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Biochemical Characteristics of the Pigmentation of Mesopelagic Fish Lenses

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Abstract. We analyzed the biochemical, anatomical, and spectrophotometric characteristics of lens pigmentation in representatives of two mesopelagic fish families, the Opisthoproctidae and the Scopelarchidae. In small and large specimens of the opisthoproctid *Macropinna microstoma* and in the larval scopelarchid *Benthalbella infans,* the lens pigment was present in all layers of the lens as a freely diffusable chromophore. In contrast, the lenses in the adult specimens of the scopelarchid *Benthalbella dentata,* which have lenses averaging 6.7 mm in diameter, had a 2.4 mm-diameter pigmentless core. In this species, the chromophore was bound to one of the major structural proteins of the lens, gamma crystallin. Because the lens grows by the layering of new cells over older ones, such a pattern in *B. dentata* suggests that the lens pigment is not present in larvae of this species. The chromophores of all specimens were characterized by a single broad peak in the shorter-wavelength blue, near-UV portion of the spectrum.

Introduction

Pigmentation in the tissues of vertebrate and invertebrate visual systems is a common phenomenon, expressing itself most often in the cornea, lens, and retinal-associated cells (Muntz, 1972; Heinermann, 1984). In the lens, pigmentation occurs: (1) in many diurnally active animals (Walls and Judd, 1933; Cooper and Robson 1969a; Muntz, 1974), including both terrestrial and shallow water aquatic species; (2) as an age-related yellowing of the lens, which occurs in vertebrate species of long lifespan, independent of habitat (Cooper and Robson, 1969b; Zigman, 1971; Villermet and Weale, 1972); and (3) as a rare occurrence among animals in the mesopelagic zones of the ocean (from about 100–1000 m depth) (see for review, Heinermann, 1984).

The three types of lens pigmentation differ in their biochemical characteristics, their ontogenic pattern in the animal, and perhaps also in their function. In diurnally active animals, the lens pigment is present at all stages in the animal's life history as a soluble component of the lens cells; *i.e.,* it is freely diffusible through the numerous gap junctions between adjacent cells in the lens. Lenticular coloration in these species is thought to increase visual acuity by reducing chromatic aberration produced by the shorter wavelengths. In age-related pigmentation of the lens, while young lenses may be nearly colorless, yellowing intensifies with age. The older central portion of the lens is yellower than the younger, more peripheral lens layers (Mellerio, 1987). This age-associated yellowing of the lens probably results from accummulated, UV-induced modifications of the lens structural proteins, or crystallins, and may have no adaptive value (Zigman, 1971). The chromophores that occur in the lenses of diurnal animals and in the aging lens have simple spectra with absorbances in the short-wavelength blue and near-UV region of the spectrum. A recent study of the spectroscopy, ontogeny, and biochemistry of lens pigmentation in deep-sea hatchetfishes (Family Sternoptychidae) revealed a completely different pattern of pigmentation from those patterns reported for the more common types of lens pigmentation (McFall-Ngai *et al.,* 1986). Lens pigmentation in the deep-sea hatchetfish has an abrupt, age-dependent onset. It begins when the fish is about 38 mm SL (maximum SL about = 90 mm), and is restricted

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Table I

Mesopelagic fish species analyzed in the present study

Family	Species	Capture site	n	SL range (mm)	Orbit diameters (mm)	Lens diameters (mm)
Opisthoproctidae	*Macropinna microstoma*	SCB[1]	6	37–140	6.3–18.5	3.7–10.7
	Dolichopteryx sp.	SCB	1	70	ND[3]	~2
Scopelarchidae	*Benthalbella dentata*	SCB	2	202, 218	7.9, 8.1	6.6, 6.8
	B. infans	PRT[2]	2	39, 55	2.0, 2.8	1.4, 2.0
	Scopelarchus sp.	SCB	1	121	10.4	5.7

[1] San Clemente Basin.
[2] Puerto Rican Trench.
[3] Not determined.

to the peripheral cells of the lens that are laid down after the 38 mm stage. Further, the chromophore has a complex spectrum resembling that of carotenoid pigments and is associated specifically with alpha crystallin. Thus, it cannot diffuse between the cells. The biological significance of the pigment is unclear, although it certainly affects the optical function of the lens as a component of the visual system.

