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PHOTOSENSITIVE PIGMENTS FROM THE RETINAE OF CERTAIN DEEP-SEA FISHES

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The bizarre morphology of deep-sea fishes has fascinated students and led to the inference of functional as well as structural modification. Although the strange eyes of deep-sea fishes have been the subject of histological studies (e.g. Brauer, 1908) and of scientific speculation for many years, experimental work was not attempted until very recently. Then light-sensitive retinal pigments of several species of deep-sea fishes were examined by measuring the gross changes in optical density following exposure of the retinae to light (Denton & Warren, 1956). The spectral location of these pigments was reported to be about 20 mμ less than that of rhodopsin, thus placing the peak absorptions at about 480 mμ. The name chrysopsin was proposed for these pigments.

In recent years the analysis of retinal photosensitive pigments has been greatly advanced by the method of partial bleaching, which makes it possible to test the homogeneity of the photolabile component present in retinal extracts (Dartnall, 1952). The present investigation was undertaken to explore the retinal pigments of a number of deep-sea fishes using these modern methods. A preliminary note of this study has already appeared (Munz, 1957).

METHODS AND APPARATUS

Collection and identification of material

Through the kindness of Dr C. L. Hubbs, of the Scripps Institution of Oceanography, the eyes of bathypelagic fishes collected on an expedition of the Scripps vessel *Paolina-T*, in which the author took part, were made available for this study. Organisms were collected in a 10 ft. Isaacs-Kidd mid-water trawl on the nights of 9 and 10 February 1957, at depths from 280 to 380 fathoms near Guadalupe Island, Baja California, Mexico. The net was brought up on deck by moonlight, and its luminescent blue-glowing contents were dumped into a bucket, which was carried into the ship's laboratory. Here by dim red light Dr Hubbs sorted the collections and made field identifications of the fishes. The author then removed the eyes and, where possible, the retinae by red light, placed them in distilled water or 4% potassium alum solution and kept them frozen in light-

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proof containers until returning to the laboratory (8–9 days). The fishes were preserved after removal of their eyes and were identified by Mr A. W. Ebeling of the Scripps Institution of Oceanography. Table 1 lists the species examined in this study and certain details of preparation of the retinal extracts.

TABLE 1. Spectral characteristics of retinal extracts

Species	Family	No. of eyes	Extract sample	Density (700 mμ)	Maximum density	$\lambda_{max.}$ (mμ)	Ratio	Adjusted $\lambda_{max.}$ (mμ)
Bathylagus wesethi	Argentinidae	8*	1A	0·004	0·460	483	0·39	484
			1B	0·004	1·060	485	0·37	486
			1C	0·028	0·672	482	0·56	485
Argyropelecus affinis	Sternoptychidae	2	2A†	−0·003	0·562	477	0·43	478
			2B	−0·001	0·372	477	0·43	478
			2C	0·002	0·143	476	0·54	478
Sternoptyx obscura	Sternoptychidae	6*	3A	0·006	0·552	484	0·48	485
			3B	0·001	0·594	484	0·50	486
			3C	0·001	0·148	484	0·59	487
Stomias atriventer	Stomiatidae	4	4A	0·020	0·202	475	0·95	—‡
Lampanyctus mexicanus	Myctophidae	16§	5A	0·047	1·056	486	0·65	490
			5B	0·093	0·553	468	0·98	—‡
Melamphaes bispinosus	Melamphaidae	16§	6A	0·050	1·121	485	0·63	488
			6B	0·035	0·552	484	0·68	488

* Retinal material frozen in 4% potassium alum solution; otherwise in distilled water.
† After the absorption spectrum was measured, this extract was divided and diluted into samples 2B and 2C.
‡ The high ratio prevented estimation of the true $\lambda_{max.}$.
§ Whole eyes extracted; otherwise retinae were removed before extraction.

Preparation of retinal extracts

Digitonin extracts of the whole retinal material were prepared with methods used previously (Munz, 1956). Where the eyes were small, the whole eyes were mashed, then washed and extracted in the same manner as the retinae. Retinae which had been frozen in distilled water were thawed and then hardened for a few minutes in 4% potassium alum solution (this step was omitted with the material which had been frozen in alum solution). They were then washed twice in distilled water, being centrifuged lightly each time to allow separation from the supernatant fluid. After a further wash in alkaline (pH 8·6) borate-KCl buffer, the retinal material was extracted with two successive portions of 2% aqueous digitonin solution made up in borate-KCl buffer (pH 8·3). The extracts were stored in darkness at 10° C.

Measurement of absorption spectra and bleaching apparatus

After centrifugation the retinal extracts were analysed in a Beckman DU spectrophotometer with photomultiplier attachment. Two per cent digitonin solution was used as a blank; the temperature was regulated at 20±1° C. Optical density of the extracts (1 cm light path) was measured from 700 to 320 mμ in 20 mμ intervals, followed by an interlaced return series of measurements, also at 20 mμ intervals. A Bausch and Lomb grating monochromator with interference filters placed in the exit light path provided narrow-band coloured light (half band-widths between 10 and 15 mμ) which was used in bleaching the extracts. Following exposure to light the extracts were again placed in the spectrophotometer and the absorption spectrum measured as before. In this way the effects on each extract of several bleaching wave-lengths were studied successively. White light from a 60 W tungsten bulb was also employed as a bleaching source.

