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For whales and seals the ocean is not blue: a visual pigment loss in marine mammals*

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Abstract

Most terrestrial mammals have colour vision based on two spectrally different visual pigments located in two types of retinal cone photoreceptors, i.e. they are cone dichromats with long-to-middle-wave-sensitive (commonly green) L-cones and short-wave-sensitive (commonly blue) S-cones. With visual pigment-specific antibodies, we here demonstrate an absence of S-cones in the retinae of all whales and seals studied. The sample includes seven species of toothed whales (Odontoceti) and five species of marine carnivores (eared and earless seals). These marine mammals have only L-cones (cone monochromacy) and hence are essentially colour-blind. For comparison, the study also includes the wolf, ferret and European river otter (Carnivora) as well as the mouflon and pygmy hippopotamus (Artiodactyla), close terrestrial relatives of the seals and whales, respectively. These have a normal complement of S-cones and L-cones. The S-cone loss in marine species from two distant mammalian orders strongly argues for convergent evolution and an adaptive advantage of that trait in the marine visual environment. To us this suggests that the S-cones may have been lost in all whales and seals. However, as the spectral composition of light in clear ocean waters is increasingly blue-shifted with depth, an S-cone loss would seem particularly disadvantageous. We discuss some hypotheses to explain this paradox.

Introduction

A prerequisite for colour vision is the presence of at least two types of photoreceptors with different visual pigments (opsins) and thus different spectral sensitivities. Most terrestrial mammals are cone dichromats, having long-to-middle-wave-sensitive (L-) cones (green- to red-sensitive, depending on species) and short-wave-sensitive (S-) cones (blue- to near-UV-sensitive, depending on species); for reviews see, e.g. Jacobs (1993) and Ahnelt & Kolb (2000). In humans and some other primates alone, cone trichromacy has evolved by duplication of the L-opsin gene, resulting in red and green cones in addition to the blue cones (see, e.g. Jacobs, 1993; Nathans, 1999). Among terrestrial mammals, cone monochromacy and hence the absence of cone-based colour vision is rare. Of the many species studied, only two primates (owl monkey and bushbaby; Wikler & Rakic, 1990; Jacobs *et al*., 1993, 1996), three carnivores (common raccoon, crab-eating raccoon and kinkajou; Jacobs & Deegan, 1992; Peichl & Pohl, 2000) and a few rodents are cone monochromats lacking S-cones (summaries in Jacobs, 1993; Szél *et al*., 1996; Crognale *et al*., 1998; Peichl & Moutairou, 1998; Ahnelt & Kolb, 2000). They all have a nocturnal lifestyle, and their colour blindness probably does little harm to their fitness. Nevertheless, cone dichromacy is the rule also among nocturnal terrestrial mammals.

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Our interest in the cone types of marine mammals originated from our and colleagues' recent isolated observations that the harbour seal, ringed seal and bottlenose dolphin have no S-cone opsin (Crognale *et al*., 1998; Fasick *et al*., 1998; Peichl & Moutairou, 1998). The present survey of a larger range of marine mammals, including toothed whales, eared seals and earless seals, demonstrates the consistent absence of S-cones. Unlike the terrestrial cone monochromats listed above, many marine mammals have daylight activity phases, and the cone opsin deficit may be of more serious bearing. On the other hand, many species forage in deep water, where little light is available even on a sunny day. Furthermore, absorption and scattering of light in the water column narrows the available spectrum with increasing depth. Depending on the type of water, the wavelengths penetrating deepest may be short (clear, blue ocean water) or long (turbid, brownish coastal or estuarine water). The variety and variability of the populated habitats invite speculation about the evolutionary conditions that have led to the loss of S-cone opsins in two groups of marine mammals. A preliminary account of the pilot whale findings has been given in abstract form (Peichl & Behrmann, 1999).

Materials and methods

Animals and tissue preparation

The study includes seven species of toothed whales (order Cetacea, suborder Odontoceti), namely six marine dolphins (family Delphinidae) and one porpoise (family Phocoenidae). Among the pinnipeds (now included in the group Caniformia of the order

TABLE 1. Retinal cone populations in marine mammals and close terrestrial relatives

Taxonomic group and species*	Common name	L-cone density† (1000/mm^2)	S-cone density† (1000/mm^2)	Habitat‡
Cetacea	Whales			
Odontoceti	Toothed whales			
Delphinidae	Marine dolphins			
Delphinus delphis (1)	Common dolphin	3.7–4.6	None	Open sea & coast
Tursiops truncatus (4)	Bottlenose dolphin	3.7–4.0	None	Coast & open sea
Lagenorhynchus albirostris (1)	White-beaked dolphin	3.0–4.5	None	Coast & open sea
Grampus griseus (1)	Risso's dolphin	6.3	None	Open sea
Globicephala melaena (1)	Long-finned pilot whale	4.5–6.5	None	Open sea (& coast)
Globicephala macrorhynchus (1)	Short-finned pilot whale	6.3	None	Open sea (& coast)
Phocoenidae	Porpoises			
Phocoena phocoena (4)	Harbour porpoise	7.0–12.8	None	Coast
Artiodactyla	Even-toed ungulates			
Bovidae	Cattle, sheep, etc.			
Ovis musimon (1)	Mouflon	16.4–33.4	1.0–2.9	Terrestrial
Hippopotamidae	Hippopotamuses			
Choeropsis liberiensis (1)	Pygmy hippopotamus	5.1–7.1	0.5–1.8	Amphibious
Carnivora	Carnivores			
Otariidae	Eared seals, sealions			
Arctocephalus pusillus (1)	Australian fur seal	3.0–4.4	None	Coast
Callorhinus ursinus (1)	Northern fur seal	1.4–5.4	None	Open sea (& coast)
Otaria byronia (1)	Southern sealion	4.0–4.7	None	Coast
Phocidae	Earless seals			
Halichoerus grypus (1)	Grey seal	3.3	None	Coast
Cystophora cristata (1)	Hooded seal	5.3	None	Open sea (& coast)
Phoca vitulina (3)§	Harbour seal	7.0	None	Coast
Phoca hispida (1)§	Ringed seal	5.0–11.0	None	Coast
Canidae	Dogs, foxes, etc.			
Canis lupus (1)	Wolf	5.7–19.0	0.4–1.5	Terrestrial
Mustelidae	Weasels, otters, etc.			
Mustela putorius furo (2)	Domestic ferret	5.7–18.7	0.2–1.5	Terrestrial
Lutra lutra (2)	European river otter	2.2–5.6	0.3–0.5	Amphibious

