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Changes in the visual pigments of trout[1]

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The proportions of two visual pigments (rhodopsin and porphyropsin) were examined in four species of trout under experimental and natural conditions. Brook trout (*Salvelinus fontinalis*), rainbow trout (*Salmo gairdneri*), and brown trout (*Salmo trutta*) have different relative proportions of visual pigments in their retinae. The visual pigment balance in wild cutthroat trout (*Salmo clarki*) is related to forest canopy (access to light) and season. The brown trout have a more red-sensitive and less labile pair of visual pigments than brook or rainbow trout, which respond to photic conditions by increasing the proportion of porphyropsin (in light) and increasing rhodopsin (in darkness). The brown trout have a high percentage of porphyropsin, regardless of experimental conditions. This result does not reflect an inability to form rhodopsin but rather may relate to a consistently high proportion of 3-dehydroretinol in the pigment epithelium. The possible advantages and mechanisms of environmental control of trout visual pigment absorbance, as currently understood, are discussed.

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On a déterminé les proportions de deux pigments visuels (la rhodopsine et la porphyropsine) chez quatre espèces de truites vivant dans des conditions naturelles ou expérimentales. Chez la truite de ruisseau (*Salvelinus fontinalis*), la truite arc-en-ciel (*Salmo gairdneri*) et la truite brune (*Salmo trutta*), les proportions relatives des pigments visuels sont différentes. Chez *Salmo clarki*, l'équilibre des pigments visuels est fonction de la couverture des arbres (accès à la lumière) et de la saison. Chez la truite brune, les deux pigments visuels sont plus sensibles au rouge et moins labiles que chez la truite de ruisseau et la truite arc-en-ciel; ces deux dernières espèces réagissent à la lumière par une augmentation relative de la porphyropsine, et à l'obscurité, par une augmentation de la rhodopsine. Le pourcentage de porphyropsine est toujours élevé chez la truite brune, quelles que soient les conditions expérimentales: cela ne signifie pas pour autant que l'animal soit incapable de synthétiser la rhodopsine, mais ce résultat est probablement relié à la concentration toujours élevée de 3-déhydrorétinol dans l'épithélium pigmentaire. La discussion porte sur l'absorbance du pigment visuel chez les truites, en ce qui a trait à ses avantages et à son contrôle par les facteurs de l'environnement. [Traduit par le journal]

Introduction

Recently, considerable interest has been focused on the ecological meaning of the spectral positions of visual pigments from various animals, especially fishes (Lythgoe 1972; Crescitelli 1972). Spectral position is defined by the maximum of the absorption band (λ_{max}), and is

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usually species specific. Two main hypotheses concerning the ecological significance of visual pigments have been proposed: (1) λ_{max} matches the more prevalent wavelengths of available light, to yield maximal visual sensitivity (Munz 1958, 1965; Bridges 1965*a*), or (2) λ_{max} is somewhat offset from the more prevalent wavelengths, to enhance visual contrast (Lythgoe 1966). Maximal visual sensitivity seems to prevail in deep-sea fishes, in which most visual pigments have λ_{max} near 485 nm (Denton and Warren 1957; Munz 1958). But in other aquatic habitats, fishes possess a very diverse array of visual pigments, and it has been difficult to determine whether improvement of sensitivity or of contrast has been the primary factor in the evolution of visual systems (Munz and McFarland 1973).

A promising area of study is the analysis of changes in the proportions of visual pigments in those fishes that possess "paired pigments." All known visual pigments utilize the aldehyde of vitamin A_1 (retinal) or vitamin A_2 (3-dehydroretinal) as a prosthetic group of the visual protein (opsin) to form distinctive molecules called rhodopsin and porphyropsin, respectively. In paired-pigment species of fishes both prosthetic groups are present in the retina and either can combine with the opsin, resulting in a mixture of rhodopsin and porphyropsin visual pigments. In this case, the porphyropsin pigment absorbs light at longer wavelengths than its rhodopsin counterpart. Therefore, a change in the proportions of a mixture of rhodopsin and porphyropsin will change the total absorption spectrum of the mixture, and may alter visual sensitivity (Northmore and Muntz 1970; Muntz and Northmore 1973). Isolating the factors which control the proportions of paired pigments in a fish should improve our understanding of how visual function is related to the photic environment.

Many freshwater fishes have paired pigments (Schwanzara 1967). Metamorphic or successional changes in the proportions of rhodopsin–porphyropsin mixtures have been found in the lamprey *Petromyzon marinus* (Wald 1947, 1957; Crescitelli 1956), the eel *Anguilla anguilla* (Carlisle and Denton 1959), some amphibians (Wald 1947; Crescitelli 1958; Wilt 1959), and some migratory salmon (Beatty 1966). Seasonal changes in the proportions of paired pigments were first recorded by Dartnall *et al.* (1961) in a strictly freshwater cyprinid, *Scardinius erythrophthalmus*. This species has a low proportion of porphyropsin in summer and a high proportion in winter. Experimental light induces a high proportion of rhodopsin, but continuous darkness increases the proportion of porphyropsin (Dartnall *et al.* 1961; Bridges and Yoshikami 1970*b*). This suggests that *Scardinius* may respond to some change in light quality in nature. Age may be an important factor in *Scardinius*; older fish have more porphyropsin (Bridges and Yoshikami 1970*b*). Two other species of fish exhibit seasonal changes and light-induced changes in rhodopsin–porphyropsin ratio similar to those occurring in *Scardinius*: a poeciliid, *Belonesox belizanus* (Bridges 1965*b*), and a cyprinid, *Notemigonus chrysoleucas* (Allen and McFarland 1973). However, Allen (1971) found that a western cyprinid, *Richardsonius balteatus*, showed the same seasonal changes in visual pigments as did *Scardinius*, but that light had an entirely different effect. In *Richardsonius* light favors formation of porphyropsin and darkness favors rhodopsin! An apparently simple situation has become more complex, since different responses can occur in different species.

Salmonids possess paired pigments (Bridges 1956; Bridges and Yoshikami 1970*a*), and changes in the rhodopsin–porphyropsin ratio are known to occur in some of the migratory species (Beatty 1966). Questions arise, therefore, about whether pigment changes occur in nonmigratory salmonid populations, and, if so, what functions they might serve. Dartnall (1962) alludes to a switch toward rhodopsin in *Salmo gairdneri* (rainbow) and *Salmo fario* (= *trutta*, brown trout) in response to light. Increased brightness favored rhodopsin in juvenile coho and king salmon, (*Oncorhynchus kisutch* and *O. tshawytscha*) (Beatty 1966). However, the evidence is fragile, and Beatty implies that light is not a dominant factor in the paired-pigment changes which occur during the euryhaline migrations of salmonids. In this paper we report on the paired-pigment shifts in the following species: *Salmo gairdneri* (rainbow trout), *S. clarki* (cutthroat trout), *S. trutta* (brown trout), and *Salvelinus fontinalis* (brook char or trout). The investigation included a survey of seasonal changes in wild and hatchery populations living in defined photic zones, and experimental studies on the effects of light on the proportions of rhodopsin and porphyropsin.