RESULTS

The photosensitive pigment of Argyropelecus

Two specimens of the hatchet-fish *Argyropelecus affinis* Garman were obtained, and a digitonin extract was prepared from the right retinae. The absorption maximum of this extract was 477 mμ (see Table 1 and curve 1 of Fig. 1*A*). The ratio of optical densities of the minimum (at 390 mμ) to maximum was 0·43. This $D_{min.}:D_{max.}$ ratio is related to the absorption maximum of visual

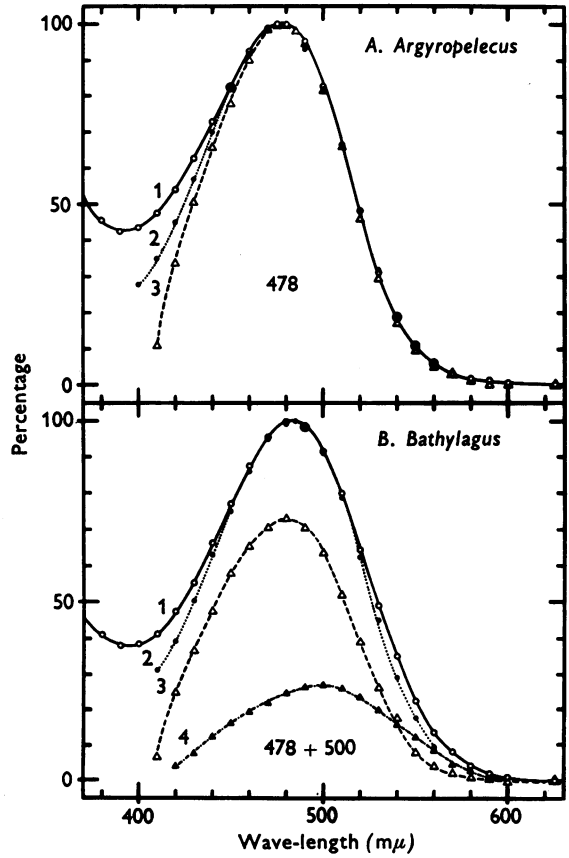

Fig. 1 *A*. Curve 1, absorption spectrum of extract sample 2 A of *Argyropelecus*; curve 2, constructed from Dartnall's nomogram, assuming a maximum at 478 mμ; curve 3, NH_2OH experiment, difference spectrum after exposure of sample 2 C to green light (560 mμ).

Fig. 1 *B*. Curve 1, absorption spectrum of extract sample 1 A of *Bathylagus*; curve 2, constructed from Dartnall's nomogram, assuming a maximum at 485 mμ; curves 3 and 4 from NH_2OH experiment with sample 1 C. Curve 4 is difference spectrum obtained by exposure to red light (631 mμ); curve 3 is difference spectrum after exposure to 560 mμ. Curves 3 and 4 are the same as curves 1–2 and 6–7 of Fig. 3*B* but scaled so as to approximate in sum the total difference 1–7.

pigment extracts (Crescitelli & Dartnall, 1954, Fig. 8). From this curve true $\lambda_{max.}$ of the hatchet-fish pigment was estimated to be 478 mμ. Curve 2 of Fig. 1*A* is the theoretical curve constructed for a visual pigment maximal at 478 mμ from Dartnall's nomogram (1953). The nomogram is based on his observation that the difference spectra of all known visual pigments have the same shape when plotted on a frequency scale. The theoretical curve and absorption spectrum (curve 1) coincide except at wave-lengths less than 450 mμ, where curve 1 lies above the theoretical curve, presumably owing to the presence of blue-absorbing impurities. Hydroxylamine (NH_2OH) combines with retinene to form the oxime (Hubbard & Wald, 1952), which has a $\lambda_{max.}$ at a shorter wave-length than retinene, thus minimizing the effect on the positive portion of the difference spectrum of the product of bleaching (Crescitelli, 1956). The NH_2OH difference spectrum (curve 3) of the hatchet-fish is also in close agreement with curve 2 except in the short wave-length portion, where the NH_2OH difference spectrum falls more rapidly owing to the presence of the product. These three sources of information, i.e. $\lambda_{max.}$ of absorption spectrum, agreement with theoretical curve for a visual pigment maximal at 478 mμ, and $\lambda_{max.}$ of the NH_2OH difference spectrum, all combine to show that $\lambda_{max.}$ of the hatchet-fish retinal pigment is 478 ± 1 mμ.

Partial bleaching with lights of several wave-length compositions demonstrated the photosensitivity and homogeneity of the *Argyropelecus* extract (see Table 2). No evidence for a mixture of light-sensitive pigments was obtained; each stage of the bleaching indicated that the same component had been affected by exposure to light. The difference spectrum without NH_2OH was maximal at 481 or 482 mμ and at 478 mμ with NH_2OH. Fig. 2 shows the results of a partial bleaching experiment with extract 2C. The difference spectra (Fig. 2*B*) have the same $\lambda_{max.}$ except for the last, which showed a small shift toward shorter wave-lengths caused by exposure to isomerizing light. Attention has been called to this effect before (Crescitelli, 1956; Munz, 1956), and it is not believed to indicate the bleaching of any additional pigment of the same type as the one maximal at 478 mμ.

