1965). All samples were stored at 4°C until further processing back on land. After thorough washing in buffer, the pineals were postfixed in 1% osmium tetroxide (2 h), blockstained with 1% uranyl acetate and embedded in Epon. Short series of 1 μm sections were cut alternating with ultrathin (80 nm) sections. Digital micrographs were obtained with a Zeiss Axioskop and a LEO EM912.

Microspectrophotometry

All procedures were carried out under dim red light. Pineal organs were recovered by removal of the dorsal surface of the cranium and, in most cases, the whole piece of cranium including the pineal organ was stored. Tissue was lightly fixed in a 2% glutaraldehyde solution for ~15–30 s, washed in saline and then stored at 4°C in saline containing antibiotic and antimycotic agents (streptomycin and amphotericin; Sigma Chemical Co.). In London, the lightly fixed pineal organs were removed from the skull and teased apart on a coverslip with fine needles. The dispersed tissue was mounted in marine saline containing 5% or 10% dextran and squashed with a second coverslip, which was sealed with wax.

Microspectrophotometric recordings were made in the conventional manner using a Liebman dual-beam microspectrophotometer (Bowmaker et al., 1991; Liebman and Entine, 1964; Mollon et al., 1984). Spectra were recorded at 2-nm intervals from 750 to 350 nm and from 351 to 749 nm on the return scan. The outward and return scans were averaged. A baseline spectrum was measured for each cell, with both beams in an unoccupied area close to the cell, and this was subtracted from the intracellular scan to derive the final spectrum. Two baseline scans were recorded for each cell and averaged. All cells were bleached with white light for 2 min, and post-bleach spectra were recorded. The λ_{max} of both the absorbance spectra and difference spectra were determined by a standard computer programme that best fits a visual pigment template to the right-hand limb of the spectra (Bowmaker et al., 1991; Mollon et al., 1984). Selection criteria were used to discard records either with low absorbance or in which the difference spectrum was clearly distorted.

Results

Histology

In the isolated brain of the hatchet fish *Sternoptyx diaphana*, the pineal has a mushroom-like morphology and is connected to the diencephalon *via* a delicate stalk (Fig. 1a). The flattened upper side is located directly underneath a ‘window’ of the skull where the cartilage is translucent and the skin lacks melanophores. The wall of the pineal vesicle consists of a neural epithelium between 20 and 30 μm thick and a dense plexus of blood vessels surrounding it (Fig. 1b). The luminal side of the epithelium is formed mostly by photoreceptor and non-photoreceptor cells, often referred to as supporting cells (Ekström and Meissl, 1997). Photoreceptor inner and outer segments protrude into the lumen. Radial sections show a stack of up to 20 curved disks that form a cap-like cover of the inner segment (Fig. 1c). Cells on the basal aspect of the pineal vesicle show irregular indentations.

The low-power micrograph of the pineal of another hatchet fish, *Argyropelecus affinis*, shows the flattened vesicle in close apposition to the cartilaginous skull (Fig. 1d). The vesicle wall is formed by a folded epithelium of irregular width. The basal lamina shows a number of infoldings, and the spaces thus created are occupied by numerous blood vessels (Fig. 1e). On the apical (luminal) side, numerous elongated profiles protrude into the vesicle lumen; sometimes they show an hourglass-like constriction in the middle, separating the inner and outer segments of the photoreceptors. Apart from photoreceptors that border the lumen and have mostly spherical and lighter nuclei, at least one additional cell type is present in this epithelium, which is characterised by a darker-staining cytoplasm and nucleus. The outer segments contain a stack of ~30 discs that overall form a conical structure with an extension of the inner segment in the centre (tangential section: Fig. 1f). On the external (basal) surface of the pineal vesicle, cells with a more electron-dense cytoplasm are found, containing bundles of neurofilaments and lobulated nuclei with conspicuous clumps of heterochromatin.