Materials and Methods

1. Preparation of Retinal Extracts

All trout were dark-adapted for 6 to 12 h, and the following procedure was performed in the presence of deep-red light (Wratten series 2 filters): eyes were removed, enucleated, and placed in a petri dish with 4% potassium alum solution. The retinae were removed and stored in darkness at −20°C in 4% alum. Later, the retinae were thawed, washed, and lightly centrifuged 3 times in cold, distilled water. After the final rinse, 0.4 ml of 2% digitonin (Merck) was added to the retinal pellet and the preparation was sonified at 0°C. The cell fragments were incubated for 2 h at 20°C, centrifuged at 12 000 × *g* for 10 min, and the supernatants were transferred to vials, to which 0.04 ml of saturated sodium borate solution had been added. The extracts were then stored in darkness at −20°C for later spectrometric analysis.

2. Spectrometric Analysis of Visual Pigments

The extracts were thawed, shaken and centrifuged to remove sediment, and transferred to optical cuvettes to which hydroxylamine had been added (final concentration 0.02 M). Spectrometric analysis was performed at 20°C on a specially equipped Cary 14 recording spectrophotometer, which not only provided analog presentation of absorbance data, but also stored wavelength and absorbance values on magnetic tape at 1-nm intervals. The spectrometric data in this digital form were later analyzed by computer (Munz and Allen 1968).

Each extract was partially bleached in a special apparatus (Munz and Beatty 1965), using a standard experimental procedure (for a general discussion of this technique, see Dartnall 1962). In each experiment, we recorded the absorbance spectrum initially (curve 1) and again after exposure to deep-red light (675 nm) for 1.0 min (curve 2), to red light (660 nm) for 4.0 min (curve 3), and to orange light (610 nm) for 6.0 min (curve 4, see Fig. 1, top). This protocol removed the more red-sensitive porphyropsin pigment first, leaving more of the less red-sensitive rhodopsin pigment for the last bleach. Subtraction yielded difference spectra (curves 1−2, 2−3, and 3−4, Fig. 1, bottom) which represented the absorbance of the mixture of the two visual pigments in the extract.

The basic method for determining the proportions of two visual pigments in a mixture was devised by Dartnall *et al.* (1961). This method was applied to the visual pigments of salmonids by Munz and Beatty (1965) and adapted to computer analysis by Munz and Allen (1968). It requires that the difference spectrum of the rhodopsin and porphyropsin each be known. Several species of salmonid fishes have mixtures of the same rhodopsin and porphyropsin pigments 503_1 and 527_2; i.e., the rhodopsin pigment has its λ_{max} at 503 nm and the porphyropsin at 527 nm (Munz and Beatty 1965; Bridges 1972). The difference spectra of these two components were normalized (rescaled on a percentage basis) and added together in varying proportions to produce a template of curves that represented difference spectra of various mixtures (0, 10, 20, ... 100% of the 527_2 pigment). Any normalized experimental difference spectrum which contains the 503_1 and 527_2 pigments can be matched to this template, and by interpolation their proportions determined with precision.

3. Visual Pigments of Salmo trutta

When we analyzed the brown trout extracts routinely on the assumption that they were mixtures of 503_1–527_2 visual pigments, the absorbance values of the difference spectrum resulting from the initial bleach (curve 1−2) often fell to the longer wavelength side of the absorbance curve for pure 527_2. We felt certain, therefore, that brown trout porphyropsin was different from other trout porphyropsins. However, since the extracts always contained a large proportion of porphyropsin, we could not destroy the more red-sensitive porphyropsin by preferentially bleaching it with deep-red light, and still retain enough of the rhodopsin pigment for accurate analysis of its absorbance spectrum. Contributing to our trouble was the fact that rhodopsins are more photosensitive than their porphyropsin analogs (Dartnall 1968).

For the brown trout, we resorted to a different method for analyzing the rhodopsin–porphyropsin mixture. This method is independent of precise knowledge of spectra of the pure rhodopsin and porphyropsin pigments. The products of bleaching (left-hand absorbance bands Fig. 1)

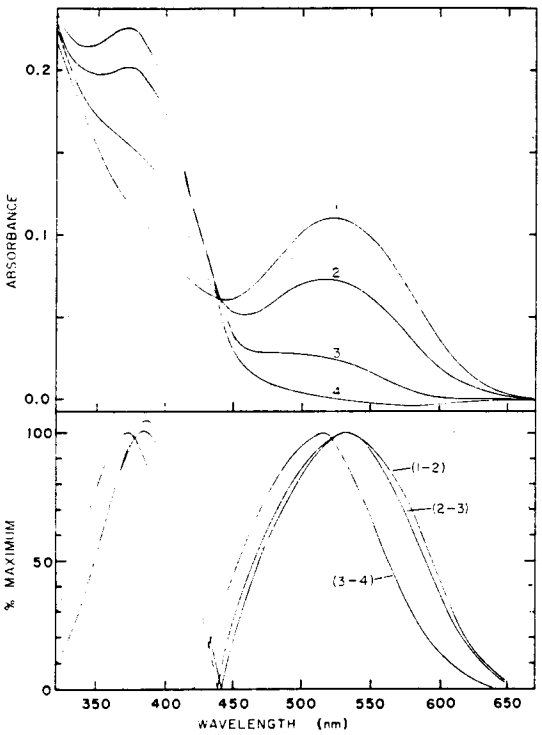

FIG. 1. Difference spectra for the extractable visual pigments from the retina of the brown trout, *Salmo trutta*. Upper curves are tracings of spectrophotometer recordings; lower curves the normalized difference spectra. The series of curves above 420 nm result from bleaching of the visual pigment. Curves below 420 nm are the retinal-oxime products generated by bleaching. Bleaching protocol was as follows: curve 1, not bleached; curve 2, 1 min at 675 nm; curve 3, 4 min at 660 nm; curve 4, 6 min at 610 nm. The percentage of porphyropsin is 87.8 ± 0.5 (see text for details).

are stable and have different absorbance spectra: for the retinal-oxime, λ_{max} is 367 nm, and for the 3-dehydroretinal-oxime, λ_{max} is 385 nm. The same approach devised by Dartnall *et al.* (1961) for rhodopsin–porphyropsin mixtures was applied to the spectra of the products of bleaching. A set of 0, 10, 20, . . . 100% dehydroretinal-oxime difference spectra was constructed from the pure oximes as before. A subroutine in our computer analysis (Munz and Allen 1968) evaluates the normalized product spectrum (left-hand absorbance band, curve 1–4) and reports the corresponding percentage of porphyropsin in the extract. One disadvantage of this method is that the oximes absorb in the near ultraviolet, where extract impurities are also likely to absorb. Sometimes, there is also appreciable instability in this spectral region.