The nature of the carotenoid chromophore of a visual pigment molecule is suggested by the spectral position of the product of bleaching, and this relationship has been used by Crescitelli (1956) to characterize the carotenoid systems of amphibian and gecko visual pigments. The product of bleaching of the *Argyropelecus* pigment absorbed light maximally at 375–379 mμ (pH 8·3) and 365–367 mμ when NH_2OH was added to the extract (Table 2). These maxima are in accord with the data of Crescitelli and strongly suggest that retinene$_1$ is the chromophore of the photosensitive pigment of *Argyropelecus*. NH_2OH was added to a digitonin solution (pH 8·3) of crystalline all-trans retinene$_1$ to form the oxime, and the absorption spectrum of this preparation was measured (data of Dr Crescitelli). In Fig. 2 the points of this curve are

plotted in scale for comparison with the NH_2OH difference spectrum. The close agreement of these curves is further evidence that retinene$_1$ oxime was formed in the NH_2OH experiment. The retinal pigment of *A. affinis* is a 478_1 photopigment. The subscript 1 means that retinene$_1$ is the chromophore of the pigment molecule.

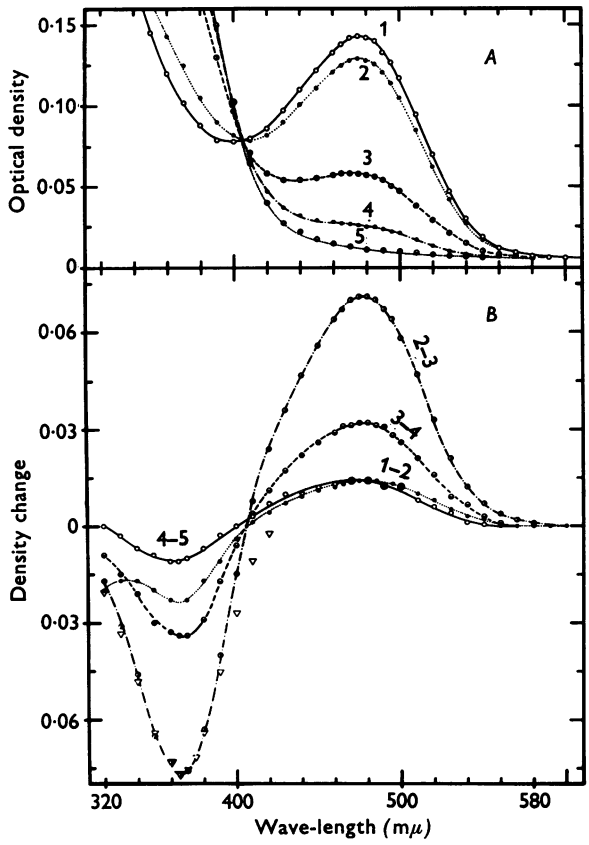

Fig. 2*A*. A partial bleaching experiment with *Argyropelecus* sample 2C (NH_2OH added). Curve 1, absorption spectrum of unbleached extract; curve 2, after 1 hr exposure to orange light (606 mμ); curve 3, after 90 min further exposure to yellow light (580 mμ); curve 4, after 30 min further exposure to green light (560 mμ); curve 5, after 10 min exposure to white light.

Fig. 2*B*. Difference spectra of experiment with sample 2C. Upward changes indicate loss of density; downward changes gain in density. Curve 1–2 is the result of the 606 mμ bleach; 2–3 the 580 mμ bleach, 3–4 the 560 mμ bleach, and 4–5 the white light bleach. The inverted triangles are the spectral densities for a digitonin solution (pH 8·3) of crystalline all-trans retinene$_1$ to which was added NH_2OH to yield the oxime. These points were scaled for plotting with the difference spectrum.

