Notes

VISUAL PIGMENT SENSITIVITY IN THREE DEEP DIVING MARINE MAMMALS

Morphological examination of both pinnipeds and cetaceans indicate that they are well suited to see in dim light conditions (Walls 1942, Jamieson and Fisher 1972). Some interspecies differences in scotopic (rod-based) visual sensitivity, however, have been observed that may reflect visual adaptations for functioning in variable underwater photic environments. These differences are determined primarily by the absorptive characteristics of the visual pigments (rhodopsins) located in the rod outer segments (ROS) of the retina, although other factors such as tapetal reflectance may also be important (Walls 1942, Collins and Morton 1949).

While shallow-diving animals tend to encounter ambient light spread across much of the visual spectrum, deep-diving species experience a photic environment that is increasingly dominated by a narrow band of relatively short (blue) wavelengths of light centered around 475 nm (Kirk 1994). Avian and terrestrial mammalian rod spectral sensitivities typically fall within a narrow range of 492–506 nm (Dartnall 1962, Bowmaker et al. 1997), whereas those of fish and marine mammals are more broadly distributed (McFarland 1971, Lavigne and Ronald 1975, Lythgoe 1979). The "sensitivity hypothesis" posits that the wide range of sensitivity observed in aquatic animals is a consequence of different species maximizing sensitivity to the broader range of light conditions available in various marine and freshwater environments (Clarke 1936).

Accordingly, species active in deep marine environments are typically sensitive to the comparatively short wavelengths that predominate at depth (Lythgoe 1979). Short wavelength shifts (blue-shifts) in visual pigment sensitivity have been documented in a variety of meso- and bathypelagic fishes (Munz 1964, Knowles and Dartnall 1977a). For those marine mammals that have been studied, the limited number of deep-diving species examined tend to be more blue-shifted than shallower divers, suggesting a similar relationship between foraging depth and visual pigment sensitivity (Lythgoe and Dartnall 1970, McFarland 1971, Lavigne and Ronald 1975). California sea lions (Zalophus californianus) and harbor seals (Phoca vitulina), both shallow divers (Feldkamp et al. 1989, Boness et al. 1994), have peak sensitivities of 502 nm and 497 nm, respectively (Cresticelli 1958, Lavigne and Ronald 1975). In contrast,

southern elephant seals *(Mirounga leonina),* which are deep divers (see Le Boeuf and Laws 1994), have a blue-shifted sensitivity peak of 487 nm (Lythgoe and Dartnall 1970).

We determined the rod visual pigment sensitivities of three of the deepest-diving marine mammal species: northern elephant seal *(Mirounga angustirostris),* sperm whale *(Physeter macrocephalus)* and Cuvier's beaked whale *(Ziphius cavirostris).* Eyes were collected from one stranded sperm whale and one stranded Cuvier's beaked whale approximately six to eight hours after death. The eyes of two elephant seals were obtained from stranded individuals that died in captivity at the Marine Mammal Center, Sausalito, California. Elephant seals and sperm whales regularly dive to depths of 500 m or greater while foraging and/or travelling (see Le Boeuf and Laws 1994, Papastavrou *et al.* 1989); anecdotal evidence suggests that most beaked whale species are deep divers as well (see Berta and Sumich 1999).

The cetacean eyes were removed in bright, sunlit conditions and then wrapped in aluminum foil and placed on ice. The beaked whale eyes were frozen within one hour of removal. The eyes of the sperm whale were maintained on ice until the retinae were removed and analyzed 14 h later. For the elephant seals, enucleation was performed several minutes postmortem in dim light and eyes were frozen within one hour. Therefore, the elephant seal eyes were probably more dark-adapted than the cetacean eyes. After enucleation, samples were kept on ice and handled exclusively in dim red light to reduce further photopigment bleaching and provide conditions suitable for postmortem regeneration of rod photopigments (Knowles and Dartnall 1977*b*).

Extraction of visual pigments followed the general procedures of van Kuijik *et al.* (1991). Each extracted retina was divided in half and placed in a light-tight container containing 2 ml of 43% sucrose in tris-buffered saline (TBS) solution. Thawed retinae were manually shaken for 2 min to detach the ROS. Samples were then placed in a filter cup and masticated with a glass rod to facilitate passage of the ROS through a Nitex filter. Twenty-five nmol of 11-cis retinal was added to one of the two samples from each cetacean and incubated at 37°C with the ROS for 45 min to regenerate the visual pigment. No regeneration was performed with the elephant seal samples. The filtrate was diluted 1:2 with TBS and sedimented by centrifugation. Rinsing/washing with TBS followed by sedimentation was then performed several times to remove soluble impurities, principally hemoglobin. Following these rinses, the supernatant was poured off and 1.2 ml of freshly prepared 2% digitonin solution was added to the pellet. One sperm whale sample and one elephant seal sample were extracted with 2% lauryl maltoside instead of digitonin for comparative purposes. The pellet was vigorously resuspended and thoroughly mixed at 5-min intervals for 30 min to solubilize the ROS in the detergent. After 30 min the sample was sedimented by centrifugation. The pigment residing in the supernatant was pipetted into a spectrophotometer cuvette. The remaining cellular debris formed a pellet and was discarded.