To test the adequacy of the product method, we conducted partial bleaching experiments on visual pigment extracts from *Salmo clarki* (503_1–527_2), *S. gairdneri* (503_1–527_2), and *Richardsonius balteatus* (506_1–531_2). After finding the rhodopsin–porphyropsin ratio by each method (product and pigment), we corrected both results to allow for the fact that porphyropsins are two-thirds as photosensitive as rhodopsins (in the case of the product, the dehydro-oxime is about 0.52 as photosensitive, based on our data). Correcting for these differences in absorptivity gave us an estimate of the proportion of porphyropsin based on the number of molecules (molar basis). (For a discussion of molar absorptivity, see Dartnall 1968).

We compared the molar percentage of 527_2 (or 531_2) pigment in the extract with the molar percentage of the porphyropsin product (3-dehydroretinal-oxime). In extracts that ranged from 9.9 to 99.4% porphyropsin, the mean estimate of the porphyropsin product was only 0.2% lower than the mean percentage of porphyropsin visual pigment. In general, visual pigment and product estimates were in close agreement, but the product estimates seemed somewhat less precise. Experiments with a variety of species having either pure rhodopsin or nearly pure porphyropsin were used to check the ends of the scale. In 32 pure rhodopsin extracts, the mean estimate given by the product method was 1.0% porphyropsin. In 25 porphyropsin extracts production estimate was 92.2% porphyropsin (range 81 to 99%). A small amount of rhodopsin was present in some of these extracts.

The product method not only allowed us to determine the proportion of brown trout porphyropsin, but also made it possible for us to determine the spectrum of brown trout rhodopsin. First, we averaged several normalized experimental difference spectra resulting from the initial exposure of *S. trutta* extracts to deep-red light. This gave us an experimental difference spectrum for pure *S. trutta* porphyropsin, with λ_{max} 533.8 nm (nomogram of Munz and Schwanzara 1966). We then took a total normalized difference spectrum for which the proportion of porphyropsin was known from the product analysis, and subtracted the proportionate fraction of the pure porphyropsin spectrum from it at each nanometer. The remaining absorbance, when rescaled, was an estimate of the pure *S. trutta* rhodopsin spectrum. We repeated this process for six experimental extracts which had different proportions of porphyropsin. The mean rhodopsin spectrum from these six operations served as our estimate of the *S. trutta* rhodopsin. This spectrum, fitted to Dartnall's (1953) nomogram gave a λ_{max} at 508 nm.

Armed with the estimated difference spectra of the pure *S. trutta* pigments, we constructed a template of curves for the intermediate mixtures as before (Methods 2), and used it when the absorbance of products of bleaching was not suitable. The 508_1–534_2 pigment pair of *S. trutta* represents a λ_{max} displacement toward the red of 5 and 7 nm, respectively, from the visual pigments of other trouts.

We should point out that the new method of product analysis is potentially useful for determining the proportions of rhodopsin and porphyropsin visual pigments in any mixture.

4. Experimental Site and Design

Rainbow, brook, and brown trout were raised in a single outdoor raceway at the Tunison Laboratory of Fish Nutrition, Cortland, New York (water temperature 8 ± 0.5°C all year). These same three species were also held at various times and for different lengths of time under conditions of cyclic, continuous light, or continuous darkness at both the Tunison Laboratory and our Cayuga Lake Station. At the Tunison Lab, some brook trout were raised in conditions of seasonal cyclic light, continuous light, or continuous darkness from October 1966 (hatched) to April 1969. We measured the proportion of porphyropsin in the retinae of fish sampled during experimental periods. Other experiments were performed at the Cayuga Lake facility, which was specially equipped for holding fish under different light conditions at cold-water temperatures (4–7°C yearly). Light sources were tungsten lamps. All of the rainbows, brooks, and browns used in New York were from the same stocks that have been maintained at the Tunison Laboratory for several years.

Wild cutthroat trout were sampled by hook and line from a swift-flowing mountain stream (Lookout Creek, H. J. Andrews Experimental Forest) east of Eugene, Oregon. Because of the logging practice called "clear-cutting," there were stream sites available that were either intensely shaded, partially shaded, or completely open to sunlight. These photic zones, which are about 500 m across, exist throughout the year because of the dominance of conifers. Fish were sampled in the central portion of each photic zone from well-defined holes in the stream. We did not mark fish to determine what movement, if any, occurred between collecting sites. The water temperature was never more than 1°C different between adjacent collecting sites (measured at the time of sampling). Our opportunities to sample were limited in the winter by snow. Therefore, we sampled over several seasons in an attempt to derive a clear picture of the seasonal changes of rhodopsin–porphyropsin ratio in the different photic zones.

5. In Vitro Regeneration of Visual Pigments

In one of our experiments we used a regeneration technique that is a test-tube modification of the method of Collins *et al.* (1953), and based on our understanding of techniques used by Reuter *et al.* (1971) on whole retina regeneration. Our aim was to regenerate visual pigment from a combination of bleached, washed retina (opsin

source), with pigment epithelium (P.E.) (retinol or 3-dehydroretinol source).

We first completely bleached whole retinae with amber light. We then rinsed the retinae with phosphate buffer (pH 7.3) 3 times to remove free prosthetic groups and prevent spontaneous regeneration (see Cone and Brown 1969). We then extracted the bleached visual pigment in 0.2 ml of 2% digitonin solution for 1 h. We centrifuged, added 0.05 ml saturated sodium borate, and stored the extracts in a freezer (−20°C) overnight. The pigment epithelium from the same eye was also removed, sonicated in 0.2 ml phosphate buffer (pH 7.3), and stored overnight at 4°C. We then thawed the different opsin preparations and combined them with pigment epithelial preparations from different fishes. In this way we hoped to show how the opsin regenerated when supplied with prosthetic groups from a source other than its own pigment epithelium. The recombinant preparations were allowed to stand in the dark for 30 min at 22°C and were then transferred to 4°C for another 30 min. The newly regenerated visual pigment was separated from the cell fragments by centrifugation, NH_2OH was added, and spectrometric analysis was performed as before (Methods 2). We were able to determine the amount of regenerated visual pigment, the proportion of porphyropsin, and the type of visual pigment (opsin) present in each preparation. Contralateral eyes were used to determine what proportion of porphyropsin was present in the eyes that were used to supply the opsin and pigment epithelium preparations. We assumed that the proportion of 3-dehydroretinol in the pigment epithelium would reflect the proportion of porphyropsin in the retina of the contralateral eye. In salmonids, there normally seems to be little difference between each eye in regard to the proportion of porphyropsin (Munz and Beatty 1965). Other investigations have indicated that the pigment epithelium would be a good source of retinol or 3-dehydroretinol (Wald 1939; Dowling 1960; Bridges and Yoshikami 1970*c*). Reuter *et al.* (1971), working with bullfrog eyes, were able to show that the proportion of porphyropsin regenerated by whole retina laid upon an intact P.E. (retinol or 3-dehydroretinol source) depends on the rhodopsin–porphyropsin ratio in the eye from which the P.E. is taken. Thus, the use of intact retinae and P.E. and (or) the corresponding retinal homogenates should serve as useful tools in reclaiming previously bleached visual pigments for analysis.