TABLE 2. Details of bleaching experiments

Species	Extract sample	Bleaching wave-length (mμ)	$\lambda_{max.}$ of difference spectrum (mμ)	Density loss at maximum	$\lambda_{max.}$ of product (mμ)	Density gain of product at maximum
Bathylagus wesethi	1A	640	—*	—	—	—
		606	504	0·049	375	0·063
		606	497	0·047	373	0·070
		580	487	0·109	375	0·101
		580	483	0·101	376	0·082
		580	480	0·110	380	0·090
		W†	—‡	0·014	—‡	—
	1B§	631	—‖	0·145	368	0·188
		606	498	0·108	368	0·081
		580	482	0·587	367	0·578
		580	480	0·122	367	0·098
		W	475	0·018	—‡	—
	1C§	631	500	0·057	368	0·068
		606	497	0·075	365	0·080
		606	485	0·063	366	0·072
		580	480	0·133	367	0·144
		580	478	0·133	365	0·152
		560	478	0·114	367	0·110
		W	474	0·020	360	0·021
Argyropelecus affinis	2B	640	482	0·013	—*	—
		606	483	0·060	379	0·012
		580	481	0·060	376	0·046
		580	482	0·082	375	0·072
		580	481	0·119	375	0·089
		W	474	0·030	—‖	0·038
	2C§	606	480	0·014	365	0·023
		580	478	0·071	367	0·077
		560	478	0·032	367	0·034
		W	475	0·014	363	0·011
Sternoptyx obscura	3A§	606	487	0·096	365	0·089
		580	484	0·209	367	0·216
		580	486	0·116	367	0·110
		560	484	0·100	368	0·093
	3B	631	490	0·030	375	0·020
		606	485	0·067	378	0·053
		580	488	0·142	378	0·104
		580	487	0·154	375	0·114
		580	487	0·148	379	0·101
		W	385‡	0·056	—‡	0·004
Stomias atriventer	4A§	631	—*	—	—	—
		606	491	0·013	363	0·047
		580	490	0·061	368	0·091‖
Lampanyctus mexicanus	5A	631	500‖	0·059	373	0·060
		606	490‖	0·063	380	0·070
		580	490	0·348	378	0·275
		580	491	0·231	382	0·170
		560	492	0·096	377	0·066
	5B§	631	—*	—	—	—
		580	490	0·093	367	0·170
		560	490	0·114	365	0·145
Melamphaes bispinosus	6A	631	492	0·035	373	0·070
		580	490	0·320	375	0·266
		580	490	0·300	374	0·231
		580	490	0·197	374	0·151
		W	404	0·087	—‡	—

* Difference small, with no clear peak. † 60 W tungsten bulb used as bleaching light. ‡ Isomerization. § 0·1 ml. of 0·1 M-NH_2OH (pH 7·0) added to extract. ‖ Thermal instability of extract; difference spectrum not reliable.

A mixture of photosensitive pigments in a retinal extract of Bathylagus

A digitonin extract prepared from both eyes of four adult *Bathylagus wesethi* Bolin was analysed in the same manner as *Argyropelecus*. When corrected for the $D_{min.}:D_{max.}$ ratio, the absorption maximum of the unbleached pigment

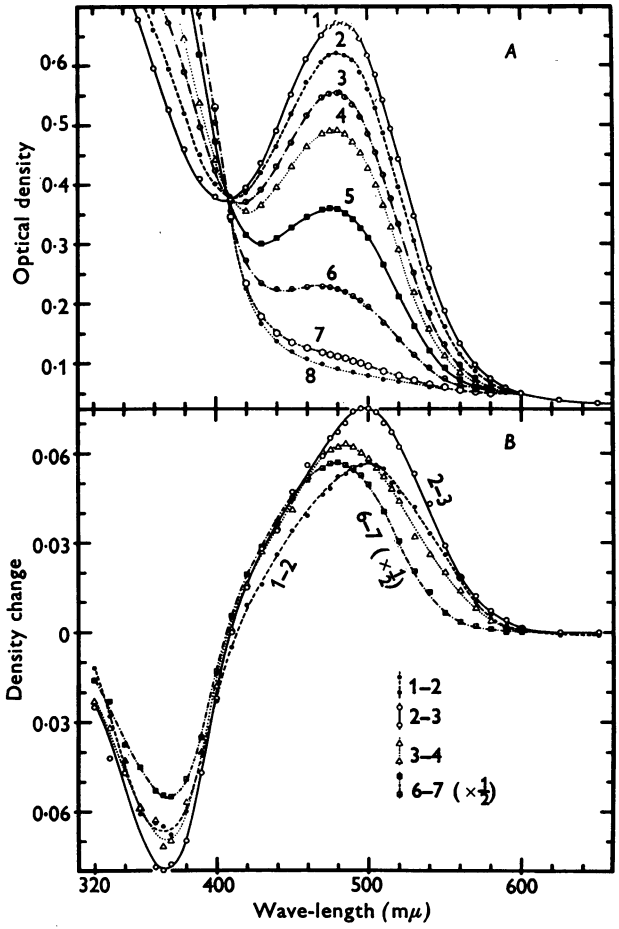

Fig. 3*A*. A partial bleaching experiment with *Bathylagus* sample 1C (NH_2OH added). Curve 1, absorption spectrum of unbleached extract; curve 2, after 1 hr exposure to red light (631 mμ); curve 3, after 30 min further exposure to orange light (606 mμ); curve 4, after 1 hr further exposure to 606 mμ light; curve 5, after 25 min further exposure to yellow light (580 mμ); curve 6, after 45 min further exposure to 580 mμ light; curve 7, after 40 min further exposure to green light (560 mμ); curve 8, after 10 min exposure to white light.

Fig. 3*B*. Difference spectra of experiment with sample 1C. Upward changes indicate loss of density; downward changes, increase in density. Curve 1–2 is the result of the 631 mμ bleach; curves 2–3 and 3–4, 606 mμ bleaches; and curve 6–7 (drawn to one-half scale), a 560 mμ bleach. Curves 4–5 and 5–6 (580 mμ bleaches) and 7–8 (white light bleach) are omitted for clarity of figure.