*Species listed according to order, suborder and family; numbers of individuals studied in parenthesis; †densities sampled at selected locations; topographic density gradients and peak densities not assessed systematically; ‡includes seasonal changes of habitat (Leatherwood & Reeves, 1983; Reeves *et al*., 1992); §Phoca data from Peichl & Moutairou (1998).

Carnivora; see Ledje & Arnason, 1996) we studied three species of eared seals (family Otariidae) and two species of earless seals (family Phocidae). For comparison, we also included close terrestrial relatives of the whales and pinnipeds. The wolf (family Canidae), ferret and European river otter (family Mustelidae) are caniform carnivores (see Ledje & Arnason, 1996). The order Artiodactyla, represented here by the mouflon (ancestor of domestic sheep, family Bovidae) and the pygmy hippopotamus (family Hippopotamidae), is now considered to form a monophyletic group with the order Cetacea; in fact, hippopotamuses may be the closest terrestrial relatives of the whales (for an overview, see Luo, 2000). The amphibious river otter and hippopotamus are of particular interest because of potential adaptation to their partly aquatic lifestyle. The species and their taxonomic groupings are listed in Table 1.

Eyes were obtained from stranded animals, and animals that died or had to be euthanized in zoos and wildlife sanctuaries (for sources, see Acknowledgements). All material was obtained in compliance with the respective government regulations. The animals were adult or adolescent, all with mature retinae. For some species we compared the data of several individuals, but for most only one individual was available (Table 1). Eyes were enucleated, cut open and placed in fixative. *Post mortem* times until fixation varied from a few hours to two days. Fixation was in 4% paraformaldehyde (in physiological saline or in 0.1 M phosphate buffer, pH 7.4). Fixation times ranged between one day and several years.

Immunocytochemistry

We used antisera JH 492 (dilution 1 : 2000) and JH 455 (dilution 1 : 5000), provided by J. Nathans, to label L-cones and S-cones, respectively (Wang *et al*., 1992). Antibody OS-2 (dilution 1 : 10 000), provided by Á. Szél, was used as a further marker for S-cones (Szél *et al*., 1986, 1988, 1996). These antibodies have been shown to specifically and reliably label the respective cone types in a wide range of mammalian species across orders (summaries in Szél *et al*., 1996; Peichl & Moutairou, 1998). In our experience the antibodies also recognize the opsins in rather poorly preserved tissue, and in tissue that was kept in fixative for many years. Standard immunocytochemistry was performed on isolated pieces of retina, and immunoreactive cones were visualized via the peroxidase–antiperoxidase technique and 3,3′-diaminobenzidine (for details, see Peichl & Moutairou, 1998). Reacted tissue was then flat-mounted for analysis. For the assessment of rod densities, pieces of retina were flat embedded in Epon 812, horizontally sectioned at 1 μm and stained with toluidine blue (Peichl & Moutairou, 1998). Rod densities were corrected for tissue shrinkage during embedding.

Results

L-cones

In all terrestrial and marine species studied here (see Table 1 and Materials and Methods), immunostaining with the L-opsin-specific