Results

1. Rhodopsin Porphyropsin Ratio. Effect of Season

Visual pigments from brown, rainbow, and brook trout were sampled from fish held in a single raceway at the Tunison Laboratory (Cortland, New York) from May 1968 to December 1969. Initially, these fish were of the 0-year class (spawned in autumn, 1967) and weighed between 100 and 200 g.

There was a constant relative difference in the proportions of porphyropsin pigment among the three species (Table 1). Brown trout mostly maintained the highest proportion, brook trout were consistently lowest, and rainbow trout were intermediate. Surprisingly, the proportion of porphyropsin declined in both rainbow and brook trout in the last few samples, as the fish were becoming sexually mature. Brown trout did

TABLE 1

Percentage of porphyropsin visual pigment in fish sampled from the Cortland raceway. Values are mean ± standard deviation (sample size). No differences were detected between sexes

Date of sample	% 527_2 brooks	% 527_2 rainbows	% 534_2 browns
5-31-68	58.2±8.2 (6)	78.9±6.5 (6)	84.6±6.4 (6)
7-2-68	53.2±15.8 (6)	69.5±7.8 (5)	—
8-13-68	58.9±13.4 (6)	74.3±9.0 (6)	—
9-11-68	50.2±17.8 (6)	62.1±22.3 (6)	79.1±6.2 (6)
10-7-68[a]	52.8±7.1 (6)	73.5±6.7 (6)	86.4±5.9 (5)
10-28-68	55.5±6.9 (6)	63.6±12.4 (5)	79.0±3.7 (4)
12-17-68[b]	48.4±13.2 (6)	63.1±13.7 (6)	72.3±5.9 (4)
1-16-69	44.6±6.9 (6)	72.3±8.4 (5)	81.7±2.9 (5)
2-19-69	50.1±8.9 (6)	67.0±5.3 (6)	76.3±4.2 (2)
3-11-69	51.0±7.2 (6)	73.4±12.7 (6)	81.2±3.8 (4)
5-5-69	51.2±9.4 (5)	72.6±10.3 (5)	81.4±4.2 (4)
6-3-69	58.7±12.8 (5)	73.5±14.9 (5)	76.4±8.1 (5)
7-17-69	53.9±5.8 (5)	72.7±3.1 (5)	70.4±8.2 (5)
8-20-69[c]	52.1±4.6 (5)	59.5±15.9 (5)	85.1±9.6 (5)
9-29-69[d]	44.6±12.2 (4)	53.0±8.6 (4)	78.6±14.8 (4)
11-6-69[e]	31.0±15.7 (4)	31.3±14.7 (5)	83.3±9.0 (4)

[a]Both female and male brook trout were ripe, but not brown and not rainbow.
[b]Some brown and rainbow males had semen, but not *all* of them.
[c]Some rainbows had semen. Eggs 3 to 4 mm in all three species, but *not* ripe.
[d]Semen in brooks and rainbows, no brown males sampled. Ripe eggs in brown and rainbows but *not* in brooks.
[e]Ripe eggs and semen in all three species.

not display a comparable decline (Table 1). Since further samples were not available after November 6, 1969, we are uncertain whether this drop in proportion of porphyropsin was a temporary or permanent aspect of sexual maturation and spawning condition. It is interesting that the direction of this change (towards rhodopsin) is opposite to the increase in porphyropsin associated with age in the rudd, *Scardinius* (Bridges and Yoshikami 1970*a*), and also opposite to the changeover to a porphyropsin-dominated retina that occurs in some spawning salmon (Beatty 1966). We are unable to relate this change to rhodopsin to any significant change in ambient light or to ambient temperature, which was constant at 8 ± 0.5°C throughout the study.

Seasonal changes were studied in native cutthroat trout from different photic zones in a Cascade mountain stream (see Methods 4). Trout were sampled from the middle of each photic zone (open, deeply shaded, and partially shaded) from May, 1967 to February, 1970. There was a seasonal shift in porphyropsin from a high percentage during late winter and spring to a low percentage during midsummer and fall (Table 2 and Fig. 2). This shift is most evident in cutthroat collected from the open areas, which achieved a higher proportion of porphyropsin during late winter and spring than did fish from the shaded areas (Fig. 2). Fish sampled from intermediate, partially shaded areas had an intermediate proportion of porphyropsin when the difference between open and shaded fish was maximal (Table 2). If allowance is made for a seasonal lag in temperature-dependent processes, then there appears to be a seasonal temperature effect on the rhodopsin–porphyropsin ratio, which is manifest during late summer (Fig. 2).

In the February–March collections, the fish were in spawning condition, as judged by gonadal development. Reaching the spawning condition clearly did not cause a shift to rhodopsin in these fish. Also, we did not observe any significant relationship between length (12.0- to 23.0-cm range) and percentage of porphyropsin, within or among the different samples.

TABLE 2

Seasonal changes during 1967–1970 in porphyropsin (% 527_2) in cutthroat trout (*Salmo clarki*) from different photic zones in Lookout Creek, H. J. Andrews Experimental Forest, Oregon

Date	% 527_2 (mean, SD, *N*)	Photic zone[a]	Significance tests[b]
9-18-67	43.4±13.2 (13)	Open	ns[c]
	47.6±13.4 (12)	P. open	
	50.1±9.0 (16)	Shaded	
3-3-68	74.7±7.4 (7)	Open	Open vs. p. open*
	60.3±10.2 (10)	P. open	P. open vs. shaded*
	45.4±17.7 (5)	Shaded	Open vs. shaded***
5-6-68	73.4±5.9 (8)	Open	Open vs. shaded***
	54.1±6.7 (8)	Shaded	
7-20-68	57.9±13.6 (11)	Open	Open vs. shaded***
	44.7±9.0 (7)	Shaded	
10-24-68	59.6±7.4 (11)	Open	Open vs. shaded***
	46.7±9.5 (13)	Shaded	
6-19-69	65.0±7.3 (17)	Open	Open vs. shaded***
	54.5±9.0 (10)	Shaded	
8-7-69	56.5±7.3 (9)	Open	ns
	49.1±11.8 (16)	Shaded	
9-24-69	45.7±6.1 (6)	Shaded	Fish were taken from
	53.1±5.5 (6)	P. open	single holes in each
	47.6±4.5 (6)	Open	photic zone, ns be-
	48.0±6.7 (12)	P. open	tween groups
2-12-70	53.0±10.5 (8)	Open	Open vs. shaded, ns
	42.6±4.0 (2)	Shaded	
2-22-70	55.4±8.9 (8)	Shaded	

[a]Open: fish collected from middle of clear-cut area. P. open: collected in partially shaded area, usually thinned or salvaged logged. Shaded: light intensity 1/100 of open (fish taken from stream within mature stand of Douglas fir, cedar, hemlock, and big leaf maple). Blue/red intensity ratio (475 nm/600 nm): for shade 1.2, for open 0.8 during midday. Water is clear and shallow; readings above and below water surface did not alter ratio between the two zones.

[b]Significance tests conducted on a priori assumption, using mean square of ANOVA. Data first transformed from percent to arcsin.

[c]No significant difference (ns).