(Fig. 1*B*, curve 1) was estimated to be between 484 and 486 mμ. This curve does not coincide with the theoretical curve constructed from Dartnall's nomogram (curve 2), but is broader. This suggests what was later shown in partial bleaching experiments; namely, that two components were present in the extract and were summed in the absorption spectrum. The data of Table 2 show that bleaching with red light first removed a red-sensitive component ($\lambda_{max.} = 500$ mμ) and that with continued step-wise bleaches the difference spectra maxima gradually shifted to shorter wave-lengths, finally reaching 478 mμ. This information is shown graphically in Fig. 3, which gives the results of the experiment with sample 1 C. In Fig. 3*B* the difference spectra show the selective bleaching of a rhodopsin-like component (curve 1–2, with $\lambda_{max.} =$ 500 mμ) by red light. Curves 2–3 (497 mμ), 3–4 (485 mμ) and 6–7 (478 mμ) gradually shift toward lower wave-lengths. Curves 4–5 (480 mμ) and 5–6 (478 mμ) are omitted for clarity of the figure. Curve 1–2 nearly fits the nomogram curve for a visual pigment with $\lambda_{max.} = 500$ mμ, and curve 6–7 is near the theoretical curve for $\lambda_{max.} = 478$ mμ. Both curves are slightly broader than the nomogram curve, indicating contamination of each with the other. The products of bleaching of the components were spectrally the same and resembled the bleaching product of *Argyropelecus*. Both pigments of *Bathylagus* are therefore believed to have retinene$_1$ as the chromophore. In Fig. 1*B* the difference spectra curve 4 (1–2 of Fig. 3) and curve 3 (6–7 of Fig. 3) are drawn in approximately the correct proportions to represent the total difference spectrum of the *Bathylagus* extract. Between 20 and 25% of the total bleaching was due to the 500_1 pigment and the remainder to the 478_1 photosensitive pigment.

Photosensitive pigments of Sternoptyx and Melamphaes

A retinal extract of another hatchet-fish, *Sternoptyx obscura* Garman, did not contain the 478_1 pigment. Instead, there was a 485_1 photosensitive pigment (see Tables 1 and 2, Fig. 4*A*). Partial bleaching experiments demonstrated the substantial homogeneity of this extract (possibly some red-sensitive pigment was present as a very small proportion of the whole). A whole-eye extract of the right eyes of sixteen *Melamphaes bispinosus* Gilbert contained a single light-sensitive pigment with $\lambda_{max.} = 488$ mμ. In this case the NH_2OH difference spectrum is lacking, owing to instability of the sample when NH_2OH was added. The retinal pigment, however, was shown to be homogeneous by a partial bleaching experiment (Table 2) without NH_2OH. The absorption spectrum (Fig. 4*B*) does not fit the theoretical curve for a visual pigment with $\lambda_{max.} = 488$ mμ. The slightly opalescent extract had an optical density of 0·050 at 700 mμ. Even after subtraction of 0·050 from all the readings, the gradually increasing absorption due to opalescence disturbed agreement with the nomogram curve as the short-wave end of the spectrum was approached. The

difference spectrum without NH_2OH (curve 3) is also interesting, for it shows the distortion caused by the appearance of the bleaching product. The curve is narrower and shifted slightly toward longer wave-lengths; it is not as good an approximation of the true absorption spectrum as the NH_2OH difference spectrum. While the evidence for the 488 pigment of *Melamphaes* is not as precise as for the other pigments, Fig. 4 *B* is instructive in the methods used to locate $\lambda_{max.}$ of a photosensitive pigment of this type. As before, the product of bleaching (Table 2) suggested that retinene$_1$ is the chromophore of the pigment, which is a 488_1 mμ photosensitive pigment, location of the $\lambda_{max.}$ being within ± 2 mμ of this figure.

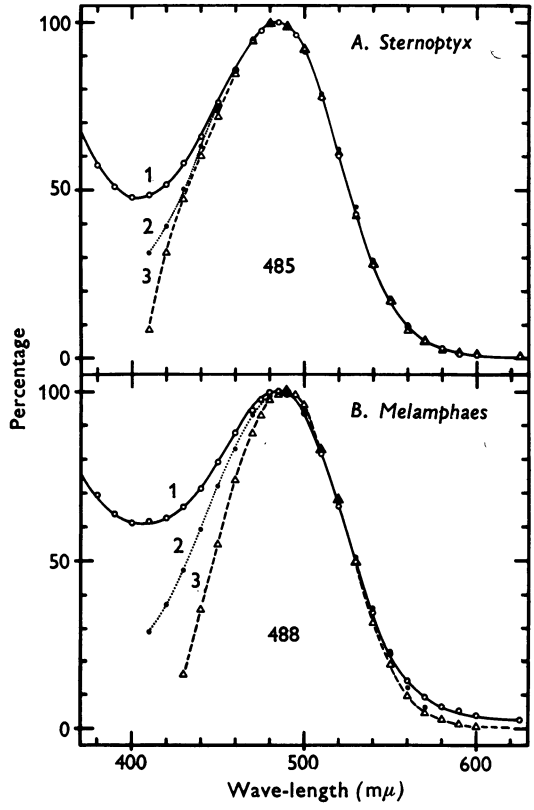

Fig. 4 *A*. Curve 1, absorption spectrum of extract sample 3 A of *Sternoptyx*; curve 2, constructed from Dartnall's nomogram, assuming a maximum at 485 mμ; curve 3, NH_2OH experiment, difference spectrum after exposure of sample 3 A to yellow light (580 mμ).