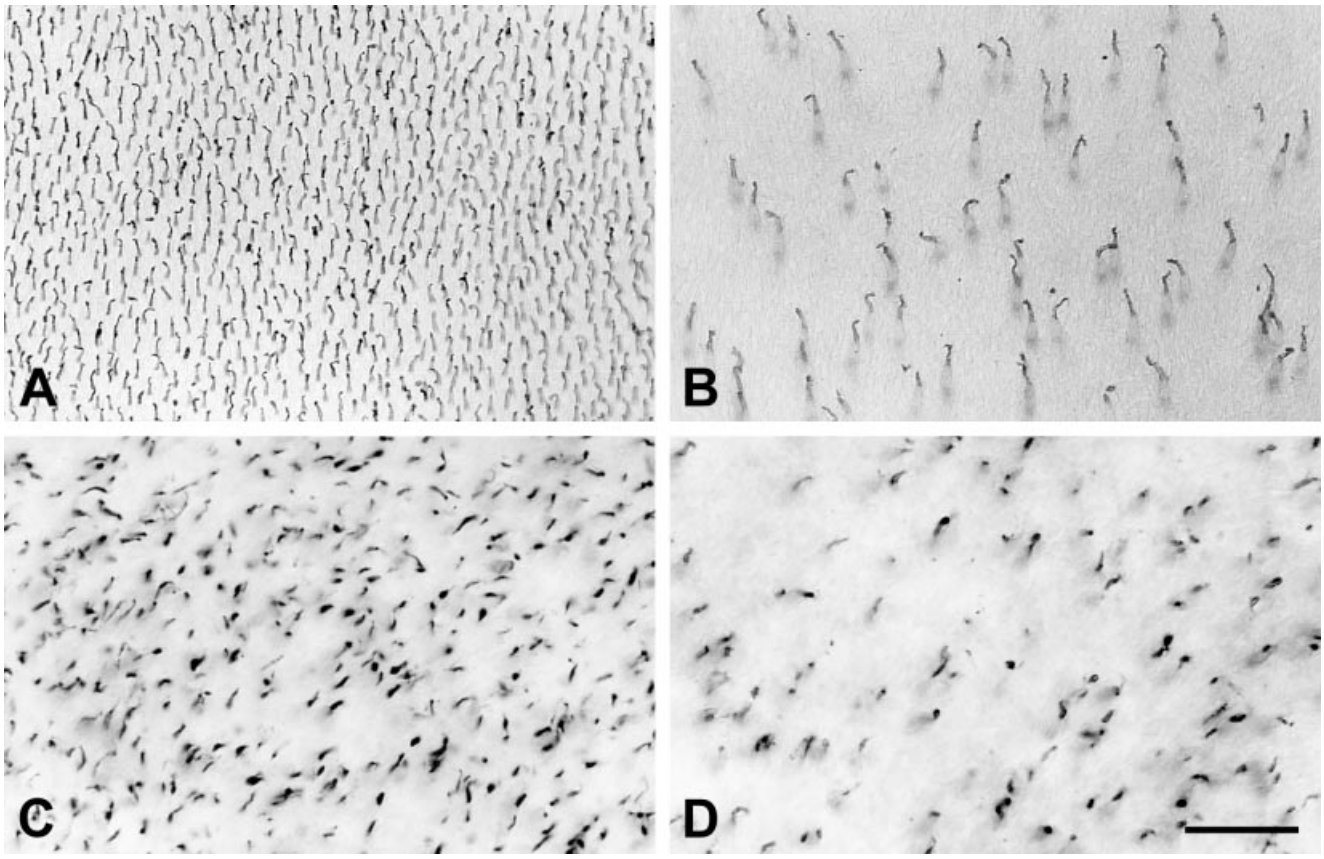

FIG. 1. Cone photoreceptor types in the retinae of artiodactyls, close terrestrial relatives of the whales. (A and B) Mouflon and (C and D) pygmy hippopotamus, immunolabeled for (A and C) L (green) cone opsin by antiserum JH 492 and (B and D) S (blue) cone opsin by antiserum JH 455. Both the terrestrial mouflon and the amphibious hippopotamus have a substantial population of L-cones and a sparser population of S-cones. Micrographs show flat views of the retinal photoreceptor layer; the opsin-containing cone outer segments are intensely labelled. In the mouflon, the S-cone antiserum in addition lightly labels the S-cone inner segments (B). This is due to a species-dependent staining property of the antisera (see also Fig. 3B, D and E). The structural preservation of the cones is very good in the mouflon and less good in the hippopotamus. The spaces between the cones are occupied by the rods. Scale bar, 50 μm (A–D).

antiserum JH 492 revealed a substantial population of cones across the retina (left panels in Figs 1–3). L-cone densities varied between species and also showed topographic density gradients across each retina. These are standard features of mammalian retinae (see, e.g. Ahnelt & Kolb, 2000). In the present survey, L-cone densities were determined at selected locations; peak densities and density gradients were not systematically assessed. Among the terrestrial species, the predominantly diurnal mouflon has the highest L-cone densities (16 400–33 400/mm^2; see Table 1) The crepuscular to nocturnal wolf and ferret have lower densities (some 5000–19 000/mm^2). Lowest densities are found in the predominantly nocturnal pygmy hippopotamus and river otter (some 5000–7000/mm^2 and 2200–5600/mm^2, respectively). The L-cone densities of most marine mammals studied here are in the range of 3000–7000/mm^2; in the harbour porpoise and ringed seal they rise above 10 000/mm^2 (see Table 1). Even though cone density maxima may have been missed in our analysis, the cone density ranges observed in the marine mammals are close to those of nocturnal terrestrial mammals.

S-cones

For the labelling of S-cones, two independent S-opsin-specific markers were used, namely antiserum JH 455 and antibody OS-2. Both markers revealed low density populations of S-cones in the terrestrial species, including the amphibious pygmy hippopotamus and river otter (right panels in Figs 1 and 3, Table 1). This is the typical mammalian pattern. Across mammals, S-cones usually represent a minority of some 10% of the cones, and their spacing commonly is as irregular as illustrated here.

Neither S-opsin marker, however, labelled any cones in any of the toothed whales and pinnipeds (right panels in Figs 2 and 3F, Table 1). This indicates that the retinae of all marine mammals studied here lack the S-opsin and hence possess no functional S-cones. We confirmed the absence of S-opsin immunoreactivity at several locations across the retina in each species, so that regional specializations can be excluded. The stained and analysed retinal pieces were large enough (up to ≈ 0.5 cm^2) to detect even sparse populations of S-cones if present. Depending on the particular circumstances of acquisition and fixation, preservation of the retinae varied considerably between animals (see Materials and methods), and we were concerned about compromised immunoreactivity due to *post mortem* damage or decomposition of the cone opsins. To control for integrity of the tissue, we used adjacent retinal pieces for the labelling of L- and S-cones across each eye. Positive results of L-cone labelling established that cone outer segments and opsins were present in an immunoreactive form. Hence the conspicuous absence of S-cones in the marine