In contrast to the two previous species, in the bristlemouth *Gonostoma elongatum*, the pineal epithelium is composed of a continuous sheath of tall columnar cells (Fig. 1g). The photoreceptor outer segments resemble those in the other mesopelagic fish. Blood vessels are located outside the smooth basal surface. In the vicinity of the capillaries, the surface of the basal plasma membrane of the external cells is greatly enlarged by numerous infoldings. In the dragonfish *Chauliodus sloani*, the thin wall of the pineal vesicle appears to be perforated by numerous dilated capillaries, around which the sensory epithelium is wrapped (Fig. 1h). The concentric whorls of outer segment discs contain about 20 lamellae. Basal cells are more electron dense and contain lobulated nuclei with numerous clumps of heterochromatin. Their basolateral aspect is smooth and shows little infolding.

Microspectrophotometry

Measurements began a few weeks after collection and, in this ‘early’ tissue, high densities of pigments were measured in the pinealocytes, but with rising absorbance at short wavelengths (Fig. 2A). The data were all best fitted to a vitamin A_1 (rhodopsin) template. Estimates of λ_{max} from both the absorbance spectra and the difference spectra were similar, within about 3 nm of each other. With increasing storage times, the density of pigment decreased with a significant rise in shortwave absorbance (Fig. 2B), making use of the absorbance

spectra for estimates of λ_{max} somewhat unreliable. However, even after 12 months of storage, clear difference spectra could still be obtained after bleaching and no 'empty' pinealocytes were identified. The absorbance at short wavelengths had triple peaks characteristic of carotenoids and it is assumed that the yellow pigmentation in the antimycotic/antibiotic storage medium had become bound to the pineal tissue. Because of the need to use difference spectra to obtain reliable λ_{max} from the

