THE VISUAL SYSTEMS OF EURYHALINE FISHES

By GEORGE WALD*

(From the Biological Laboratories of Harvard University, Cambridge)

(Received for publication July 8, 1941)

The red photosensitive pigment rhodopsin participates with the carotenoids retinene$_1$ and vitamin A_1 in a cycle found almost universally in the rods of vertebrate retinas. All terrestrial vertebrates and all marine fishes so far examined except the Labridae possess this system. The true freshwater fishes, however, have evolved a different retinal cycle, compounded of the purple photopigment porphyropsin, with retinene$_2$ and vitamin A_2 (Wald, 1936–39).

For this reason a special interest attaches to the situation in the euryhaline fishes, which, though usually restricted in spawning either to the sea (catadromous) or to fresh water (anadromous) can exist otherwise in both environments. One may expect a distinctive distribution of visual systems within this group; and these animals alone, since they pass readily from one environment to the other, permit a test of whether the visual system is fixed genetically or varies with the history of the individual.

A survey of the eye and liver vitamins A of several euryhaline fishes has already been reported (Wald, 1938–39*a*). The retinas contain either mixtures of both vitamins A, in which that commonly associated with the spawning environment predominates; or exclusively the spawning type of vitamin A. The vitamin A configurations of both eye tissues and liver are primarily determined genetically, and are relatively independent of the environment.

The present paper is concerned with an examination of the visual systems of certain euryhaline fishes: the anadromous salmonids, white perch and alewife; the catadromous eel; and the killifish, which verges on the catadromous condition.

I. Methods

The methods used in this investigation are identical with those described previously (Wald, 1936–39). Photopigments were extracted with aqueous digitonin solution from dark adapted retinas previously hardened with alum and neutralized with phosphate buffer. The absorption spectrum of rhodopsin prepared in this way is maximal at about 500 mμ, that of porphyropsin at about 522 mμ.

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* This research was supported in part by a grant from the Milton Fund of Harvard University. I am indebted also to Mr. J. Arthur Kitson of the Division of Fisheries and Game, Department of Conservation of the Commonwealth of Massachusetts, for gifts of live pond and hatcheries fishes.

The carotenoid components of the retinal cycle were examined in a standardized procedure, which yielded the data shown in Figs. 1, 2, 4, and 6. Retinas were extracted by shaking with petroleum ether (benzine) in three singular states: (1) dark adapted; (2) immediately on exposure to bright light, which bleaches the photopigments to orange products; and (3) after 1 hour at room temperature following irradiation, during which the retinas fade to colorlessness. In stenohaline marine or fresh water fishes respectively, the dark adapted tissues yield small residual amounts of either vitamin A_1 or A_2. The just irradiated retinas yield large quantities of retinene$_1$ or retinene$_2$, liberated in the bleaching of photopigment. Extracts of bleached and completely faded retinas contain large amounts of vitamins A_1 or A_2, formed from the retinenes during the fading process. In each case, the benzine extract was transferred to chloroform and tested by mixing with antimony chloride reagent. In this reaction, the retinenes and vitamins A yield blue colorations due to absorption bands in specific positions: retinene$_1$ at 664 mμ, retinene$_2$ at 705 mμ, vitamin A_1 at 615–620 mμ, and vitamin A_2 at 693–696 mμ.

All spectra but those of Fig. 7 were recorded automatically with Hardy's photoelectric spectrophotometer at the Massachusetts Institute of Technology. The spectra of Fig. 7 are transcriptions of similar recordings. Absorptions are expressed as percentage absorption, $1 - I/I_0$, in which I_0 is the incident and I the transmitted intensity; or as extinction or optical density, $\log I_0/I$.

II. Salmonids

Members of three different genera of the family Salmonidae have been examined: the brook trout, *Salvelinus fontinalis*; the rainbow trout, *Salmo irideus*; and the chinook or king salmon, *Oncorhynchus tschawytscha*. All these fish are anadromous and hence potentially migratory. All of them, however, can, and on occasion do pass their entire life cycles without detriment in fresh water. They are to be regarded as fresh water fishes which—since euryhaline—can migrate seaward. The chinook salmon performs this migration commonly, the brook trout more rarely, becoming in the process a sea trout, and the rainbow trout most rarely of all. It should be noted, however, that the fish used in the following experiments were obtained from a hatchery, and had never left fresh water.