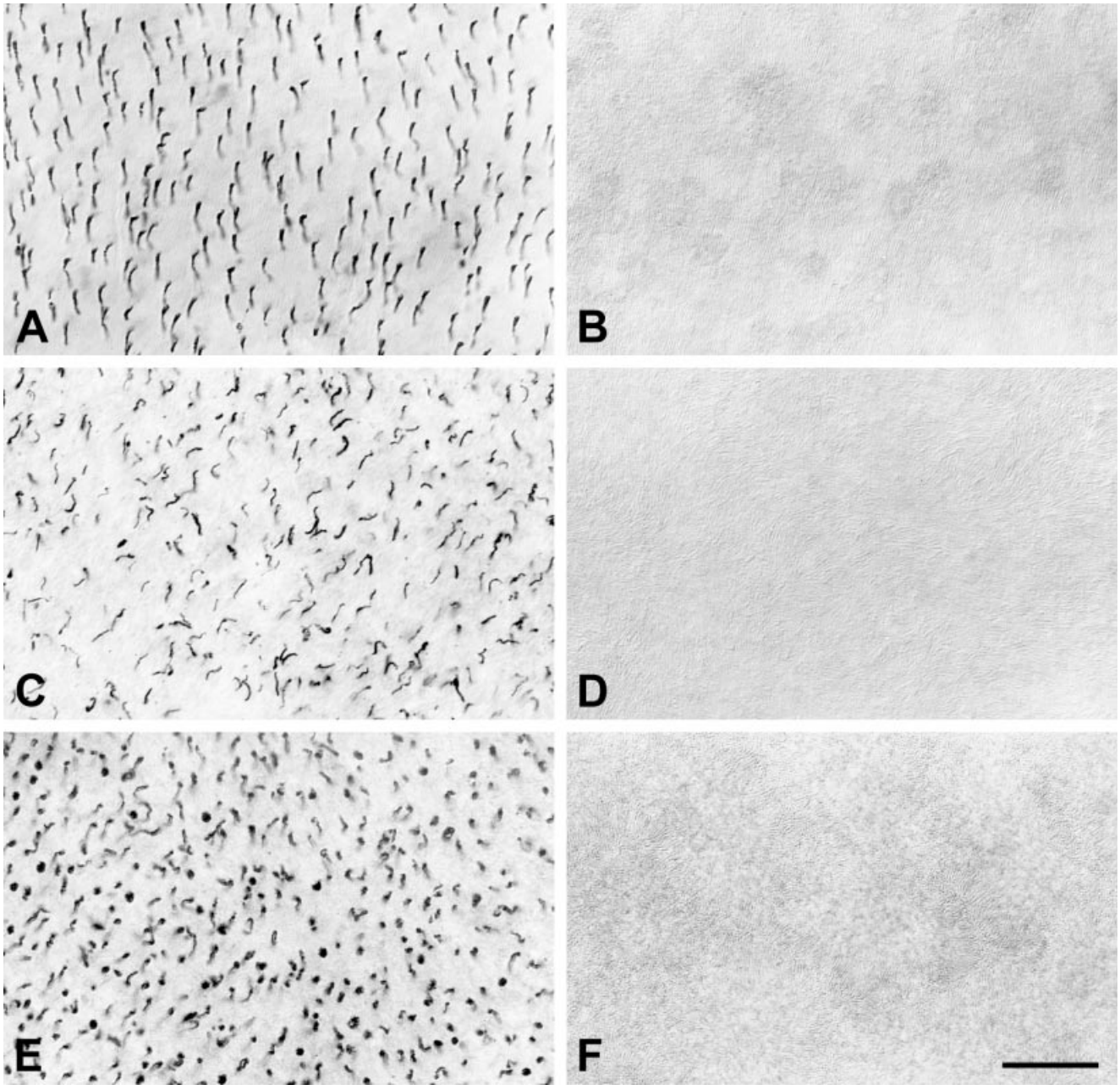

FIG. 2. Cone types in marine toothed whales (Odontoceti). Long-finned pilot whale (A and B), common dolphin (C and D) and harbour porpoise (E and F), immunolabeled for L-cones by antiserum JH 492 (left column A, C and E) and S-cones by antiserum JH 455 (right column B, D and F). All these whales possess a substantial population of L-cones but no S-cones. Tissue preservation is better in the pilot whale and harbour porpoise than in the common dolphin. Scale bar, 50 μm (A–F).

mammals is unlikely to be attributable to artefactual tissue conditions.

Rods and cone : rod ratios

In all species reported here, the cones are greatly outnumbered by the more sensitive rod photoreceptors, which are operative at low light levels. Figure 4A shows a transverse section through the retina of the long-finned pilot whale. The majority of photoreceptor somata in the outer nuclear layer are those of rods; the cone somata form a small minority. Horizontal sections at the level of the rod and cone outer segments (Fig. 4B) were used to assess the densities of rods and cones. For this the cones were labelled with the L-opsin antiserum, and the sections were counterstained with toluidine blue to reveal the rods. In some of the retinae of marine mammals, preservation of the rod outer segments was good enough to reliably count rods and calculate cone : rod ratios, another indication that poor tissue preservation cannot be the cause for the absence of S-cone opsin immunoreactivity in those retinae.

The long-finned pilot whale has some 330 000 and the short-finned pilot whale some 400 000 rods/mm^2. In both species $\approx 1.5\%$ of the photoreceptors are cones. The common dolphin has some 200 000 rods/mm^2 and $\approx 2\%$ cones. The Northern fur seal has some