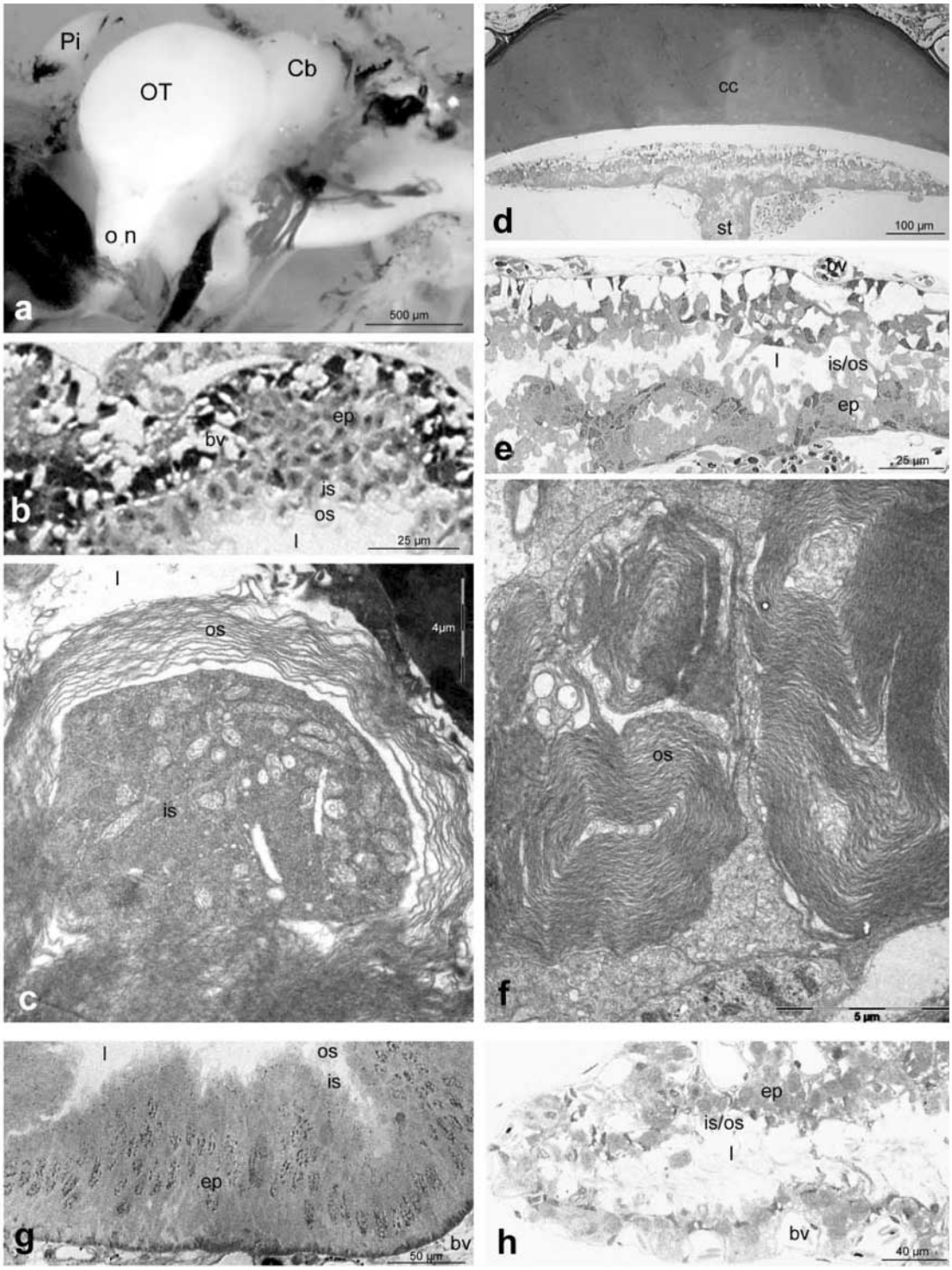

Fig. 1. Pineal morphology and microanatomy of *Sternoptyx diaphana* (a–c), *Argyropelecus affinis* (d–f), *Gonostoma elongatum* (g) and *Chauliodus sloani* (h); light micrographs, except c and f (electron micrographs). Abbreviations: bv, blood vessel; Cb, cerebellum; cc, cranial cartilage; ep, pineal neuroepithelium; is, photoreceptor inner segment; l, lumen of pineal vesicle; on, optic nerve; os, outer segment; OT, optic tectum; Pi, pineal gland; st, stalk.