These fish all possess mixtures of the rhodopsin and porphyropsin systems, predominantly the latter. This is shown most clearly in tests of the retinal carotenoids. Data from the brook trout and salmon are shown in Figs. 1 and 2; those from the rainbow trout are very similar in appearance. Extracts of dark adapted retinas, tested with antimony chloride, yield low absorption spectra, displaying a small vitamin A_1 inflection at about 620 mμ and a much higher vitamin A_2 band at about 696 mμ (Figs. 1 and 2, curves *a*). Similar extracts of just irradiated retinas in which the photopigments had been bleached to orange products yield high, peculiarly broad bands, maximal at 686–691 mμ, due to fusion of the absorption bands from retinene$_1$ and retinene$_2$, at 664 and 705 mμ respectively (curves *b*). Bleached retinas which had been allowed to fade before extraction yield high double-banded absorptions, displaying a large vitamin A_2 and a smaller vitamin A_1 component (curves *c*). The location of the vitamin A_1 band on the shoulder of the vitamin A_2 absorption shifts its maximum somewhat toward the red. The proportions of both vitamins in these extracts have already been reported (Wald, 1938–39*a*). Clearly both

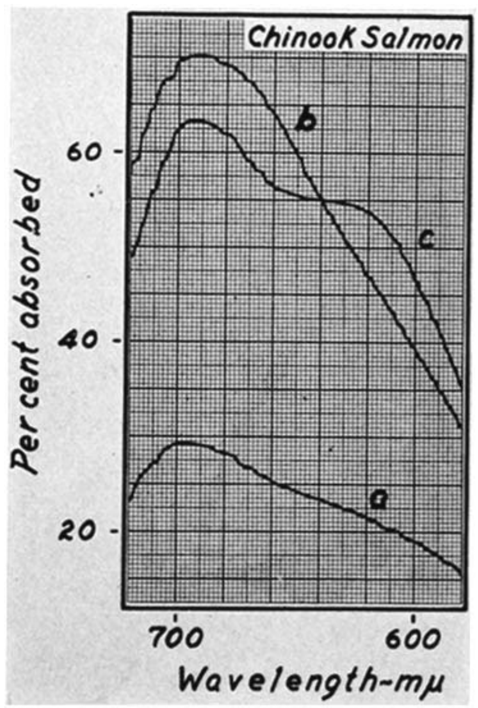

FIG. 1. Spectra of the antimony chloride reaction with benzine extracts of seven chinook salmon retinas: (*a*) dark adapted; (*b*) on irradiation with bright daylight; and (*c*) after 1 hour at room temperature following irradiation.

of them, but predominantly vitamin A_2, participate actively in the retinal cycles of these fishes.

The duplex character of these visual systems is reflected also in the absorption spectra of their photopigments (Fig. 3). The photopigment absorption maxima occur at 510–515 mμ, approximately halfway between those of rhodopsin (500 mμ) and of porphyropsin (522 mμ). They represent mixtures of both pigments.

The bleaching of brook trout photopigment is also shown in Fig. 3. This is intermediate at all stages between the bleaching of rhodopsin and of porphyropsin described previously (Wald, 1937–38; 1938–39*b*).

III. Eel and Killifish

The so called freshwater eel, *Anguilla rostrata,* is a typically catadromous fish. It spawns in sea water of great depth and salinity, and ordinarily enters fresh water after more than a year of marine existence. Its history therefore is directly the reverse of that of the salmonids—it is essentially a marine fish which euryhalinity permits to migrate into fresh water.