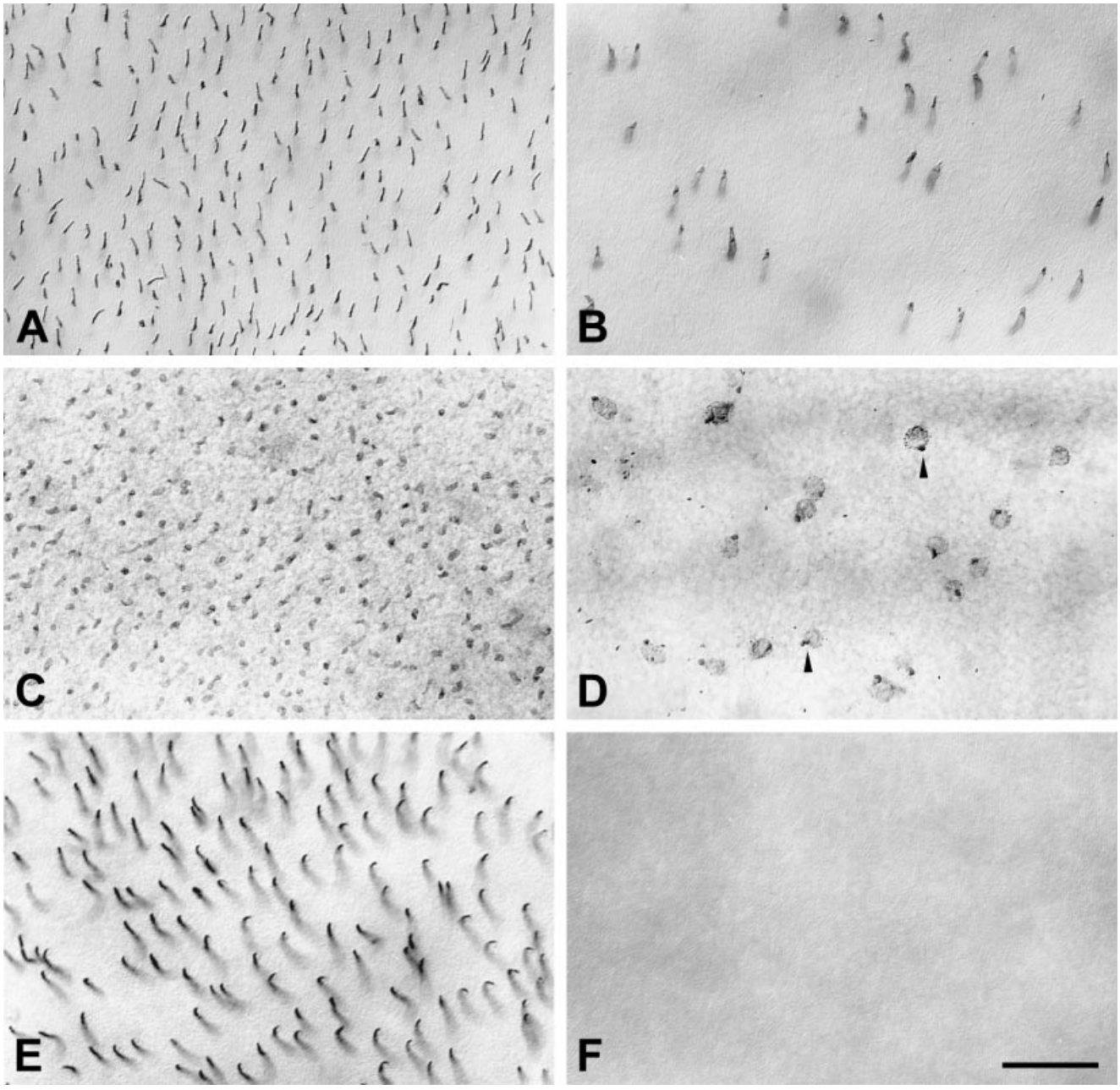

FIG. 3. Cone types in terrestrial and marine carnivores. (A and B) Wolf, (C and D) European river otter and (E and F) Northern fur seal, immunolabeled for L-cones by antiserum JH 492 (A, C and E) and S-cones by antiserum JH 455 (B, D and F). Both the terrestrial wolf and the amphibious river otter have L- and S-cones, whereas the fur seal has only L-cones. Tissue preservation is better in the wolf and fur seal than in the otter. Wolf S-cones, otter S-cones and fur seal L-cones appear larger than wolf and otter L-cones because parts of the inner segments are labelled in addition to the outer segments (see also Fig. 1B). In the otter, S-cone inner segments are particularly large and globular; the strongly labelled outer segments (two arrowed) may have been partly torn off during preparation (D). Scale bar, 50 μm (A–F).

310 000–800 000 rods/mm^2 and 0.4–1.0% cones, and the Australian fur seal some 400 000 rods/mm^2 and ≈1% cones. Earlier studies report similar ranges for the ringed seal (some 400 000–520 000 rods/mm^2, 1.5–1.8% cones; Peichl & Moutairou, 1998) and the bottlenose dolphin (some 290 000–390 000 photoreceptors/mm^2; Dawson, 1980). A comparable range of rod densities and cone proportions is found in terrestrial mammals (such as rats, mice, rabbits and cats) that are either crepuscular or polyphasic with diurnal and nocturnal activity phases. Indeed, many marine mammals are also polyphasic (Leatherwood & Reeves, 1983; Reeves *et al*., 1992). Furthermore, the observed cone : rod ratios appear well suited for visually guided foraging during daylight hours at various water depths, i.e. varying light intensities.

Discussion

We here report an absence of S-cones in seven species of toothed whales and five species of pinnipeds. Previous studies had observed an S-cone lack in two Phoca species and in the bottlenose dolphin (Crognale *et al*., 1998; Fasick *et al*., 1998; Peichl & Moutairou, 1998). The inclination then was to regard that as a rare and perhaps