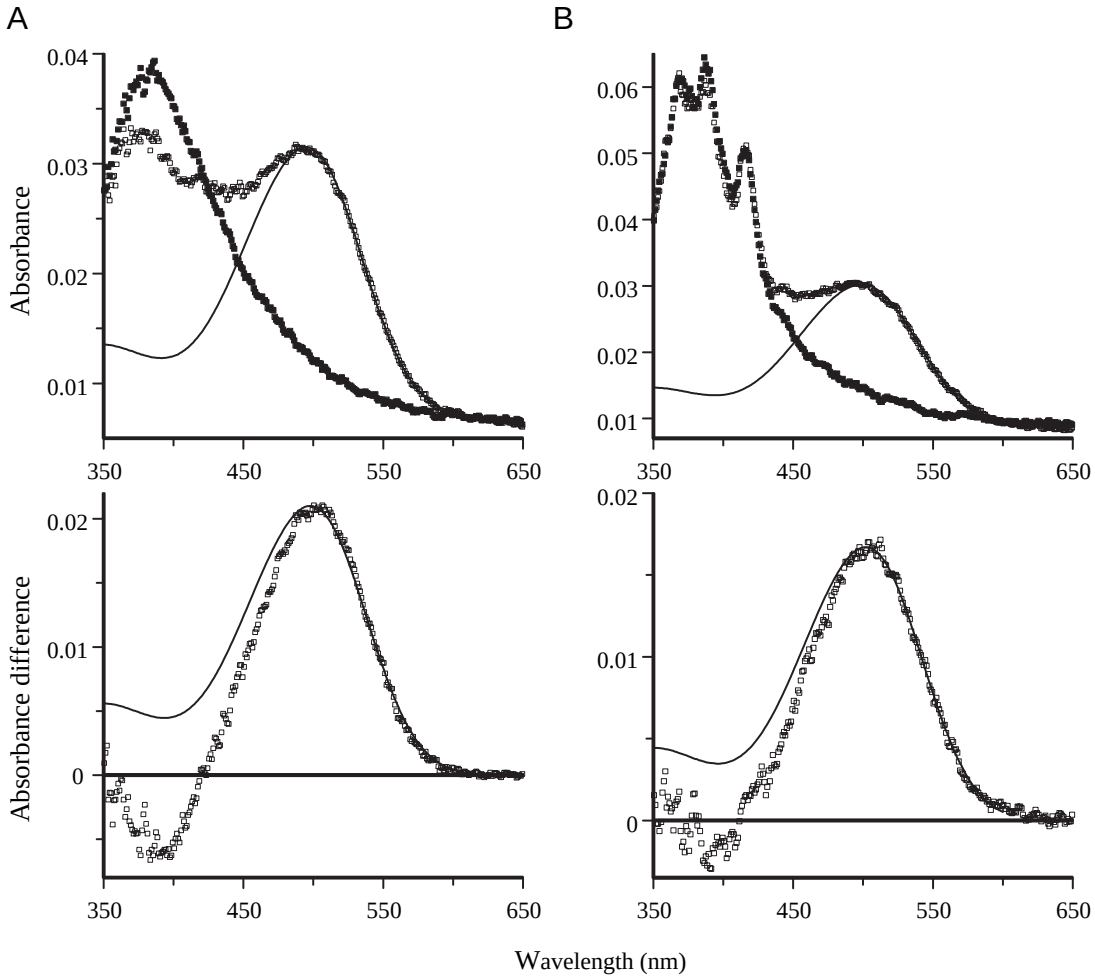

Fig. 2. (A) Mean absorbance and difference spectra from pinealocytes of *Argyropelecus affinis*. Upper traces: open squares, before bleach; filled squares, after complete bleach; solid line, 495 nm template. Lower trace: open squares, difference spectrum; solid line, 498 nm template. (B) Mean absorbance and difference spectra from pinealocytes of *Gonostoma elongatum*. Upper traces: open squares, before bleach; filled squares, after complete bleach; solid line, 499 nm template. Lower trace: open squares, difference spectrum; solid line, 502 nm template. The visual pigment templates are 'Govardovskii' spectra (Govardovskii et al., 2000).

older tissue, data from all the species have been tabulated in this form to aid comparisons.

Photosensitive pigments were measured from the pinealocytes from all eight species. Full details of the number of fish and the λ_{max} of the pigments are listed in Table 1, along with previously published data for the rod visual pigments (with reference sources). Histograms of the distribution of the λ_{max} of individual cells are presented in Fig. 3. In every case, the pineal pigments had λ_{max} at longer wavelengths than the published data for retinal rod visual pigments. In seven of the species, only a single pineal pigment was detected. However, in one species, *Argyropelecus affinis*, the distribution of the λ_{max} of the individual cells (Fig. 3) indicated a bimodal population with spectrally distinct pigments recorded from separate populations of pinealocytes, although no morphological differences could be identified. This division was apparent in four of the five individuals studied. The distribution of λ_{max} has been arbitrarily divided at 490 nm (the minimum point in the histogram and the region of greatest spectral separation), yielding two pigments with λ_{max} at ~486 and 498 nm (Fig. 4). Both pigments have λ_{max} at longer wavelengths than the retinal rod visual pigment (Table 1).

Within the hatchet fish, the genera *Sternoptyx* and *Argyropelecus* have retinal rod pigments with λ_{max} at ~478 nm, whereas the λ_{max} of the pinealocytes are close to 486 nm and 497 nm. A further species, *Polyipnus polli*, has a rod pigment with λ_{max} at 483 nm and a 500-nm pinealocyte pigment. The bristlemouth *Gonostoma elongatum* has pigments very similar to those of *P. polli*, as does the scaly dragon-fish, *Chauliodus sloani*, with the rod and pineal pigments at 485 and 503 nm, respectively (Table 1).

In marked contrast, the deep-sea demersal eel, *Synaphobranchus kaupi*, although having a typical deep-sea rod pigment with λ_{max} at ~478 nm, possesses a pineal pigment displaced some 37 nm to longer wavelengths, close to 515 nm.