We analyzed the biochemical and spectrophotometric properties of the lens pigmentation in two other fish families, the Opisthoproctidae and the Scopelarchidae, that also occur in the mesopelagic zone. Our results indicate that representative species of two families have patterns of lens pigmentation unlike not only the type described in diurnally active animals and the age-related lens coloration, but also unlike those of the deep-sea hatchetfish. These differing patterns suggest that the lens pigmentation is a character that has arisen independently a number of times among the mesopelagic fishes.

Materials and Methods

Animals

Fishes were collected in the Eastern Pacific from the San Clemente Basin (Southern California) during cruises of the RV Velero IV in February and July 1984 and August 1985, and from the Caribbean Sea in the Puerto Rican Trench during a cruise of the RV Knorr in December 1985. The methods of capture and handling of the fishes were as previously described (McFall-Ngai *et al.*, 1986). The species analyzed in the present paper are: (1) Family Opisthoproctidae, *Macropinna microstoma* Chapman and *Dolichopteryx* sp.; (2) Family Scopelarchidae, *Benthalbella dentata* (Chapman), *B. infans* Zugmayer, and *Scopelarchus* sp. (Table I). All specimens were adults except those of *B. infans*, which were translucent larval fish. The lenses were removed through a superficial cut made around the edge of the cornea with a scalpel.

To compare the pigmentation patterns of these species with those of diurnally active animals and those with age-related lens pigmentation, we used lenses of *Spermophilus beecheyi*, the California ground squirrel, and normal human lenses, respectively. We obtained specimens of *S. beecheyi* from the laboratory of Dr. S. Fisher at the University of California, Santa Barbara; normal human lenses were from donors to the Jules Stein Eye Institute eye bank.

Spectral analyses of lenses and lens extracts

To determine the spectral characteristics of whole lenses, freshly dissected lenses were positioned in a quartz cuvette of 1-cm pathlength along the axis of the beam of a Shimadzu UV-160 spectrophotometer (for details, see McFall-Ngai *et al.*, 1986). Briefly, lenses were placed in holders that fit snuggly into the cuvette. The holders consisted of two pieces of black lucite with hemispherical depressions of various sizes to hold lenses of differing diameters. One-mm holes were drilled through the lucite at the center of the depressions to permit transmission of the beam. This configuration insured that the beam would go directly through the center of the lens. The lens was surrounded by fish ringer's solution to prevent optical aberrations. Spectral absorbances of a given whole lens were determined between 300 and 800 nm. The spectra of a given intact lens pair were then compared with extracts of the same lenses. One whole lens of each pair was homogenized and other lens was dissected into core and periphery, which were homogenized separately. Lens tissue was homogenized with a Wheaton tissue homogenizer in 50 m*M* sodium phosphate buffer (pH 7.4 at 20°C). The homogenate was spun at 4°C in 1.5 ml eppendorf tubes at 14,000 × *g* for 15 minutes in an Eppendorf 5414 tabletop centrifuge to pellet the insoluble material. The color of the pellet was noted and the spectrum of the supernatant fluid from each extract was determined between 300 and 800 nm. Lens extracts from

other specimens of the same species were frozen to determine the effect of freezing on the spectral characteristics.

During cruises in which a spectrophotometer was not available to measure whole lens spectra, lenses were removed from the eyes of specimens within one hour of trawl recovery, placed in vials with enough fish Ringers solution to cover them, and immediately frozen at −20°C. Within 1 to 2 weeks of collection, they were placed in a −80°C freezer until use. When analyzed, these lenses were thawed and extracted in the same manner as fresh lenses. The spectral absorbance of each supernatant fluid was determined from 300–800 nm using a Kontron Uvicon or a Shimadzu UV-160 spectrophotometer.