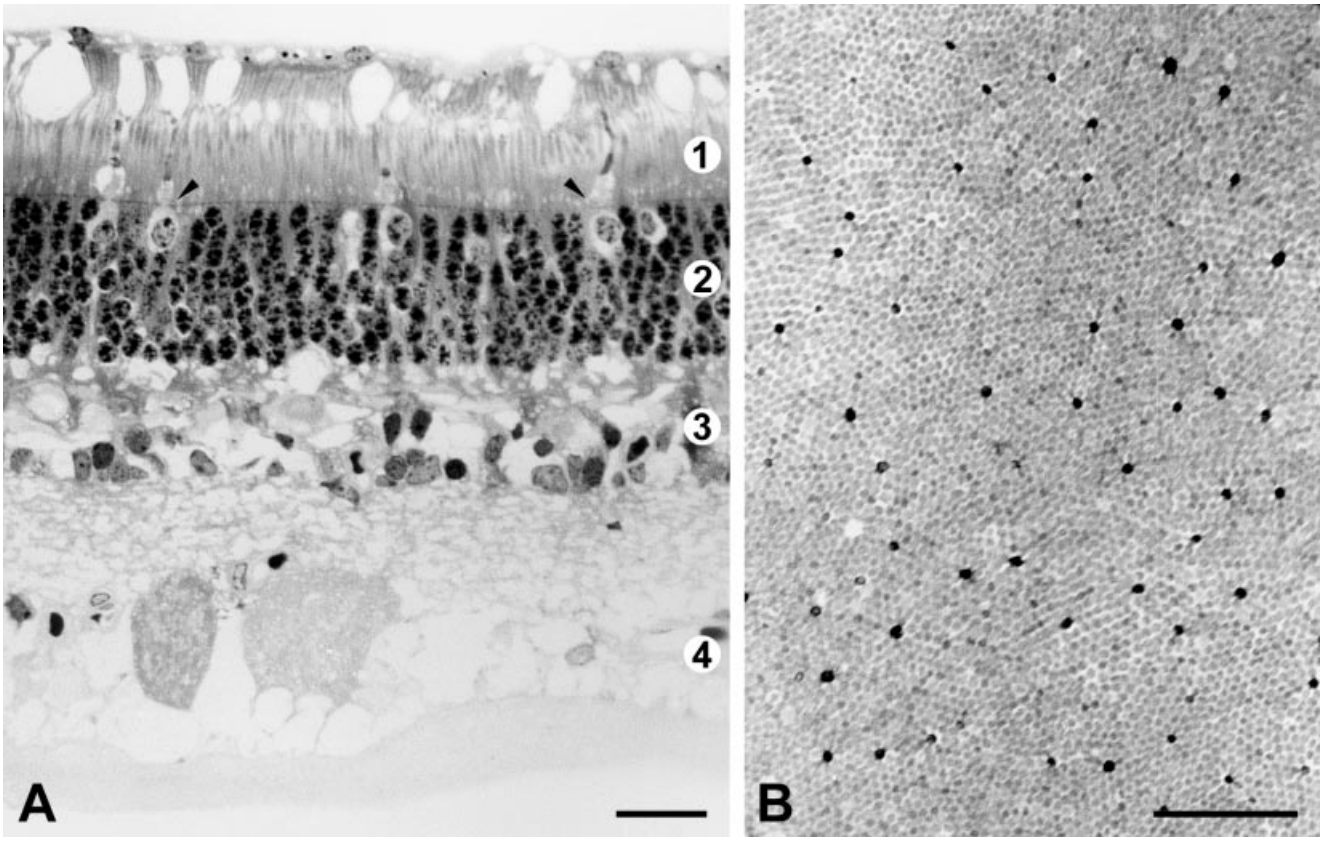

FIG. 4. Cones and rods in the long-finned pilot whale. Semithin sections of a retina immunolabeled for L-cones and counterstained with toluidine blue to reveal the rods and other retinal cells. (A) Vertical section. The outer nuclear layer contains many more rod somata (smaller, darker profiles) than cone somata (larger, lighter profiles). Two of the labelled L-cones with their fat inner segments are marked by arrowheads. 1, photoreceptor outer and inner segments; 2, outer nuclear layer; 3, inner nuclear layer; 4, ganglion cell layer with two large ganglion cells. (B) Horizontal section at the level of the rod and cone outer segments, clearly showing the intensely labelled L-cones and the densely packed rods. Scale bars, 20 μm.

insignificant defect, comparable to the absence of S-cones in a few isolated terrestrial nocturnal species (see Introduction). The present larger sample of species from several whale and pinniped families does not contain a single species that has S-cones, whereas their closest terrestrial relatives possess S-cones. This strongly favours the view that the deletion of S-cones has some adaptive advantage in the marine visual environment. In the following we address the functional implications of our findings and discuss the apparent paradox of a loss of blue cones in an underwater environment that is often represented in the literature as dominated by blue light.

Pinniped and cetacean cone monochromacy

The immunocytochemical demonstration of an S-opsin deficit in marine mammals is reliable. Both S-opsin antibodies used here have been shown to specifically label the S-cones in a wide range of mammalian species across orders, including blue-sensitive and near-UV-sensitive cones (summaries in Szél *et al*., 1996; Peichl & Moutairou, 1998). Clearly, both antibodies recognize highly conserved epitopes of the S-opsin. Specifically, we here demonstrate that these antibodies recognize the S-opsins in terrestrial species that are closely related to the marine mammals. Moreover, where a methodologically independent check has been done, it was consistent with the immunocytochemical data. In the harbour seal the immunocytochemical demonstration of an S-cone deficit (Peichl & Moutairou, 1998) has been confirmed by electrophysiological evidence (Crognale *et al*., 1998), and in the bottlenose dolphin the present finding corroborates the report of a deleterious S-opsin gene (Fasick *et al*., 1998). We conclude that all marine mammals reported here have no functional S-cones.

We assume that the labelled L-cones represent a single (green-sensitive) spectral type. This has been established for the harbour seal and bottlenose dolphin (Crognale *et al*., 1998; Fasick & Robinson, 1998; Fasick *et al*., 1998). One could speculate that some of the marine mammals may have evolved two spectral L-cone types to re-establish some form of colour vision. This would not be detectable with the antiserum JH 492 used here, which recognizes both the red and green cone opsin in trichromatic primates (Wang *et al*., 1992). However, excepting primates, no mammal has yet been found to possess two spectral types of L-opsin (reviewed in Jacobs, 1993; Nathans, 1999) or the retinal circuitry necessary to process red vs. green as a chromatic signal (see Wässle, 1999). Hence our provisional conclusion is that all whales and pinnipeds reported here are L-cone monochromats and lack cone-based colour vision. In photopic (i.e. cone-stimulating, rod-saturating) lighting conditions we suppose them to be colour-blind. Some colour discrimination may be possible by interactions of L-cones and rods in mesopic lighting conditions, where both photoreceptor types are operative. This has been demonstrated for the nocturnal New World monkey *Aotus* (owl monkey), an L-cone monochromat (Jacobs *et al*., 1993). In fact, behavioural studies have reported some capacity for colour discrimination in several pinniped species (harp seal, Lavigne & Ronald, 1972; larga (spotted) seal, Wartzok & McCormick, 1978; South

American and Australian fur seal, Busch & Dücker, 1987; California sealion, Griebel & Schmid, 1992). However, the spectral sensitivity characteristics reported in these studies are unusual. They are not consistent with the expected performance of two spectral cone types, nor can they be easily explained by the interaction of L-cones and rods. Hence these behavioural findings have met with criticism (Jacobs, 1993; Crognale *et al*., 1998). Should the pinnipeds be able to discriminate colours, then the underlying mechanisms are still obscure. Behavioural tests in the bottlenose dolphin found no indication of an ability to discriminate colours (Madsen & Herman, 1980).