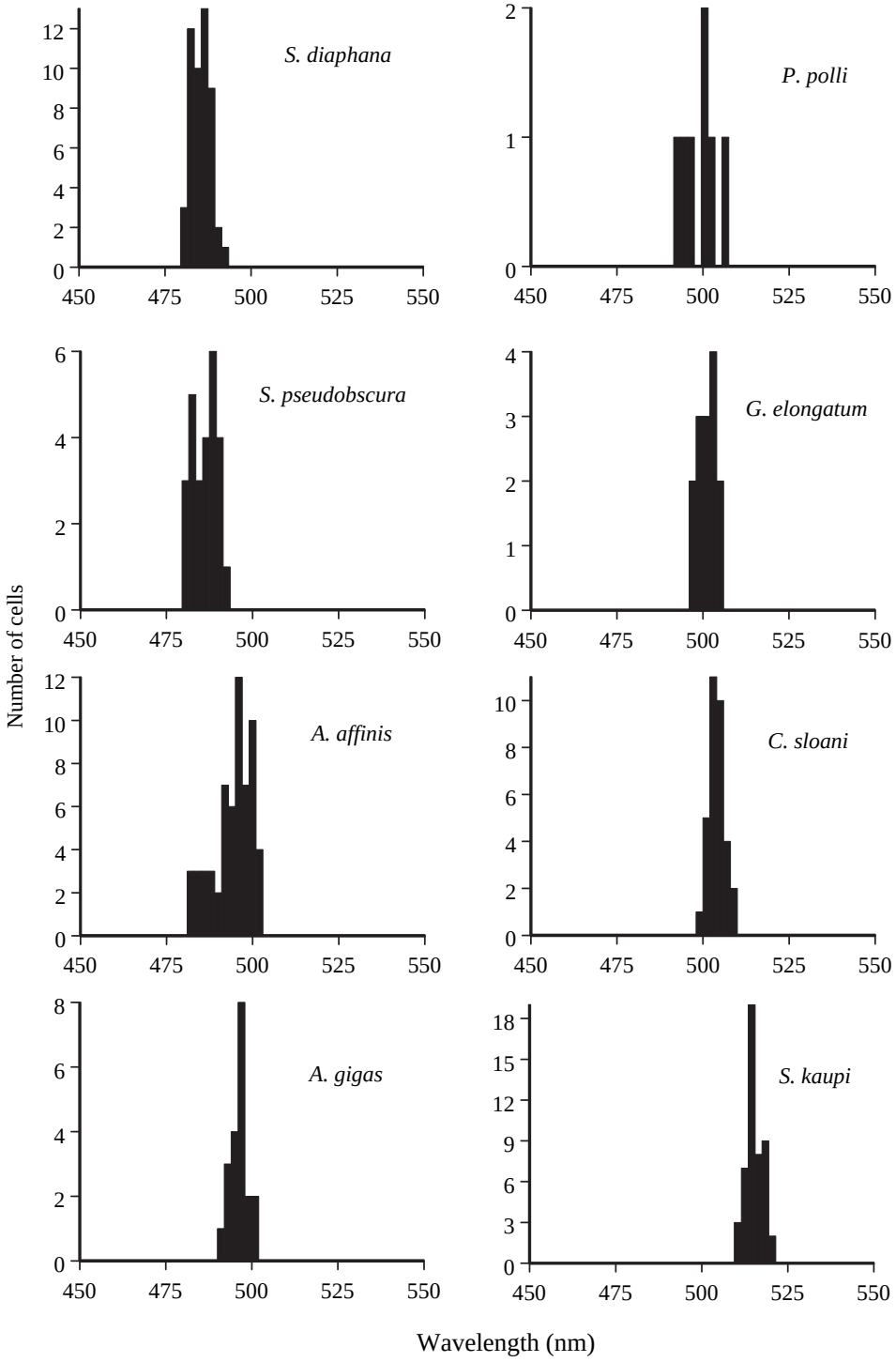

Fig. 3. Distribution histograms of the λ_{max} of individual difference spectra of pinealocytes from all species, as labelled. For the mean λ_{max} of each distribution, see Table 1. Note the indication of a bimodal distribution for *A. affinis*. Bin size is 2 nm.

Discussion

The morphological organisation of pineal organs in deep-sea fish is remarkably similar to that of surface-dwelling fish. Previous studies at the light and electron microscopic level have shown photoreceptors with varying numbers of discs in their outer segments, along with other typical attributes such as synaptic ribbons and other cell types, some of which contain dense bundles of intermediate filaments (McNulty and Nafpaktitis, 1976, 1977; Wagner and Mattheus, 2002). In the mesopelagic fish from the upper 800 m of the water column, McNulty's studies indicated a trend of increasing outer segment volume with depth. Since the present samples from this habitat show no significant variation of depth distribution, it is not surprising that such a trend is not apparent. It is, however, clearly demonstrated when taking the deep-sea eel *S. kaupi* into consideration. The adult specimens used in this and