Biochemical analyses

Gel filtration of soluble extracts from frozen lens samples was performed at room temperature on a Pharmacia FPLC system with a Pharmacia HR-6 column in 20 m*M* Tris buffer with 0.1 *M* NaCl (pH 7.9 at 20°C) at a flow rate of 1 ml/min. The absorbance of each resulting fraction was determined between 300 and 800 nm on the Shimadzu UV-160. Samples in which the pigment occurred in the lowest molecular weight fraction resolved by the HR-6 column were subjected to further gel filtration on a Sephadex G-25 column (2.5 × 5 cm), at a flow rate of 1 ml/min with the same elution buffer as used on the FPLC HR-6 column. Fractions obtained by gel filtration were subjected to polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS-PAGE) (Laemmli, 1970). Protein concentrations were determined spectrophotometrically using the difference in absorbance between 235 and 280 nm (Whittaker and Granum, 1980).

Results

General description of the visual system

All mesopelagic fish species analyzed in the present study have tubular, upwardly directed eyes. Such eyes, which are characteristic of certain families of fishes that occur in the lower portions of the mesopelagic zone (500–1000 m), are generally large relative to body size of the fish and have relatively large lenses, a much-reduced iris, and two photoreceptive areas—the ventral, main retina and the medial, accessory retina (Munk, 1966). Both visual and spectrophotometric analyses revealed yellow coloration in the lenses of *Macropinna microstoma, Benthalbella dentata,* and *B. infans,* but not in the lenses of *Dolichopteryx* sp. and *Scopelarchus* sp. Thus, not all tubular eyes have yellow lenses.

Table II

Peak wavelengths (λ_{max}) of the lens pigment of Macropinna microstoma *under different experimental conditions*

Specimen standard length (mm)	λ_{max} Intact lens	λ_{max} Lens total-soluble extract	λ_{max} Pigment-bearing gel filtration fraction
37	387	371	ND[1]
115	396	380	ND[1]
126	398	386	371

[1] Not determined.

Spectral characteristics of the lens pigments

Whole lenses. Macropinna microstoma was the only species with pigmented lenses that was obtained when a spectrophotometer was available for fresh whole lens spectral analyses. To determine whether the absorbance characteristics of the *M. microstoma* lens pigment changes during extraction, we compared the spectrum of an intact fresh lens to that of: (1) the total soluble lens extract and, (2) the partially purified pigment fraction of the total soluble extract, which had been obtained by gel filtration. Although the overall shapes of all of the spectra were the same, showing a single broad peak, this peak was shifted toward shorter wavelengths in the intact lenses of smaller specimens (Table II). In addition, the extracts and partially purified fractions of a given *M. microstoma* lens were also shifted several nanometers toward the shorter wavelengths relative to the intact lens spectrum of that same lens (Table II). Extracts and purified fractions that had been frozen had the same spectra as those that had not been frozen. Prolonged freezing did not affect pigment intensity or spectral quality.

Lens extracts

In the deep-sea fish and ground squirrel lenses, all the pigment was in the total soluble extract; the pellet of the insoluble lens material was white. However, in human lenses, because the pigment is associated with all proteins and these proteins gradually become insoluble with age, both pellet and supernatant fluid were a light yellow in older lenses.