Fig. 4 *B*. Curve 1, absorption spectrum of extract sample 6 A of *Melamphaes*; curve 2, constructed from Dartnall's nomogram, assuming a maximum at 488 mμ; curve 3, difference spectrum without NH_2OH after exposure of sample 6 A to yellow light (580 mμ).

Difference spectra of Lampanyctus and Stomias

Two other species of deep-sea fishes were examined. The results suggested that both possess 490_1 retinal pigments. A whole-eye extract of the right eyes of sixteen adult *Lampanyctus mexicanus* Gilbert and a retinal extract of four large *Stomias atriventer* Garman both appeared to contain homogeneous light-sensitive pigments when subjected to partial bleaching experiments (Table 2). Since the extracts had high $D_{min.}:D_{max.}$ ratios (extract 5A of *Lampanyctus* excepted), greater reliance had to be placed upon the NH_2OH difference spectra. These are shown in Fig. 5 along with difference spectra of *Argyropelecus* and *Sternoptyx* for comparison. The products of bleaching were the same spectrally as in the other species examined. The photosensitive pigments of *Lampanyctus* and *Stomias* are tentatively considered 490_1 pigments.

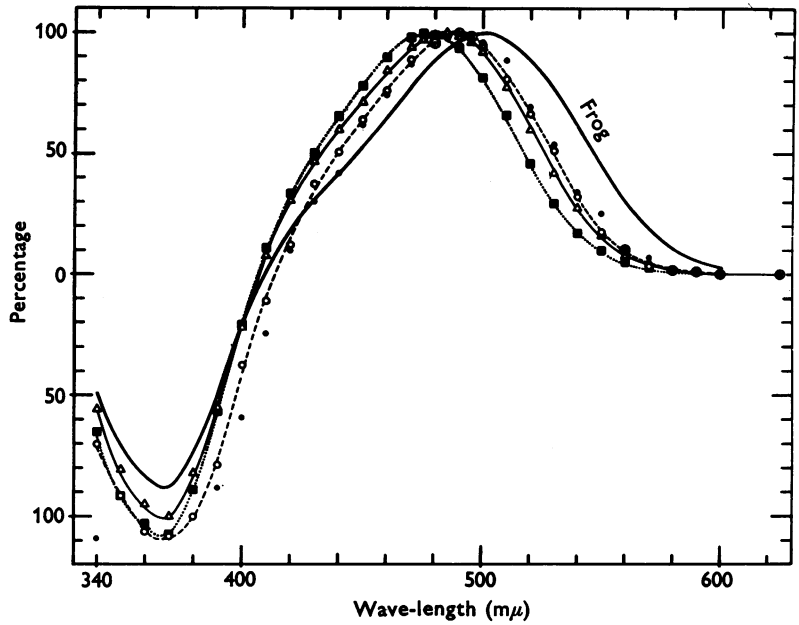

Fig. 5. Hydroxylamine difference spectra of deep-sea fish photosensitive pigments plotted as percentages of maximum density loss. ○, *Lampanyctus* sample 5B was bleached with 560 mμ light (maximum density loss 0·120). ●, *Stomias* (4A) was bleached with 560 mμ (maximum loss 0·066). △, *Sternoptyx* (3A) with 580 mμ (maximum loss 0·325), and ■, *Argyropelecus* (2C) with 580 mμ (maximum loss 0·071). The hydroxylamine difference spectrum of the frog *Rana pipiens* is also shown for comparison (data of Dr Crescitelli).

DISCUSSION

From the foregoing data it is clear that the technique of extraction and spectrophotometric analysis of the retinal pigments indicated general agreement with the results of Denton & Warren (1956) and of Walker (1956), who found in the

conger a homogeneous visual pigment with $\lambda_{max.} = 487$ mμ. There was no one photosensitive pigment to be found in the six species of fishes examined, but rather a different situation in almost every species. All six, however, did have light-sensitive pigments with absorption maxima less than 500 mμ as Denton & Warren have stated (at least two of the genera were common to both studies). The six species of the present investigation represent three different orders of fishes and are not members of a single closely related group. *Bathylagus* belongs to one clupeoid subgroup and *Argyropelecus*, *Sternoptyx* and *Stomias* to another. *Lampanyctus* is a member of the Iniomi, and *Melamphaes* is a berycoid fish. Even in such an incomplete survey of deep-sea fishes as this, considerable diversity of retinal pigments is present.

Although no direct evidence is available that these retinal pigments of the bathypelagic fishes are visual pigments, they would appear to be such because of their photosensitivity, great concentration in the retina, characteristic absorption curves (compared with the theoretical curves constructed from Dartnall's nomogram), and generation of a product of bleaching resembling that of known retinene$_1$ visual pigments.