Marine mammals have developed impressive nonvisual abilities for orientation and prey location, such as echolocation in odontocetes (review, Norris, 1969) and turbulence tracking in pinnipeds (Dehnhardt *et al*., 1998), but vision also plays a significant role (Dawson, 1980; Schusterman, 1981; Nachtigall, 1986; Davies *et al*., 1999), in spite of the fact that many species experience low light intensities when diving or when foraging at night. The rod dominance illustrated here, rapid dark adaptation especially in deep-diving species (Levenson & Schusterman, 1999), and reflective tapeta which increase the probability of photon catch (Dawson, 1980) are traits indicating that many marine mammals use vision primarily at low light levels, hence colour vision may be of secondary importance. As demonstrated in primates, signals from the sparse population of S-cones contribute little to visual sensitivity or spatial or temporal resolution; their primary role is in colour vision (Mollon, 1991). However, as most terrestrial nocturnal mammals have retained the S-cones and hence cone dichromacy despite little opportunity for colour vision, the loss of S-cones appears to be related to the marine environment and not to low light levels *per se*. This presents us with a paradox.

Adaptation to the marine visual environment?

Without S-cones, marine mammals have low sensitivity in the blue part of the spectrum, because they are constrained by the spectral sensitivity of the L-cones and rods, both having their maximum in the green part of the spectrum. This seems disadvantageous, because the underwater light field in the open ocean is predominantly blue-green and turns blue with increasing depth (see, e.g. Dartnall, 1975; Jerlov, 1976; Munz & McFarland, 1977; Loew & McFarland, 1990). Even if colour vision is not an issue, there should be some advantage in optimally tapping the available wavelengths for brightness discrimination (sensitivity hypothesis; see Muntz, 1975). In fact, the L-cones and rods of marine mammals are sensitive to somewhat shorter wavelengths than those of terrestrial mammals (McFarland, 1971; Lavigne & Ronald, 1975; Schusterman, 1981; Crognale *et al*., 1998; Fasick & Robinson, 1998, 2000; Fasick *et al*., 1998). The spectral sensitivity curves of their L-cones actually do extend into the blue part of the spectrum, albeit with reduced sensitivity. This blue-shift has been interpreted as an adaptation to the underwater light field. Given such adaptive effort it appears odd that the well-suited blue-sensitive cone pigment was deleted. One might argue that a sparse population of S-cones would not increase short-wave sensitivity significantly above the level already provided by the short-wave tail of the spectral sensitivity curve of the more numerous L-cones. But the loss remains enigmatic if one considers that cone vision is most effective at photopic light levels, i.e. limited depths. Here the light field in clear oceanic water retains a rather broad spectral range (Dartnall, 1975; Jerlov, 1976; Loew & McFarland, 1990) and is not so different from the atmospheric spectrum that has favoured the conservation of S-cones in most terrestrial mammals.

Despite this puzzle, the fact that the S-cones are absent in marine members of two distant mammalian orders strongly argues for convergent evolution, and hence for an adaptive advantage of that trait. As several families within each order are affected, we hypothesize that the S-opsin loss has occurred early in the evolution of each of the two groups of marine mammals, has spread throughout their radiations and is shared by all pinnipeds and whales. We assume that their terrestrial ancestors were cone dichromats, because dichromacy with L- and S-cones is widespread among extant carnivores and artiodactyls (reviews: Jacobs, 1993; Ahnelt & Kolb, 2000). This is exemplified here by the wolf, ferret, river otter, mouflon and pygmy hippopotamus.

An explanation for a spectral mismatch ('offset') between visual pigments and the underwater lightfield was proposed by Lythgoe (reviews and expansions in: Lythgoe, 1975; Muntz, 1975; Munz & McFarland, 1977; Partridge, 1990). When reflective objects (e.g. fish) are viewed horizontally at short ranges in shallow water, the downwelling light reflected by them has a shorter path length in water to the observer's eye and hence is spectrally broader than the background light that has travelled a longer horizontal distance through the water's blue spectral filter. In these circumstances, offset pigments with longer-wave sensitivity than that of the blue ambient light improve the detection of contrast between an object and the background (the 'contrast hypothesis', complementing the 'sensitivity hypothesis' reviewed above). However, in all other cases – objects darker than the background, and both bright and dark objects in deep water or at longer distances or seen from below – the advantage of offset pigments disappears and matched pigments are more efficient. A further factor degrading contrast underwater is nonimageforming 'veiling' light scattered into the visual pathway, similar to the effect of fog in terrestrial vision. Depending on the positions of observer and object, the veiling light coming from all directions may be 'bluer' than the light coming from the object, and offset pigments would improve contrast detection. At present we do not know enough about what stimulus configurations are important in the life of marine mammals.

Another line of argument considers optical reasons for L-cone monochromacy. Visual animals that dive into dark depths need highly light-sensitive eyes. This requires that the eye's aperture (pupil) is large in relation to its focal length. Such eyes have a very short depth of focus, often much shorter than the differences in focal length for the different wavelengths (longitudinal chromatic aberration). If no countermeasures are taken, only a narrow band of wavelengths can be in focus on the retina. Many fishes and a number of terrestrial vertebrates have solved the problem of chromatic defocus with multifocal lenses. Each focal length of the lens creates a well-focused image for one of the spectral cone types (Kröger *et al*., 1999; Kröger, 2000). However, such a mechanism sacrifices sensitivity and contrast for the sake of colour vision. Maximal light gathering can only be achieved with monofocal lenses. With these, all photoreceptors should have similar spectral sensitivities, because other wavelengths are out of focus. The absorption spectrum of the rod pigment is much closer to that of the L-cone pigment than to that of the S-cone pigment. Hence the S-cones may have been lost because there was no useful image in their spectral sensitivity range. To test this hypothesis, the properties of the lenses of marine mammals have to be investigated. Furthermore, it remains enigmatic why strongly nocturnal terrestrial mammals, which are faced with similar demands on sensitivity, have not chosen the same solution.