The peak wavelengths of lens pigment absorbance, when corrected for scatter, were in the short-wavelength blue and long-wavelength ultraviolet portions of the spectrum in all fishes with yellow lenses (Fig. 1). The uncolored lenses of the opisthoproctid *Dolichopteryx* sp. and the scopelarchid *Scopelarchus* sp. showed no absorbance in these regions of the spectrum. In contrast to the complex, multipeaked spectrum of the hatchetfishes,

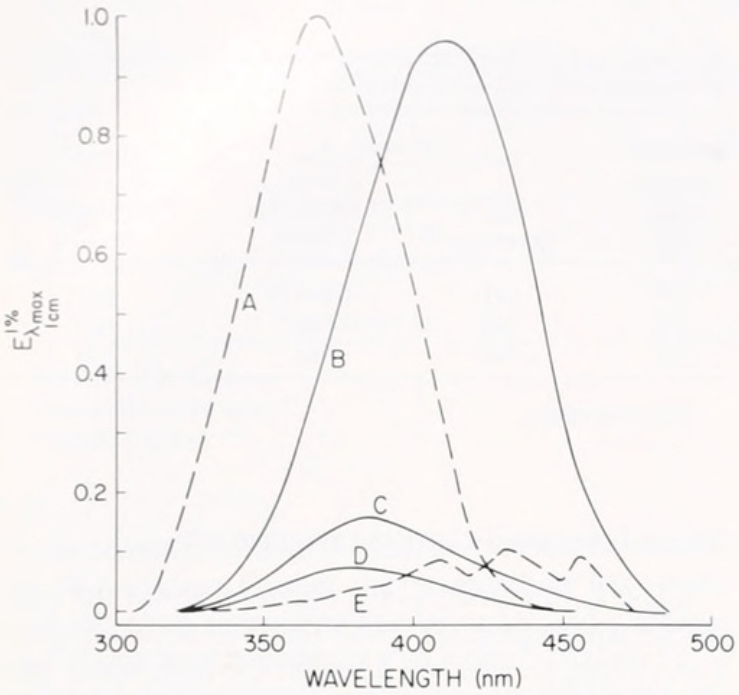

Figure 1. Comparison of the pigment intensity among the animals analyzed. A = *Spermophilus beecheyi;* B = *Benthalbella dentata;* C = *B. infans;* D = *Macropinna microstoma;* E = *Argyropelecus affinis.* Absorbances determined for a 1% solution of protein.

Argyropelecus ssp. (McFall-Ngai *et al.,* 1986), the absorbances of the lens pigments of *Benthalbella dentata, B. infans,* and, as mentioned above, *Macropinna microstoma* occur as single broad peaks, similar to that of the ground squirrel (Fig. 1). Although whole lenses of *Benthalbella* spp. were not available for spectral analysis, the whole lens extracts did not have a spectral peak markedly different from the purified fractions containing the chromophore. The age-related coloration of the human lens results in the elimination of wavelengths shorter than 350 nm. Because the scatter of the lens proteins themselves at this wavelength is so marked, no scatter-corrected pigment spectrum could be obtained for comparison.

Pigment distributions and intensities

The lenses of each species with yellow coloration were dissected to reveal pigment intensity in various layers of the lens. In the opisthoproctid, *Macropinna microstoma,* and the scopelarchid, *Benthalbella infans,* the pigment occurs in all layers of the lens. In contrast, in the scopelarchid, *B. dentata,* pigment is absent from portions of the lens core, or nucleus, measuring approximately 2.3–2.4 mm in diameter. In the 6.6 and 6.8 mm lenses of *B. dentata,* this unpigmented portion represents less than 5% of the total lens volume.

With the naked eye, *Benthalbella dentata* lens pigmentation appears more intense than that of either *B. infans* or *M. microstoma.* To quantify the intensity and compare it among the species with pigmented lenses, we determined the absorbance of the pigment at the peak wavelength for a 1% solution of the protein ($E^{1\%}_{\lambda max\ 1cm}$) (Table III). The intensity of the *B. dentata* lens pigment was similar to that of the ground squirrel, and was approximately 6–14 times stronger than that of the other mesopelagic fish lenses. The intensity differences can also be noted in the spectra (Fig. 1), when the spectra are normalized to protein concentration.