All samples were scanned with a Shimadzu 2101 PC spectrophotometer from 700 nm to 300 nm at 1-nm intervals. Before scanning, sufficient 0.5M
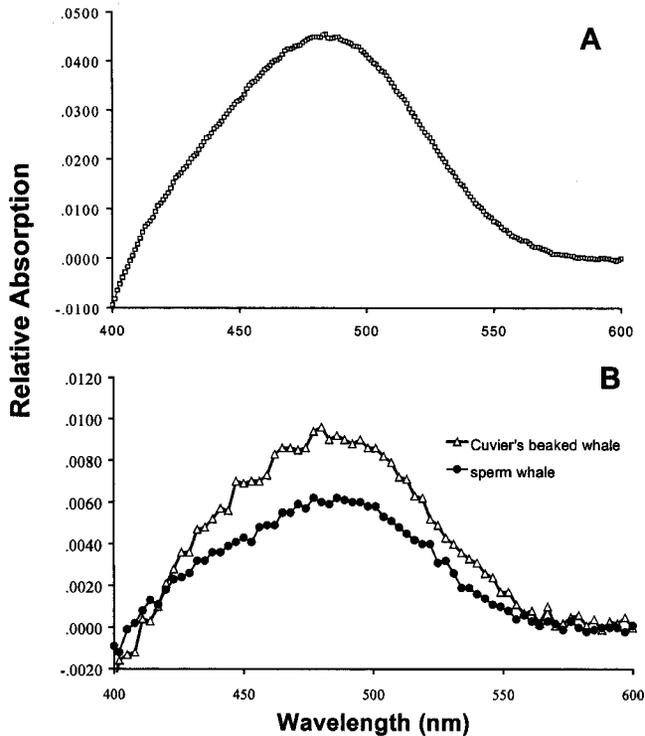
Figure 1. Spectra showing visual pigment absorption between 400 and 600 nm of retinal cells obtained from (A northern elephant seal extracted with lauryl maltoside (λ_{max} 483 nm), and (B) sperm whale and Cuvier's beaked whale extracted with digitonin (λ_{max} 483 nm for both). Absorption values determined at 1-nm intervals for each species. However, values are shown at 3-nm intervals in (B) for purposes of clarity. Differences in absorption magnitudes between (A) and (B) reflect methodological differences (see text).

NH_2OH (hydroxylamine) in TBS was added to the samples to produce a final concentration of 50 mM NH_2OH. Hydroxylamine treatment removes metastable photoproducts that can produce artifactual absorbance in the visible part of the spectrum, allowing the spectral absorbance of the extracted pigment to be more accurately determined by the spectrophotometer. After the initial absorption spectrum of the visual pigment was recorded, samples were bleached with yellow light for ~40 sec (HoyaB450 filter). Once stabilized, the absorption spectrum of the bleached pigment was recorded. The λ_{max} reported were determined directly by fitting a Gaussian function to the long-wavelength band appearing in the difference spectra; that is, the visual pigment spectrum minus the bleached pigment spectrum.

Difference spectra for individuals representing each species are presented in Figure 1. As indicated, the λ_{max} for all northern elephant seal samples was 483 nm, the λ_{max} for the two sperm whale extracts averaged 483 nm (477 and 488), and the λ_{max} for the Cuvier's beaked whale sample was also 483

nm. The elephant seal sample that was extracted with lauryl maltoside contained up to 2.3 times more pigment than those extracted with digitonin.

The reproducibility of the elephant seal λ_{max} was much better than the sperm whale samples because more pigment was present. Due to variability in the sperm whale results, and to a lesser degree in the Cuvier's beaked whale data, an association was made with poor extraction of pigment and variability. Because of this, lauryl maltoside may be a better detergent for use with eyes recovered from certain stranded marine mammal species where incomplete regeneration reduces the amount of pigment available for extraction. Digitonin was previously the most common detergent used when extracting visual pigments from retinal tissue, including those studies involving cetacean and pinniped visual pigments (Lythgoe and Dartnall 1970, McFarland 1971, Lavigne and Ronald 1975). As a natural product, digitonin is known for maintaining impurities and for batch to batch variability. Being thermally stable, it can be used with mammalian rhodopsin extracts without complications (Collins and Morton 1949, Knowles and Dartnall 1977b). Presumably the differences in extraction efficiency in this study were due to improved extraction of pigment by lauryl maltoside, although no difference was noted between the sperm whale samples. Overall, the increased yields with lauryl maltoside and successful regeneration of visual pigment with 11-cis retinal demonstrate that useful information about sensory systems may be obtained from stranded marine mammals, even hours after death. Using these techniques, we have been able to obtain visual sensitivity information from three previously untested, deep-diving mammals, using tissue samples that would likely have not yielded results with previously published methods.