The identification of an adaptive advantage is complicated by the fact that the S-opsin deficit is present in pelagic and coastal species, in deep and shallow divers, in the fully aquatic whales and in the

amphibious seals, i.e. in species living in a variety of visual environments (Table 1). But if the S-opsin deletion is a phylogenetically old event, as suggested above, its adaptive advantage may be related to an early phase in marine mammal evolution rather than to present lifestyles. During the return from terrestrial life to the sea, the ancestors of modern whales and pinnipeds most probably initially inhabited coastal waters, which is supported by the fossil record of cetacean origin (Gingerich *et al*., 1983). In many coastal marine waters, the underwater light field is red-shifted due to blue light absorption by organic and inorganic material from land drainage ('Gelbstoffe'); even at relatively shallow depths and photopic light levels, there is little blue light left (Dartnall, 1975; Jerlov, 1976; Loew & McFarland, 1990). In such conditions, a loss of blue cones would be no significant disadvantage and might even be an economical adaptation. In addition to reduced problems with chromatic aberration (see above), the complexity of information processing is reduced in a chromatically simpler retina. This would have freed cortical capacities for other sensory capabilities, which are truly remarkable in cetaceans and pinnipeds.

Some descendant species of early cetaceans and pinnipeds have stayed in coastal waters, and for them the S-opsin loss remains useful or at least neutral. Other descendant species have later conquered the open ocean in adaptive evolutionary radiation. They might now have profited from a functional S-opsin, but they could not reverse the deleterious gene defect. What they could do and have done is to shift the spectral tuning of their L-cones and rods to shorter wavelengths by changing a few functionally important amino acids (Fasick & Robinson, 1998; 2000).

This hypothesis also addresses the genetic bases for the S-opsin losses. In the bottlenose dolphin, defects of the S-opsin gene, including a frame-shift mutation with a premature stop codon, prevent expression of a functional S-opsin (Fasick *et al*., 1998). If the S-opsin loss occurred early in evolution, one would expect an associated gene defect in all whales and pinnipeds and would predict that it is different between the two orders (analogous by convergent evolution) but similar within each order (homologous by common descent). In fact, deleterious S-opsin gene mutations in a number of toothed and baleen whales were reported at a recent meeting (Levenson *et al*., 2000; D. L. Levenson, personal communication). While these preliminary data show an identical mutation within the baleen whales, they indicate that the mutation may be different in some toothed whales. This raises the possibility that within cetaceans the loss has occurred independently in the mysticete branch and in the odontocete branch. Clearly, more genetic data are required before we can retrace the phylogeny of the S-opsin loss in marine mammals.

We now have to ask why close terrestrial amphibious relatives of the pinnipeds and cetaceans, respectively, have retained cone dichromacy. The river otter and pygmy hippopotamus are here reported to possess L- and S-cones. Their freshwater habitats are shallow compared to the coastal marine habitats conquered by the first pinnipeds and whales, and the spectral composition of the underwater light field probably is closer to the broad-band spectrum at the surface. Furthermore, their considerable terrestrial activites may have supported the conservation of colour vision, because of a spectrally richer environment. In contrast, the sandy or rocky environment encountered by pinnipeds during their terrestrial activities is significantly less colourful.

L- and S-cones also appear to be present in sirenians, the third order of fully aquatic mammals. Manatees discriminate blue vs. green in behavioural experiments (Griebel & Schmid, 1996) and most likely possess L-opsin- and S-opsin-immunoreactive cones (unpublished data quoted by Ahnelt & Kolb, 2000). The herbivorous manatees live and feed in shallow rivers, swamps and estuaries, where the available light is bright and probably spectrally broad. Their need to discriminate fresh plant material from other objects may have supported the conservation of two cone types (Ahnelt & Kolb, 2000).

A final comment concerns the fact that many fish, particularly surface living diurnal species, have several spectral cone types and good colour vision (for reviews see, e.g. Bowmaker, 1990, 1995; Partridge, 1990). This compares well with the situation in the manatee and the amphibious mammals. Interestingly, deeper-living or crepuscular marine fishes usually possess blue and green cones. By contrast, deeper-living (but still diurnal) freshwater fishes possess red cones and commonly have abandoned the S-cones, perhaps reflecting their spectral surroundings as deeper freshwater, like coastal marine water, is dominated by long-wave light (see, e.g. Partridge, 1990). This would support the suggestion that marine mammals have lost their S-cones during a phylogenetically early phase of coastal marine life.

However, the parallels are limited, because lifestyle as well as retinal organization and function differ between fishes and marine mammals. In most fishes, the rods and cones are mobile and move in and out of the focal plane as required (see, e.g. Burnside & Nagle, 1983). The same fish functionally has an all-cone retina with full colour vision when light is abundant, and a colour-blind all-rod retina when light is at a premium. In mammals, the immobile rods and cones share the same retinal surface, and if one system is favoured, the other one has to be reduced. Furthermore, many fish live at a relatively constant water depth and have adapted accordingly, with the extreme of an all-rod retina in many deep-sea fish. In contrast, the air-breathing marine mammals conduct frequent excursions between the bright surface and dark depths. Cone monochromacy may have been the price mammals paid for access to the abundance of food in deeper waters.

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Abbreviations

L-cone, long-to-middle-wave-sensitive cone; S-cone, short-wave-sensitive cone.

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