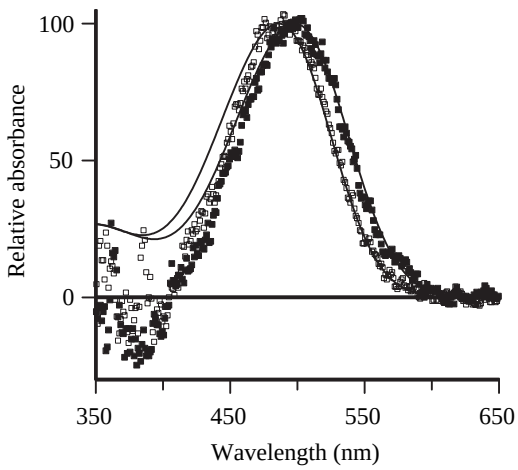

Fig. 4. Normalized difference spectra from pinealocytes of *Argyropelecus affinis*. The cells were arbitrarily divided into two classes with λ_{max} greater or less than 490 nm (see Table 1). Solid lines are pigment templates with λ_{max} at 486 nm and 498 nm (Govardovskii et al., 2000).

the previous morphological studies (Wagner and Mattheus, 2002) are bottom dwellers and have been caught between ~1000 and 2000 m. Their outer segments are roughly 2–3 times the volume of the mesopelagic species, containing up to 100 discs, in accordance with a 'deeper–bigger' trend. Interestingly, among the demersal population, the differentiation of the pineal cells was much more correlated with the differentiation of the retina than with the depth that the fish inhabited (Wagner and Mattheus, 2002).

The most striking feature of the photosensitive pigments of the pineal organs of these deep-sea fish is that the pigments are clearly spectrally distinct from the retinal rod pigments, the only visual pigment present in the pure rod retinae of these species. In all cases, the pineal pigment absorbs at longer wavelengths.

The pineal organs of these deep-sea fish do not appear to express the retinal rod opsin gene but presumably express the closely related ERrod-like opsin gene, which is expressed uniquely in the pineal (Bellingham et al., 2003; Mano et al., 1999; Philp et al., 2000). An ERrod-like opsin has not been experimentally expressed and reconstituted *in vitro* for any teleost species, so that any relationship between the λ_{max} of the rod pigment and the ERrod-like opsin has not been established. However, presumed ERrod-like opsins have been measured by microspectrophotometry in shallow-living freshwater cyprinids such as goldfish (Peirson and Bowmaker, 1999), orfe (*Leuciscus idus*; S. N. Peirson and J. K. Bowmaker, unpublished observations) and a characid, the cavefish (*Astyanax fasciatus*; Parry et al., 2003). In the goldfish, where the retinal pigments are all porphyropsins, the rods have λ_{max} at 522 nm whereas the pinealocytes appear to have a mixture of pigments based on retinal and 3-dehydroretinal with a λ_{max} close to 512 nm. By contrast, in the orfe, the pinealocytes have λ_{max} that are 15–20 nm longer (λ_{max}~534 nm) than the rods (λ_{max}=517 nm). *Astyanax* is more similar to goldfish, with pinealocytes having λ_{max} at shorter wavelengths, although the λ_{max} varies between individual fish because of variation in the ratio of A_1:A_2 chromophores. The rod and pineal pigments have λ_{max} at 511–535 nm and 503–518 nm, respectively (Parry et al., 2003). In all three species, no additional photopigments were identified in the pineal organ.

There is a superficial correlation between the λ_{max} of the rods and pineal pigments in the deep-sea pelagic species: as the λ_{max} of the rods shifts to shorter wavelengths, so do those of the pinealocytes. However, this trend is not maintained in the demersal *Synaphobranchus*, where the pineal pigment is some 37 nm longer than the P478 of the retina. It is somewhat paradoxical that the pineal pigment should be so long-wave shifted in such a deep-water species.

The λ_{max} of rod visual pigments of deep-sea fish tend to cluster at specific spectral locations (Dartnall and Lythgoe, 1965; Partridge et al., 1989) and the basis for this lies in specific amino acid substitutions within the opsins of the pigments that cause clearly defined spectral displacements (Hunt et al., 2001). Although the amino acid sequences of the pineal pigments reported here have not been determined, their spectral locations, close to cluster points of the deep-sea rod pigments, strongly suggest that they too will show similar mechanisms of spectral tuning.