Many marine mammals, including the deepest divers, appear to use vision in foraging and the extremely low levels of solar light available at depth are probably an important reason why deep divers have evolved extreme light sensitivity (e.g., Levenson and Schusterman 1999). Bioluminescent properties of certain mesopelagic organisms, including fishes, squid, and other invertebrate taxa, are also potentially important visual cues utilized by marine mammals foraging below the euphotic zone (Campagna et al. 2000). The emissions of many of these bioluminescent organisms, like that of the downwelling sunlight, are also centered near 475 nm, in the blue-green of the spectrum (Widder, in press). In view of this, the blue-shifted 483-nm rod pigment possessed by all three species examined here clearly indicates that even the deepest-diving marine mammals have rod photopigments suited for vision in their underwater foraging environments. As shown in Figure 2, these data agree well with a trend towards blue-shifted pigments in deep divers that occurs in both pinnipeds and cetaceans. It is unclear whether the short wavelength shift in sensitivity is an adaptation for detection of bioluminescence, ambient solar radiation, or other biologically significant signals that marine mammals encounter while foraging and travelling. In any case, the similar spectral absorbance of the pigments of the species examined here to those of other deep-diving marine mammals and some mesopelegic fish supports the hypothesis that these pigments have arisen several times through convergent
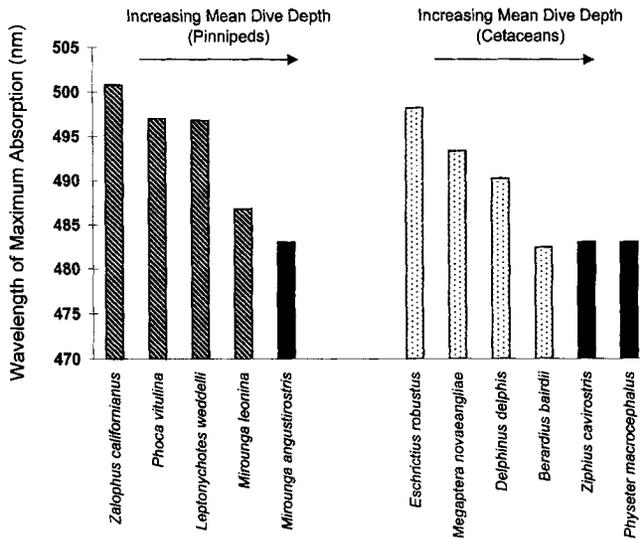
Figure 2. Adjusted[1] wavelength of maximum absorption values for rhodopsins of selected marine mammals shown in approximate order of increasing mean dive depth. Pinniped values indicated by striped bars (Lavigne *et al.* 1977). Cetacean values indicated by stipled bars (McFarland 1971). Black bars indicate data from this study. Note trend towards blue-shifted sensitivity relative to deep-diving behavior found in both pinnipeds and cetaceans (see Berta and Sumich 1999 for discussion of diving behavior).

[1] Dartnall's method of partial bleaching (Dartnall and Lythgoe 1965) has been used to estimate absorption maxima (λ_{max}) and pigment homogeniety. The λ_{max} estimates presented here were obtained by taking the difference spectra. For purposes of comparison, other previously published values were increased by 1.3 nm to compensate for previous conversions to Dartnall's nomogram (as in McFarland 1971).

evolution to maximize visual sensitivity in the deep sea. Moreover, the clear relationship between diving depth and spectral sensitivity strongly suggests that visual function is important for even the deepest-diving pinnipeds and cetaceans, as their pigments have been modified from those of shallow-diving and terrestrial species.

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LONG RANGE MOVEMENTS OF A BLUE WHALE (*BALAENOPTERA MUSCULUS*) BETWEEN THE GULF OF ST. LAWRENCE AND WEST GREENLAND

Jonsgård (1966) reviewed the distribution of the blue whale (*Balaenoptera musculus*) in the North Atlantic. In the western North Atlantic the species is known from West Greenland (Davis Strait), southwest Iceland (Denmark Strait) (Ingebrigtsen 1929, Jonsgård 1955), and eastern Canada (Jonsgård 1955, 1966). Little is known of the winter distribution but occasional sightings/strandings have been reported from as far south as the Caribbean (Harmer 1923) and the Gulf of Mexico (Baughman 1946, Lowery 1974).

Since 1980, Sears *et al.* (1990) have reported regular sightings of blue whales in the Gulf of St. Lawrence, while ice-related entrapments have occurred along the southern coast of Newfoundland during late winter–early spring (*e.g.,* Mitchell 1977, Desbrosse and Etcheberry 1987, Seton 1995). Blue whales enter the Gulf of St. Lawrence from southern Newfoundland (Cabot Strait) in March, as the ice breaks up (Mitchell 1977) and are sighted from April onward along the Quebec North Shore from the St. Lawrence Estuary to the Strait of Belle Isle (Sears *et al.* 1990). They are regularly seen in the Gulf until December, and some individuals remain until the third week of January (RS, unpublished data). Since 1992 one of the authors (RS) and observers working off