The fishes studied very probably have pure-rod retinae. With histological methods Brauer (1908) examined the retinae of each genus (different species) included in the present investigation and found only rods in each of them. The photosensitive pigments described are probably contained within the rods. The double pigment system ($\lambda_{max.}$ at 478 and 500 mμ) of *Bathylagus*, based on retinene$_1$ only, suggests that a mechanism for differentiating between wave-lengths may exist even in a pure-rod retina.

The concentration of photosensitive pigment in the extracts was very great, relative to the amount of retinal material extracted. Sample 2A of *Argyropelecus* was obtained from pieces of retina of two eyes from fish 4–5 cm long. They were extracted in 0·5 ml. of digitonin, and gave an optical density of 0·562 for a 1 cm light path. This must be related to the fact that the retinae have rods only and that the outer segments of these are very long and densely packed (Brauer, 1908). The photosensitive pigment should be able to capture a large proportion of the light which impinges on the retina. Computation of the photosensitive pigment density of the retina of deep-sea fishes would be valuable.

Photosensitivity of the retinal pigments

The actual light sensitivity of these pigments did not appear to be greatly different from that of rhodopsin. This was apparent from the length of time required to bleach the extracts with coloured light, which also has been employed with a number of rhodopsins (unpublished data). In a rough trial of this, an extract of frog (*Rana pipiens*) rhodopsin and the *Sternoptyx* extract 3C were simultaneously exposed to blue light (peak wave-length 493 mμ) about half way between their maxima of absorption. At intervals the extracts

were removed from the bleaching apparatus and their absorption spectra were measured. When the percentage of the total bleaching was plotted against log. time, it appeared that the *Sternoptyx* extract may have been somewhat, but not greatly, more sensitive to the light used. This point of the relative photosensitivity of various visual pigments would be worth careful investigation, but at least as a first approximation the sensitivity of deep-sea fish eyes seems to depend on increased concentration of light-sensitive pigments more than on an increased photosensitivity of the pigments themselves.

Nomenclature

The nomenclature of visual pigments has not yet evolved into a uniformly applied system. The photosensitive pigments discussed in this paper need to be given names to identify them and, if possible, to describe their properties. Although the names 'rhodopsin' and 'porphyropsin' have often been rigidly applied to mean that there are only two rod visual pigments (e.g. Wald, 1955), recent investigations, as Dartnall (1957, pp. 38–41) has already pointed out, indicate much more complexity. 'Rhodopsin' and 'porphyropsin', when employed to describe rod visual pigments based respectively on retinene$_1$ ($\lambda_{max.} = ca.$ 500 mμ) and retinene$_2$ ($\lambda_{max.} = ca.$ 525 mμ), may have a certain value as broad group names; but they should not be used indiscriminately. For example, Crescitelli (1956) found that visual pigments of the geckos have spectral absorption characteristic of porphyropsin, but that the product of bleaching is similar to that of rhodopsin.

The present data strongly suggest that retinene$_1$ is the chromophore of the pigment molecule in all six species of deep-sea fishes examined. These results thus spread further the already wide limits to which the name 'rhodopsin' might possibly be applied. It is doubtful, however, whether 'rhodopsin' can be usefully stretched to include photosensitive pigments with $\lambda_{max.}$ ranging from 478 mμ (*Argyropelecus* and *Bathylagus*) to 524 mμ (geckos; Crescitelli, 1956). Qualification of the term 'rhodopsin', e.g. 'mammalian rhodopsin', is also inadmissible. Unpublished data of Dr Crescitelli show that the photosensitive retinal pigments of certain mammals approach those of the deep-sea fishes in wave-length of maximum absorption ($\lambda_{max.}$ of opossum 'rhodopsin' = 493 mμ). Since the deep-sea fish pigments are evidently based on retinene$_1$ also, it would be confusing to recognize them arbitrarily by a different name. For this reason 'chrysopsin' (Denton & Warren, 1956) has been avoided in the present paper. The photosensitive pigments of the deep-sea fishes have been designated instead by the wave-length of maximum absorption and the subscript 1 (to indicate that retinene$_1$ is the chromophore).

Biological significance

It was pointed out by Denton & Warren (1956) that $\lambda_{max.}$ of the retinal pigments of deep-sea fishes seems to be well suited for maximum sensitivity to those wave-lengths which are best transmitted by clear oceanic water. This statement was based upon the work of Jerlov (1951) during the Swedish Deep-Sea Expedition of 1947–48. Jerlov studied the effect of ocean water on light of different wave-lengths by lowering a photometer with coloured filters into the ocean and measuring the percentage transmission at increasing depths. His experiments show that $\lambda_{max.}$ of the transmitted light is about 475 mμ in oceanic waters. Several other workers had earlier studied the optical properties of water samples taken from various sources into the laboratory (James & Birge, 1938; Clarke & James, 1939; Hulburt, 1945). Their conclusions were similar to those of Jerlov. The work of James (later published as James & Birge, 1938) led Clarke as early as 1936 to write (p. 453): 'These results raise the question of the possibility of a shift in the sensitivity of the eye of a deep water fish toward the blue end of the spectrum.'