Biochemical analyses

The lenses of fishes are protein-rich tissues, about 50% of which is soluble protein with the remainder being mostly water (De Jong, 1981). Most of the soluble proteins are structural proteins called crystallins, which in fishes are of three primary types: alpha, beta, and gamma crystallins. In size exclusion chromatography (Fig. 2) of total soluble lens extracts, alpha crystallin, an 800 Kdal multimer, elutes first, followed by the beta crystallins, which are several oligomeric proteins ranging from approximately 50–150 Kdal. The gamma crystallins, monomers which average 20 Kdal, are the last proteins to elute. A non-protein, low molecular weight fraction appears last and includes absorbing materials around 1.5 Kdal, such as peptides and nucleic acids.

To determine whether the lens pigment is protein-associated in the opisthoproctid and scopelarchid species considered here, as in *Argyropelecus* spp. (McFall-Ngai *et al.,* 1986), we chromatographed the pigmented lenses, collected fractions, and measured the absorbances of the resultant fractions. Lenses of *Spermophilus beecheyi,* the pigment of which is known to be freely diffusable (*i.e.,* soluble but not associated with any protein species; Cooper and Robson, 1969a) were also chromatographed for comparison. The lens pigments of the opisthoproctid *Macropinna microstoma* and the scopelarchid *Benthalbella infans* eluted with the low molecular weight fraction in a similar manner to the lens pigment of *S. beecheyi.* Therefore, they were not protein associated in these fishes. In contrast, the lens pigment of *B. dentata* eluted with the gamma crystallin fraction (Fig. 2). SDS-PAGE of the pigment-containing fraction of *B. dentata* showed bands at an apparent molecular weight of approximately 20,000, characteristic of gamma crystallin, suggesting that the pigment is protein associated.

Discussion

The present study shows that the lens chromophores of the three species of mesopelagic fishes described herein differ in one or more of the following characteristics: (1) the appearance during ontogeny; (2) the biochemical as-

Table III

Spectral characteristics of three types of lens pigmentation

Class/type of lens pigmentation	Taxonomic affiliation	Species	λ_{max} of Chromophore	1% $E\lambda_{max}$ 1 cm
Deep-sea Teleosts	Order—Salmoniformes Family—Opisthoproctidae	*Macropinna microstoma* (70 mm SL)	377	.07
	Order—Aulopiformes Family—Scopelarchidae	*Benthalbella dentata* (202 mm SL)	412	.96
		Benthalbella infans (55 mm)	385	.16
	Order—Stomiiformes Family—Sternoptychidae	*Argyropelecus affinis*[1]	432	.10
Diurnal Species	Class—Mammalia Order—Rodentia	*Spermophilus beecheyi*	366	.99

[1] Strongest peak absorbance, McFall-Ngai *et al.* (1986).

sociation with a lens structural protein (crystallin); and, (3) the spectral quality.

The lens pigmentation pattern of one species, *Benthalbella dentata,* showed some similarities to the pattern previously described in the deep-sea hatchetfish, *Argyropelecus affinis* (McFall-Ngai *et al.,* 1986). Individuals of *A. affinis* smaller than 38 mm, with lenses ≤ 2.4 mm in diameter, had no lens pigment, while in those larger than 38 mm, newly synthesized, peripheral cells containing a chromophore bound to alpha crystallin surround the pigmentless core. Although small specimens of *B. dentata* were not available, the lenses of larger specimens also had a 2.4 mm core devoid of pigment. Furthermore, the pigment in the peripheral lens cells was bound to gamma crystallin, a molecule, like alpha crystallin, too large to traverse the cellular gap junctions. Thus, the characteristic distribution of lens pigment in *B. dentata* lenses suggests a similar ontogenic pattern to that of *A. affinis.* The presence of a colorless lens core and a chromophore bound to a crystallin are features of these two mesopelagic species not reported in any other pigmented eye lenses.