It is not apparent why the pineal and rod photopigments in deep-sea fish should be spectrally distinct. It has long been argued that the rod pigments of deep-sea fish are spectrally tuned to match the maximum irradiance of the down-welling daylight and/or the maximum emission of the majority of bioluminescence (for recent reviews, see Douglas et al., 1998; Partridge and Cummings, 1999). However, it could also be argued that the pineal organs should similarly be spectrally tuned to be maximally sensitive to the down-welling light, but clearly both photopigments cannot be tuned to the same stimulus. Although we have no data on the transparency of the pineal 'window' in these deep-sea fish, it is unlikely that pre-receptoral filtering would change the spectral sensitivity greatly, since the 'window' is composed of a thin layer of bone and skin pigmentation, which will be spectrally relatively neutral, causing scattered and diffuse light to reach the pinealocytes.

There is sufficient daylight in the open ocean to support scotopic vision in deep-sea fish to depths perhaps as great as 1000 m (Clarke and Denton, 1962), where the intensity of sunlight is reduced by ~10^{-12} from that at the surface. However, scotopic vision, whether the ability to detect a moving object silhouetted against the down-welling background space light or to detect bioluminescence, is concerned with transient moving stimuli. The rod neural pathway has a relatively short integration time in the millisecond to second range, along with the ability to adapt to changes in the background illumination (e.g. Arshavsky et al., 2002; Lamb and Pugh, 1990). This appears not to be the case with the pineal. Pineal photoreceptors respond to relatively long-duration flashes in a similar manner to rods, with an

amplitude-coded hyperpolarization, although the response has a much slower time course, with increased latency, time to peak and recovery. However, they behave differently to prolonged illumination (50 s), with the photoresponse maintained at the same amplitude for the whole duration of the illumination (Kusmic et al., 1992). The system thus appears to be designed to integrate over a considerable time scale with a sustained signal output and without adaptation. Because of this, it would seem likely that the pineal organs of deep-sea fish may well be able to function at light levels similar to or even lower than the levels required for scotopic vision. Short transient bursts of bioluminescence will go undetected, but small diurnal changes in the intensity of the space light may be detected at depths equal to or greater than the limits of scotopic vision.

While it is conceivable that the pineal organs of mesopelagic fish are capable of capturing photons of solar origin, this is less likely for the bottom-living eel, which not only lives outside the reach of sunlight but also has no epidermal or cranial window. It is therefore highly unlikely that the well-developed pineal photoreceptors are exposed to any kind of light. Yet one of the main functions of the pineal organ, namely the secretion of melatonin, has been demonstrated in culture experiments in *S. kaupi* and some mesopelagic species (H. J. Wagner, K. Kemp, U. Mattheus and I. G. Priede, manuscript in preparation). One may therefore wonder why pineal organs with a 'complete' set of morphological features and functional visual pigments are found in these eel specimens and, perhaps, also in the other deep-sea fish. A possible reason for this paradox may lie in the ontogeny of these fish. As a general rule, deep-sea fish spend their early lives in the upper mesopelagic or even epipelagic zone. Typically, their larvae (leptocephali) are transparent, a camouflage strategy that only makes sense in a 'visual environment'. This is also true for the eel *S. kaupi*, the eggs of which develop off the southern east coast of North America, and the larvae of which drift at depths between 100 and 270 m over a period of up to two years towards the Eastern North Atlantic (Marshall, 1954). Since pineal photoreceptors in fish start to differentiate even prior to retinal ones (Ekström and Meissl, 1997; Negishi and Wagner, 1995), it is feasible to assume that pineal organs in the eel, but probably in deep-sea fish in general, develop in a photic environment and differentiate to assume a photosensory function in their early life history, similar to surface-dwelling fish. When they start their migration towards their non-photic adult habitats, the structural and functional features are retained and are not abandoned during metamorphosis. Although they are deprived of photic stimulation, their main role of melatonin synthesis is active, thereby synchronising the biological rhythms of various organ systems. Since solar light can no longer act as a zeitgeber, alternative temporal cues such as changing water current direction, not transduced by the pineal, may become effective.

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