2. *Effect of Artificial Illumination on Rhodopsin–Porphyropsin Ratio*

The effects of continuous light (CL), variable light (VL, seasonal photocycle), and continuous darkness (CD) on rhodopsin–porphyropsin ratio were examined for a unique population of brook trout that had been reared under these conditions since hatching (Pyle 1969; Poston and Livingston 1971). Fish in constant light grew to the largest size. Fish in the continuous dark group showed somewhat less growth, but appeared normal in all other respects. The brook trout raised in the continuous darkness had significantly less porphyropsin than either light-treated group. Porphyropsin proportions in the light-treated groups were similar to those for the open raceway population (Tables 1 and 3). The somewhat higher proportion in the April sample (Table 3) was due to two individual fish, one of which had an unusually high proportion of porphyropsin. This particular sample was strongly skewed to a low percentage of porphyropsin.

The discrepancy between the rhodopsin–porphyropsin ratios of these unique brook trout and those of the brook, rainbow, and brown trout held in the open raceway under more natural conditions was curious. We thought that, perhaps, light-induced changes occur only over long periods of time. Also, we were uncertain whether changes would occur in younger trout. A second experiment was begun, therefore, to investigate light-induced changes in the rhodopsin–porphyropsin ratio of younger trout (11 months old). Brook, brown, and rainbow trout were exposed to white tungsten lamps in continuous and cyclic (12L/12D) regimes, or in continuous darkness for a total period of 5 weeks. Water temperature was 5 ± 1°C. The irradiance at the water surface was 162 × 10^{12} photons/cm²

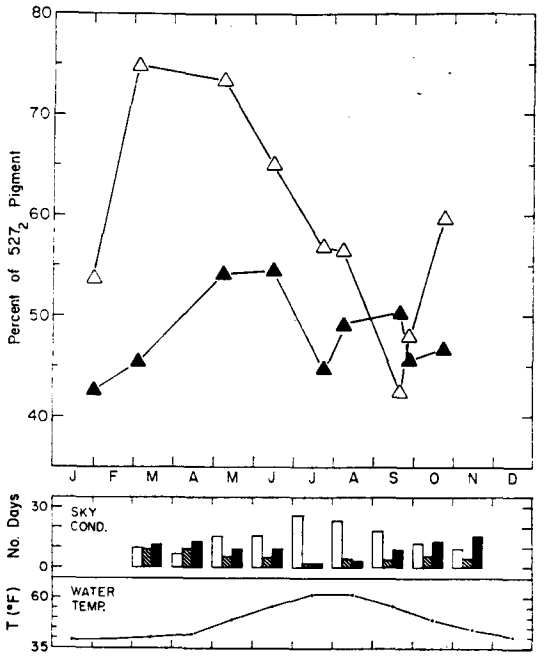

FIG. 2. A composite presentation of data on seasonal changes in percentage of porphyropsin (VP 527_2) in the retina of *Salmo clarki*, collected from Lookout Creek, Lane County, Oregon. Values plotted occurred over a period from 9/67 to 2/70 (reported also in Table 2). Open triangles are fish collected from clear-cut area, and closed triangles represent fishes taken from adjacent, shaded portions of the stream. Changes in light, compiled from data obtained from U.S.F.S. (Blue River, Oregon), shown in lower figure as number of clear (open bars), partly cloudy (hatched), or totally overcast days (darkened bars). Seasonal water temperature in lower portion of Lookout Creek was reported by U.S.F.S. in fahrenheit degrees. Values shown are mean monthly temperatures, max. in centigrade = 16.5°C, min. = 3.5°C. Temperature of stream did not differ more than 1°C between adjacent open and shaded collecting sites.

TABLE 3

The long-term effect of different photic conditions on the percentage porphyropsin in the retina of brook trout. Groups of trout were raised under either continuous light (CL), variable light (VL), or continuous darkness (CD) from the time of hatching until sampling during their 3rd year. Fishes spawned in October–November of 1966. Values are the mean percent 527_2 visual pigment ± 95% C.I., sample size, and range (*r*) of percentages. Temperature was constant at 8 ± 0.5°C

Sampling date	Photic condition		
	CL	VL	CD[a]
Jan. 1969	63.8 ± 6.4 (10) (50.2–74.0)	61.0 ± 5.2 (9) (54.4–72.5)	16.8 ± 7.1 (10) (7.7–39.8)
Apr. 1969	61.1 ± 7.6 (10) (47.1–81.0)	56.1 ± 7.8 (10) (40.1–69.2)	27.6 ± 13.9 (10) (3.5–60.7)

[a] The CD groups are significantly lower ($P < 0.05$) than the VL and CL groups.

per second (400 to 700 nm). Groups of fish from each species were sampled initially, and from each treatment at 5 weeks and 15 weeks.

The initial percentages were arranged in the same sequence (by species) as found earlier in the raceway study, i.e., brown trout were highest and brook trout lowest in percent porphyropsin. This ranking was maintained under the different light conditions (Fig. 3). The response to continuous light and continuous darkness shown by both rainbow and brook trout was similar to that shown by brook trout reared in CL and CD conditions for 3 years (Fig. 3 vs. Table 3). Darkness clearly favored rhodopsin, and light favored porphyropsin, at 5 and 15 weeks. However, in the second experiment, both brook and rainbow trout in VL light had porphyropsin levels about one-half of the level they reached in constant light, whereas, in the earlier experiment, brook trout reared in VL and CL light did not differ (VL, Fig. 3 vs. VL, Table 3). Perhaps this difference in response relates to the subtle difference between the VL treatments in the two experiments. In the earlier, long-term experiment, a seasonally changing photoperiod was used, whereas in the VL regime of the second experiment, a constant 12L/12D photocycle was given.

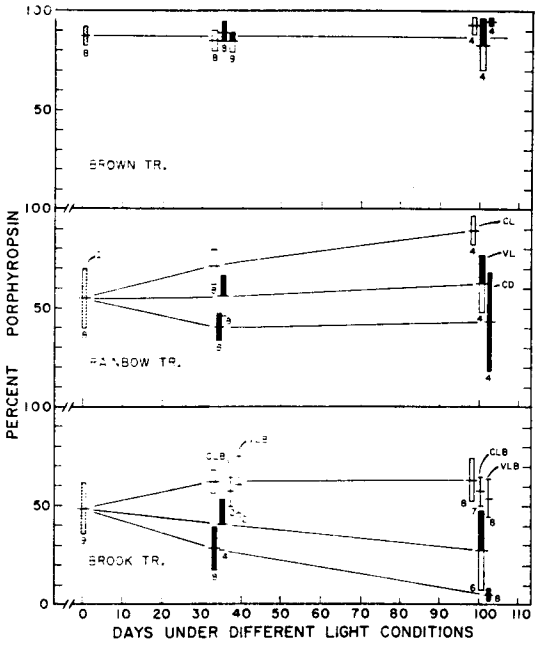

FIG. 3. Changes in percentage of porphyropsin in the retina of brown, rainbow, and brook trout held under various experimental photic conditions. Symbols represent the mean percent (horizontal line) and the 95% confidence interval (C.I.) about the mean (vertical bars or vertical lines) of each sample. Number of fish in a sample are indicated by the number at the bottom of each symbol. Lighting was incandescent. Different light treatments are indicated by different symbols for the rainbow trout and are the same for brown and brook trout: CL, continuous light; VL, 12 h light and 12 h darkness; CD, continuous darkness. Light intensity between 400 and 700 nm was 162×10^{12} photons/cm^2 per second. Two groups of brook trout were exposed to incandescent light, 56 times more intense from the above and the groups indicated as VLB (bright variable light, 12 on/12 off) and CLB (bright continuous light).