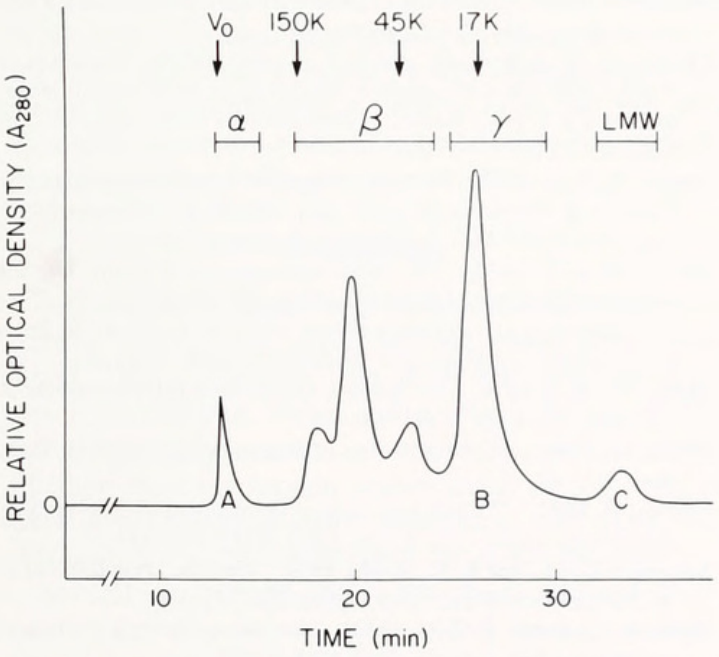

Figure 2. Typical gel filtration profile of fish lens crystallin proteins. The peak designated A, containing alpha crystallin, is the peak with which the chromophore of the *Argyropelecus affinis* lens elutes. The chromophore of *Benthalbella dentata* elutes with the gamma crystallins with the peak designated B. The chromophores of *B. infans* and *Macropinna microstoma* elute with the low molecular weight (LMW) fraction, designated C. The values across the top of the chromatogram [V_0 (void volume), 150K, 45K, and 17K] are molecular weight standards for the gel filtration column.

Our data suggest that the lens pigmentation of *B. infans,* for which only larval specimens were available, is ontogenetically and biochemically distinct from that described above for its congenor, *B. dentata.* In contrast with the unpigmented core of *B. dentata* lenses, the lenses of *B. infans* larvae, which were all 2.0 mm or less in diameter and destined to become the core of the adult lens, had an even distribution of pigment. These differences in lens pigmentation can only be reconciled within a single ontogenetic and biochemical pattern if, during the transition between larval and adult life, the pigment of *B. infans* is transported from the core to the periphery, and only there bound to gamma crystallin. In addition, the distinct spectra of the lens chromophores from these two species indicate either that the pigments are structurally distinct, or that the two species share a common pigment whose spectrum is altered in *B. dentata* by its association with the gamma crystallin.

Finally, the characteristics of lens coloration in the opistoproctid, *Micropinna microstoma,* were most like that of *Benthalbella infans.* In all specimens of *M. microstoma,* the lens pigment was a freely diffusible molecular species that was evenly distributed throughout the lens. However, since no small specimens of the opistoproctid were available, we could not determine whether the pigment is produced continuously during lens growth (as appears to be the case for larval *B. infans*) or, alternatively, is synthesized late in lens development and diffuses into the previously unpigmented core regions. Regardless of the possible similarity of ontogenic appearance, the spectral characteristics of the lens chromophores of *M. microstoma* and *B. infans* suggest that the pigments are different.