This apparent correlation between the $\lambda_{max.}$ of the retinal pigments of bathypelagic fishes and of the sunlight which reaches them suggests that the spectral absorption of the photosensitive pigments may be an adaptive characteristic, subject to natural selection. Bayliss, Lythgoe & Tansley (1936, p. 96) set out to 'inquire whether there is any correlation between the make-up of the retina of a given species and the depth which it normally frequents', but the methods then available for the investigation of retinal pigments were not sufficiently refined to establish any relation.

Barlow (1957), following a suggestion made by de Vries (1949), has proposed that the shift in maximum sensitivity of the eye toward the blue end of the spectrum at low levels of illumination (Purkinje shift) may have evolved as a means of obtaining a more favourable signal-to-noise ratio, thereby greatly increasing sensitivity of the eye to light. Such a shift in the spectral absorption of a visual pigment should be accompanied by a decreased thermal instability of the photochemical, producing the more favourable ratio. This suggestion offers an alternative explanation for the evolution of the blue-absorbing photopigments of deep-sea fishes. Fewer quanta might have to be absorbed by such more thermally stable pigments in order to give an unequivocal sensation of light (assuming the photosensitivity of these pigments to be approximately equal to that of 'rhodopsin'). This lowering of the visual threshold should be advantageous to a fish living in a low light-intensity environment. Perhaps the thermal stability factor has combined with the optical properties of oceanic water to exert a selective influence on $\lambda_{max.}$ of the deep-sea fish photopigments.

Recent studies (Clarke & Wertheim, 1956; Clarke & Backus, 1956) of

submarine light intensities in the Atlantic Ocean show that bioluminescence, coming in frequent flashes, increases with increasing depths, equalling the intensity due to sunlight at 600 m. The spectral composition of luminescence in the sonic-scattering layer was studied at night (depth 100 m) by Kampa & Boden (1957), who found the average intensity maximum to be at 478 mμ, in the spectral region best transmitted by oceanic water. Two luminescent invertebrates, *Euphausia pacifica* and *Pyrosoma atlantica*, under laboratory conditions produced light with primary intensity maxima at 476 and 482 mμ, respectively, possibly indicating adaptation of the $\lambda_{max.}$ of luminescence to the spectral transmission of oceanic water. The close agreement of these values with $\lambda_{max.}$ of the bathypelagic fish pigments suggests that the photopigments may quite as likely have become adapted to bioluminescence as to the filtered sunlight available at moderate depths.

It should be remembered that even an initial survey of deep-sea fishes disclosed several pigments, with different peaks of absorption. Perhaps it is even possible that the eyes of a deep-sea fish are most sensitive to that light which its own species produces. Of the six species examined, all but *Bathylagus wesethi* and *Melamphaes bispinosus* have light organs. *Argyropelecus* has large tubular eyes (vertically directed), *Sternoptyx* and *Bathylagus* large normally shaped eyes, and the other fishes have somewhat smaller normal eyes. It would be interesting to compare the retinal pigments of these bathypelagic fishes, which live in or near the lighted zone and most of which may come to the surface at night, with the pigments of large-eyed, benthic rat-tail fishes (macrourids), which may never enter the photic zone.

SUMMARY

1. Photosensitive extracts were prepared from six species of bathypelagic fishes, representing several major taxonomic groups, by treating either whole retinae or the crushed eyes with 2% digitonin solution. The homogeneity of these extracts was tested by the method of partial bleaching.

2. Comparison of the spectral absorption of unbleached extracts, difference spectra without and with hydroxylamine, and the Dartnall (1953) nomogram established the spectral locations of the retinal pigments.

3. The maximum absorptions of these retinal pigments was below 500 mμ, indicating general agreement with the results of Denton & Warren (1956). But there was a range of values from 478 mμ in *Argyropelecus affinis* and in *Bathylagus wesethi* to 485 mμ in *Sternoptyx obscura*, 488 mμ in *Melamphaes bispinosus*, and 490 mμ in *Stomias atriventer* and *Lampanyctus mexicanus*.

4. In addition to a pigment at 478 mμ, the *Bathylagus* extract had a minor amount of a 500 mμ pigment.

5. The product of bleaching in all extracts was characteristic of that obtained with retinene$_1$ visual pigments. The spectral absorption of the hydroxylamine difference spectrum and of all-trans retinene$_1$ oxime were compared and suggest that the retinal pigments of these deep-sea fishes have the same chromophore as that in ordinary visual purple.

6. An attempt was made to correlate these findings with the spectral transmission of oceanic water, which has been studied by several workers. In clear oceanic water the maximum transmission is in the same spectral region as the maximum absorption of the retinal pigments. Recent reports of submarine measurements of the intensity and spectral composition of bioluminescence indicate that it is relatively important and that its spectral emission maximum coincides with the pigment absorption maxima. These facts suggest that the photopigments of deep-sea fishes may have become adapted to the light available at moderate depths in the ocean.

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