No significant change was induced in the brown trout pigments and it would appear, when our other evidence is considered (Table 1), that the rhodopsin–porphyropsin ratio of the brown trout is stable under different light conditions (Fig. 3).

Is there an effect of light intensity? To answer this, we held an additional group of brook trout in bright, continuous light and one group in bright, 12L/12D light (CLB and VLB, Fig. 3). The brighter light, 56 times as intense as that given in the dimmer treatments (CL and VL, Fig. 3), eliminated the differences in porphyropsin found among the dimmer groups (Fig. 3). This suggests that intensity has an effect on the rhodopsin–porphyropsin system of brook trout held in a 12L/12D regime.

We also tested the effect of bright, artificial light on the visual pigments of cutthroat trout, which had been held for 2 weeks indoors at 12°C. In 11 days, porphyropsin in fish which were held in continuous, bright light (500-W Quartzline lamp) rose from 57.1% (SD = 10.8, N = 15; initial controls) to 70.6% (SD = 7.8, N = 8). Unfortunately, effects of continuous darkness were not investigated. However, the direction of the effect of bright light, which increased porphyropsin, is similar to the effect of bright sunlight (Table 2, open vs. shade samples).

In view of these results, it seems clear that light, or its absence, can produce significant changes in the paired pigments of some trout (rainbow, cutthroat, and brook trout) over rather short periods of time, and that these changes are reinforced by longer periods of exposure (Fig. 3). In addition, the intensity of light may influence its effectiveness in maintaining a given proportion of porphyropsin in brook trout. The visual pigments of brown trout, however, are unaffected by different light conditions.

3. Regeneration of Brown and Brook Trout Visual Pigments

We consistently found a very high percentage of porphyropsin in brown trout, regardless of experimental conditions (VL and CD, Fig. 3). Other studies have demonstrated the presence of retinol and 3-dehydroretinol prosthetic groups in the P.E. and the dependence of rhodopsin-porphyropsin ratio on a given ratio of retinol/3-dehydroretinol in the P.E. (Bridges and Yoshikami 1970*c*, *Scardinius*; Reuter *et al.* 1971, *R. catesbeiana*; Bridges 1972, Fig. 34). The consistently high porphyropsin level in brown trout retinae may indicate a lack of effect of light, or of darkness, on the dehydrogenase system in the P.E. of their eyes as compared to other trout. On the other hand, it may result from incompatibility between brown trout opsin and retinol, or an equilibrium that favors combination with 3-dehydroretinol. To test the latter possibilities, an interspecific regeneration of brown and brook trout opsin (derived from washed, bleached retinae) was performed in the presence of either high retinol concentrations or high 3-dehydroretinol concentrations (derived from dark-treated brook trout and brown trout P.E., respectively). We used retinal homogenates after the use of intact retinae and P.E. failed to produce measurable regeneration (see Methods 5 for details).

We reasoned that the brown trout opsin would show little regeneration in the presence of high levels of retinol if an incompatibility exists. Further, if the amount of regeneration was low, but favored porphyropsin, even when the relative level of retinol was high, then we could conclude that brown trout opsin favors an equilibrium biased toward combination with 3-dehydroretinol. If incompatibility does not exist in any combination of opsin and prosthetic group, then we would expect that the proportion of porphyropsin pigment regenerated would reflect the actual proportions of retinol and 3-dehydroretinol available, regardless of which opsin we used.

Retinae from brown trout that had been kept under natural light and from dark-treated brook trout (in darkness 5 months) were used as sources of opsin and P.E. The fish were dark-adapted for 8 h and a single retina of one brown and one brook trout were extracted following normal procedures (Methods 1). Analysis confirmed that the brown trout had 94.1% and the brook trout only 9.1% porphyropsin. Thus, the brown trout P.E. was presumed to contain 94% of the 3-dehydroretinol and 6% retinol; the brook trout P.E. presumably contained only 9% of the 3-dehydroretinol and 91% retinol. With this information in hand, we proceeded to perform the cross-regeneration experiments.

The contralateral eyes had been removed to serve as our opsin and P.E. preparations. Each retina was dissected out and thoroughly bleached with amber light for 15 min and immediately washed. Opsin preparations and P.E. preparations (homogenates) were made from the two eyes as described in Methods 5, except that each P.E. was halved along a dorsal–ventral plane to provide for two of each preparation from each fish. After storage, the sonicated brown and brook opsin preparations were added to the brown and brook P.E. preparations in the four desired combinations and allowed to regenerate in darkness (see Methods 5).

The amount of visual pigment regenerated in all four combinations exceeded 0.24 of the amount of visual pigments extracted previously from the contralateral eye. This is a substantial yield, and is interesting when compared to earlier studies by Collins *et al.* (1953), who achieved substantial yields of regenerated bovine rhodopsin by adding either all-*trans* vitamin A alcohol or retinene to retinal homogenates in the presence of phosphate buffer (pH 7.4). In other studies, Wald and Brown (1950) were able to achieve regeneration of rhodopsin by adding the specific 11-*cis* isomer to bleached rhodopsin solutions and Hubbard and Wald (1952) showed that all-*trans* retinol added to a solution of bleached rhodopsin produced no regeneration unless it had been isomerized to the 11-*cis* form. Our regeneration results are interesting, since they indicate that in fishes, addition of a specific isomer of retinene is not necessary; only sonified P.E. and digitonin micelles of the opsin are required to yield regeneration of either rhodopsin or porphyropsin.

The rhodopsin regenerated by the brown trout opsin/brook P.E. homogenate was clearly brown trout rhodopsin (λ_{max} = 508 nm, Dartnall's (1953) nomogram). Also, only brook trout porphyropsin regenerated from the brook opsin/brown P.E. homogenate (λ_{max} = 527 nm, nomogram of Munz and Schwanzara 1966). The expected and actual percentages of porphyropsin

obtained from the four possible combinations of opsin and P.E. are tabulated in Table 4. Clearly, there is no incompatibility of brown trout opsin toward the retinol prosthetic group (brown opsin + brook P.E., Table 4). On the other hand, the brook trout opsin showed no inability to combine with the 3-dehydroretinol molecule (brook opsin + brown P.E., Table 4). Therefore, differences in the affinity of either opsin for one of the prosthetic groups are probably not involved in the control of visual pigment mixtures (a possibility suggested by Beatty 1969*b*). It is more likely that other mechanisms are responsible for maintaining the different levels of porphyropsin which we have observed (lowest in brook trout, highest in brown trout). One very attractive possibility is the control of the proportions of retinol and 3-dehydroretinol in the P.E. by light, or some other environmental factor. A somewhat similar conclusion was reached by Bridges and Yoshikami (1970*c*): see Discussion 1.