The present study suggests that lens pigmentation has arisen a number of times in mesopelagic fishes. However, the biological function of such an adaptation remains unclear. Lens pigmentation does not correlate with the presence or absence of tubular eyes; *i.e.,* not all tubular eyes have yellow lenses (present study), while some yellow lenses occur in fishes with non-tubular eyes (Somiya, 1982). In addition, while the perception of intra- or interspecifically produced luminescence may be enhanced by the presence of yellow lenses in some species (Muntz, 1976), intraspecific communication as a function cannot be deduced from the occurrence of luminescence and lens pigmentation in the mesopelagic fishes studied thus far. For example, in *Benthalbella infans,* light organs are an adult character (Merrett *et al.,* 1973), but *B. dentata* and *Macropinna microstoma* have strong lens pigmentation and are not luminous. Furthermore, depending on the species, the lens may (Muntz, 1976) or may not (McFall-Ngai *et al.,* 1986) change the peak wavelength of visual pigment sensitivity. The only conclusion that can be made for all pigmented lenses of mesopelagic fishes is that the pigmented lens would filter out a portion of the downwelling light that impinges on the short-wavelength portion of the visual pigment curve. Thus, the function of the pigment may be similar to that suggested for the ground squirrel and other diurnal animals (Lythgoe, 1979)—to decrease chromatic aberration and increase acuity by the elimination of the higher energy wavelengths of the environment.

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Literature Cited

Cooper, G. F., and J. G. Robson. 1969a. The yellow colour of the lens of the grey squirrel. *J. Physiol.* **203:** 403–410.

Cooper, G. F., and J. G. Robson. 1969b. The yellow color of the lens of man and other primates. *J. Physiol.* **203:** 411–417.

De Jong, W. W. 1981. Evolution of lens and crystallins. Pp. 221–278 in *Molecular and Cellular Biology of the Eye Lens,* H. Bloemendal, ed. John Wiley and Sons, New York.

Heinermann, P. H. 1984. Yellow intraocular filters in fishes. *Exp. Biol.* **43:** 127–147.

Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacterial phage T4. *Nature* **227:** 680–685.

Lythgoe, J. N. 1979. *The Ecology of Vision.* Clarendon Press, Oxford. xii + 244 pp.

McFall-Ngai, M., F. Crescitelli, J. Childress, and J. Horwitz. 1986. Patterns of pigmentation in the eye lens of the deep-sea hatchetfish, *Argyropelecus affinis* Garman. *J. Comp. Physiol. A.* **159:** 791–800.

Mellerio, J. 1987. Yellowing of the human lens: nuclear and cortical contributions. *Vis. Res.* **27:** 1581–1587.

Merrett, N. R., J. Badcock, and P. J. Herring. 1973. The status of *Benthalbella infans* (Pisces:Myctophoidei), its development bioluminescence, general biology and distribution in the eastern North Atlantic. *J. Zool. Lond.* **170:** 1–48.

Muntz, W. R. A. 1972. Inert absorbing and reflecting pigments. Pp. 529–565 in *Photochemistry of Vision. Handbook of Sensory Physiology, Vol. VII/1,* H. J. A. Dartnell, ed. Springer, Berlin.

Muntz, W. R. A. 1974. The visual consequences of yellow filtering pigments in the eyes of fishes occupying different habitats. Pp. 271–287 in *Light as an Ecological Factor, Vol II,* G. C. Evans, R. Bainbridge, and O. Rockham, eds. Blackwell Scientific, Oxford.

Muntz, W. R. A. 1976. On yellow lenses in mesopelagic animals. *J. Mar. Biol. Assoc. U. K.* **56:** 963–976.

Munk, O. 1966. Ocular anatomy of some deep-sea teleosts. *Dana Rep.* **70:** 1–62.

Somiya, H. 1982. 'Yellow lens' eyes of the stomiatoid deep-sea fish, *Malacosteus niger. Proc. R. Soc. Lond. B* **215:** 481–489.

Villermet, G. M., and R. A. Weale. 1972. Age, the crystallin lens of the rudd and visual pigments. *Nature* **238:** 345–346.

Walls, G. L., and H. D. Judd. 1933. The intra-ocular colour filters of vertebrates. *Brit. J. Ophthalmol.* **17:** 641–675.

Whittaker, J. R., and P. E. Granum. 1980. An absolute method for protein determination based on difference in absorbance at 235 and 280 nm. *Anal. Biochem.* **109:** 156–159.

Zigman, S. 1971. Eye lens color: formation and function. *Science* **171:** 807–809.