Discussion

1. *Effects of Photic Conditions, Experimental and Seasonal*

When brown, brook, and rainbow trout were held under identical photic conditions in a raceway, the percentage of porphyropsin in the retinae are different (Table 1). Brown trout have the highest and brook trout the lowest percentage. Therefore, the mechanisms underlying rhodopsin–porphyropsin balance must be somewhat different in each species. That the differences exist is emphasized by the data on the effect of light and darkness on the three species. Artificial light favored porphyropsin, and darkness caused a switch to the rhodopsin pigment in both brook and rainbow trout. However, light or darkness had no effect on the rhodopsin–porphyropsin ratio of brown trout, which always remained high in porphyropsin (Fig. 3). The general picture becomes complex when comparisons are made between these effects, and the effects of light and darkness reported for other species (Table 5). Clearly, light, or its absence, may have opposite effects on the paired visual pigments of different species or, as in the brown trout *Salmo trutta*, no effect whatsoever. In four species (three cyprinids, one poeciliid) light favors rhodopsin and darkness porphyropsin (Table 5). In three species of fishes (two trout, one cyprinid) and in tadpoles of *Rana* (three species) (Bridges 1970), light favors porphyropsin and darkness rhodopsin (this paper and Allen 1971). In spite of the opposite effects of light or darkness in the various species, there is an overall trend toward increased porphyropsin in winter and increased rhodopsin in summer (Table 5). Clearly, no single mechanism can account for this complex picture. What differences in mechanisms could account for this paradoxical situation?

An hypothesis to explain the effect of light in the rudd (Bridges and Yoshikami 1970*c*) relates light absorption in the pigment epithelium (supposedly the "myeloid bodies" of Yamada 1961) to the hydrogenation of 3-dehydroretinol to form retinol. Most likely this results from a change in activation of a dehydrogenase(s) and a resultant shift in the equilibrium between free retinol and 3-dehydroretinol. With this kind of mechanism, increased light intensity would produce a shift from high levels of porphyropsin to high levels of rhodopsin in the rudd and, presumably, the

TABLE 4

Expected and actual percentages of porphyropsin visual pigment of brown and brook trout regenerated in the presence of high and low percentages of retinol and 3-dehydroretinol, as assumed from P.E. source. Expected percentages of porphyropsin (VP_2) based visual pigment represent the actual visual pigment balance measured from one retina of the brown and the brook trout used in the experiment. Actual percentages are results from analyses of the regenerated pigments following bleaching (contralateral eyes)

	Pigment epithelium source			
	Brook trout		Brown trout	
Opsin source	Expected $\%VP_2$	Actual $\%VP_2$	Expected $\%VP_2$	Actual $\%VP_2$
Brook	9.1	12.2	94.1	91.6
Brown	9.1	0.0	94.1	88.7

opposite condition in trout. It appears that the visual pigment constitution of the outer segments of the rods results from the free retinol/3-dehydroretinol ratio in the pigment epithelium (Wald 1939, fishes; Wilt 1959, bullfrogs; Dowling 1960, rats; Bridges and Yoshikami 1970*c*, on rudd; reviewed by Bridges 1972). The regeneration experiments between brown trout and brook trout opsin and pigment epithelium extracts (Table 4) reinforce this conclusion. Thus, light must act on the pigment epithelium (directly or indirectly) and, in *Scardinius*, *Notemigonus*, and *Belonesox*, favor a low free 3-dehydroretinol level. In the trouts, *Richardsonius*, and in tadpoles of *Rana*, light must favor a high free 3-dehydroretinol level (and a low retinol level) in the pigment epithelium (Table 5). Perhaps a general explanation that will encompass these paradoxical circumstances under a single bleaching–resynthesis mechanism can be found. It seems as likely, however, that two different mechanisms are involved.

Seasonal shifts in visual pigments have been attributed to seasonal changes in light quality in *Scardinius* (Dartnall *et al.* 1961) and *Belonesox* (Bridges 1965*b*). However, if light is the major factor that produces seasonal shifts in the visual pigments of cutthroat trout and *Richardsonius*, these species might be expected to show higher levels of porphyropsin in summer and lower levels in winter, in keeping with the effect of artificial light. But clearly they do not respond this way, instead having highest porphyropsin in winter and lowest in summer (Table 5). In fact, to the best of our knowledge, no species attains low porphyropsin in winter and high porphyropsin in summer. (The burbot, *Lota*, seems to be in between, Table 5). We can only conclude that where seasonal shifts are contrary to changes induced by photic treatments (trout, *Richard-*

TABLE 5

A summary of seasonal changes and light-induced changes in the paired pigments of fishes. Plus (+) or minus (−) signs indicate that porphyropsin increases or decreases relative to rhodopsin

Species	Max. for pigment pair	Mean % porphyropsin			Experimental	
		Seasonal max.	Seasonal min.	Locality	Light	Dark
Salmonidae						
Salmo gairdneri	503_1–527_2	Slightly variable 62–79%, 31% in 3 yr. olds in Nov., temp. 8 ± 0.5°C		New York	(+)	(−)
		74–88%, natural photoperiod, temp. constant 10°C		Alberta[a]		
Salmo clarki	503_1–527_2	75% Mar. – 40–50% Sept., unshaded, temp. seasonal		Oregon	(+)	(?)
Salmo trutta	507_1–534_2	Slightly variable 70–86%, temp. constant 8 ± 0.5°C		New York	(No clear effect)	
Salvelinus fontinalis	503_1–527_2	Variable 44–58%, 31% in 3 yr. olds in Nov., temp. constant 8 ± 0.5°C		New York	(+)	(−)
Oncorhynchus nerka[b]	503_1–527_2	Constant at about 15%, seasonal photoperiod, temp. 10°C, landlocked		Alberta	(Unknown)	
Cyprinidae						
Scardinius erythrophthalmus[c,d]	507_1–535_2	80% Jan.	15% Aug.	England	(−)	(+)
Notemigonus crysoleucas boscii[e]	502_1–529_2	78% Jan.	30% Aug.	Florida	(−)	(+)
Notemigonus c. aureatus[f]	504_1–530_2	96% Dec.	49% Aug.	New York	(−)	(+)
Richardsonius balteatus[g]	506_1–530_2	97% Jan.	13% Aug.	Oregon	(+)	(−)
Poeciliidae						
Belonesox belizanus[h]	498_1–521_2	83% Jan.	14% Aug.	Florida	(−)	(+)
Gadidae						
Lota lota[i]	503_1–527_2	52% Oct. Highest Sept.–Dec.	13% Mar. Lowest Mar.–June	Alberta	(Unknown)	

[a] Jacquest and Beatty (1972).
[b] Beatty (1969*a*).
[c] Dartnall *et al.* (1961).
[d] Bridges and Yoshikami (1970*a*).
[e] Bridges (1964, 1965*a*).
[f] Allen and McFarland (1973).
[g] Allen (1971).
[h] Bridges (1965*b*).
[i] Beatty (1969*b*).

NOTE: Bridges (1970) has shown that light increases porphyropsin and darkness increases rhodopsin in frog tadpoles, three species of *Rana*, but data on seasonal variation in larvae or adult frogs is not available.

sonius), light is but one of many factors involved and may have little influence on the seasonal shifts.

2. Effects of Temperature

Temperature may play a role in regulating rhodopsin–porphyropsin ratio. Rhodopsins are thermally more stable than their porphyropsin counterparts (Bridges 1972). Bridges (1956) found that porphyropsin from rainbow trout decayed at a rate 40 times as fast as rhodopsin in mixed extracts. A similar but only 10-fold difference was reported by Williams and Milby (1968) between larval porphyropsin and adult rhodopsin in *Rana catesbeiana*, but the λ_{max} of the porphyropsin was 516 nm, indicating that some rhodopsin was present. Temperature may also significantly affect activation of the retinol / 3-dehydroretinol dehydrogenase, or other factors, such as chromophore exchange in the rod outer segments. Is it possible that seasonal temperature cycles might be involved in controlling the seasonal changes in the paired pigments in fishes?

Examination of the rhodopsin–porphyropsin ratio for native cutthroat trout sampled from clear-cut areas revealed that porphyropsin is highest in late winter and spring, and lowest in late summer and early fall (Fig. 2). These shifts do follow environmental temperature when allowance for some seasonal lag is made (Fig. 2, composite of sampling over $2\frac{1}{2}$ years). In this case, the action of increasing water temperature would be to eventually favor rhodopsin, and perhaps the warming temperatures in late summer are the reason that the differences in light flux among the photic zones did not have a greater effect at this time of year. In *Richardsonius* (Oregon, Allen 1971) and in *Notemigonus* (New York, Allen and McFarland 1973), similar seasonal changes in percentage of porphyropsin follow seasonal temperature more closely. Perhaps lower wintertime temperatures in the habitat of these fishes improves the thermal stability of porphyropsin and (or) favors porphyropsin in the overall reaction sequences leading to its incorporation into the retina.

Consider the unusual findings that rainbow and brook trout, when maintained in a raceway for almost 2 years, showed little change in percentage of porphyropsin until sexual maturation occurred (Table 1). Throughout this period the fish were maintained in an open raceway where water depth did not exceed 2 ft, cover was not available, and water clarity was always high. Although total light dose changed seasonally, during each day the light intensity impinging on each fish remained quite high. Why did the percentage of porphyropsin not decline in summer as in the native cutthroat trout (Fig. 2)? The raceway water temperature remained relatively constant (7.5 to 8.5°C) in contrast to the seasonal temperature of the stream containing the cutthroat trout (3.5 to 16.5°C). The relatively high levels of porphyropsin in rainbows and brook trout under these circumstances might be expected if the bright outdoor light did favor porphyropsin and was not opposed by high temperature (see also CLB and VLB, Fig. 3).

Although consistent with the facts relating rhodopsin–porphyropsin balance to water temperature, our suggestions have been inferential, for the effect of different temperatures on paired-pigment balance in fish held under identical photic conditions has not been reported. However, in a recent experiment, we have shown that the cyprinid *Notemigonus chrysoleucas* (*boscii* and *aureatus*) reacts to high temperatures (20°C) by changing over to rhodopsin from the high percentage of porphyropsin that it maintains in colder water (7°C) (Allen and McFarland 1973). We also now have evidence that the same situation occurs in the rainbow trout (McFarland and Allen, unpublished). Since these effects occur regardless of light conditions in both species, it seems clear that water temperature may play an important role in producing shifts in the rhodopsin–porphyropsin ratio. Also of note is the fact that rainbow trout and *Notemigonus* respond differently to light and darkness (Table 5). Thus, the similarity of their response to temperature indicates that temperature may be partly responsible for the overall similarity in seasonal response displayed by the species *Richardsonius*, *Salmo clarki*, *Scardinius*, *Belonesox*, and *Notemigonus*, which respond differently to light and darkness (Table 5).

3. Adaptive Value of Seasonal Changes in Visual Pigments

What are the consequences of seasonal changes in the paired pigments of fishes? Primarily, the change to a retina with high porphyropsin during winter would increase visual sensitivity in the yellow region of the spectrum. Perhaps, during

winter, the greater path length that solar radiation must take to penetrate the atmosphere and reach the northern latitudes increases the contribution of "red" photons. This idea has been considered by Munz (1965), but it requires documentation. It has been suggested that the unique decline in yellow-orange light during twilight and the dramatic changes in behaviors of tropical coral-reef fishes which occur during this period have limited the λ_{max} of reef fish visual pigments to a narrow spectral band. This restriction, in turn, endows the fishes with high photosensitivity to available light (Munz and McFarland 1973). Similar spectral changes occur at twilight in temperate latitudes, but seasonal variation has not been measured. A precise study of the spectral distribution of light in various freshwater habitats would benefit understanding of the functional significance of seasonal changes in paired visual pigments.

Other ecological conditions that modify hue of light available to the fish are turbidity and dissolved or suspended material. Generally, these factors attenuate light and produce a shift in spectral distribution toward the red (see Bridges 1972). Therefore, shifts to porphyropsin should enhance visual sensitivity when these conditions occur during winter. Bridges (1965*a*) has suggested this as a possible advantage accruing from the higher proportion of porphyropsin he found in *Notemigonus* taken from turbid waters in Florida. Often, however, lacustrine habitats, particularly in northern regions, become clear and "bluer" during winter, not turbid and "redder." At this time, therefore, there seems to be no general explanation of the functional significance of seasonal changes in the visual pigments.

It is clear, even to the casual observer, that some streams are more turbid than others. Possibly, brown trout have adapted their visual pigment absorbance for life in more turbid streams. They have a unique 508_1–534_2 pigment pair, and the level of porphyropsin (534_2) always remains high. Therefore, the brown trout is always scotopically more sensitive to red light than other trouts. In New York, brown trout are more characteristic of warmer, turbid streams than other trout (Embody 1922; and personal observations). Interestingly, Dartnall (1962) reported finding a larger proportion of rhodopsin in european brown trout that we have observed in hatchery-reared brown trout. He also indicated that the rhodopsin–porphyropsin ratio was labile. This may be true. Brown trout are less sensitive to warm temperatures than most other trout, and, as yet, we have not investigated the effects of elevated temperatures on their paired-pigment balance. At the moment, we can only say that the visual pigment system of the brown trout is, in many respects, very different from the other trout we examined.

We cannot yet ascribe a general function to the changes in the proportions of paired visual pigments of trout or of other fishes. Because of their differences, however, trout do stress the complexity of the question of how seasonal changes in the visual pigments are controlled. Hopefully, the responses we have reported will serve to aid further research that will yield answers on the basic mechanisms and adaptive functions of changes in the visual pigments of fishes.

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