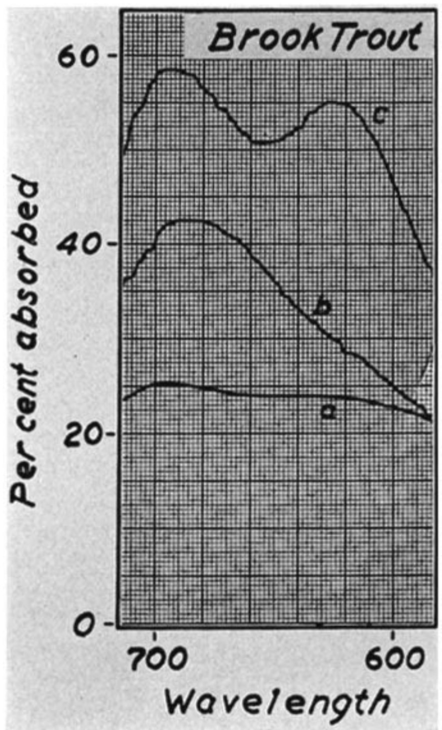

FIG. 2. Spectra of the antimony chloride reaction with extracts of seven brook trout retinas. Otherwise as in Fig. 1. Curve *a* is raised above its correct level due to turbidity of the solution.

The eel, like the salmonids, possesses a mixture of the rhodopsin and porphyropsin systems, but in reverse proportions. This is demonstrated in Fig. 4. Each of the spectra in this figure resembles a mirror-image of its analogue in Figs. 1 and 2. Curves *a* and *c* display the bands of both vitamins A, in this case with the A_1 band dominant and in the correct position, while the A_2 band is shifted toward shorter wavelengths by its location on the rising limb of the A_1 absorption. Similarly, the maximum of the fused band of the retinenes (curves *c*) occurs in this case at 672–674 mμ, close to the band of the dominant retinene$_1$.

Absorption spectra of eel photopigment solutions are shown in Figs. 5 and 7. Though these preparations contain an admixture of porphyropsin, their maxima occur at 498 and 502 mμ, within the range of rhodopsin maxima. The bleaching of eel photopigment, shown in Fig. 5, also closely resembles that of rhodopsin.

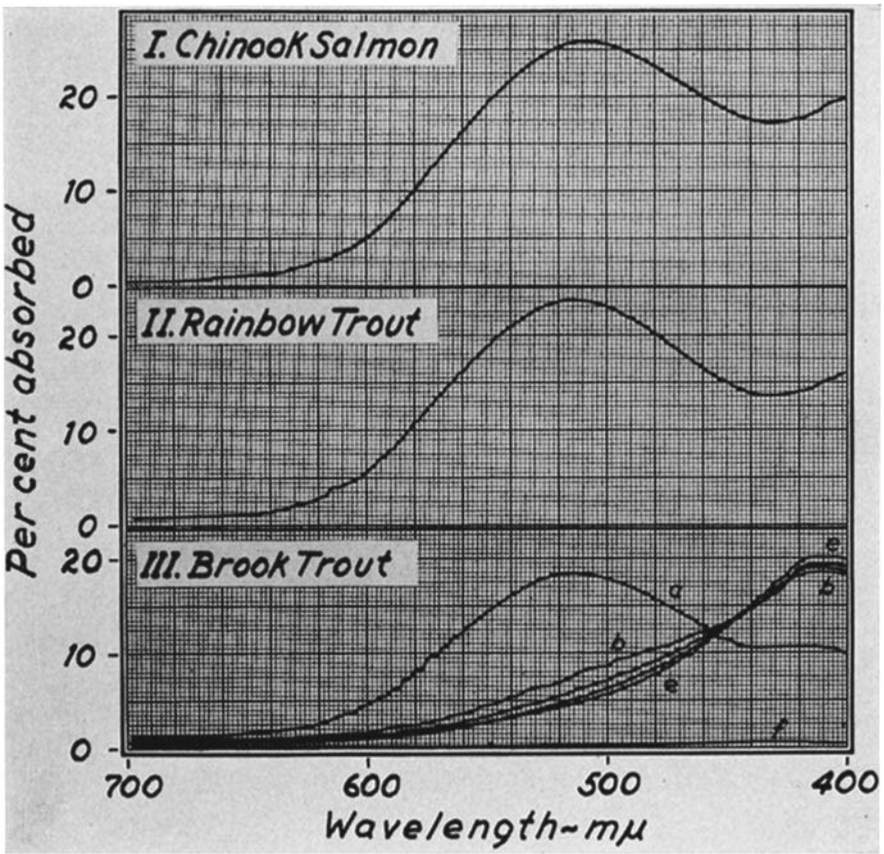

FIG. 3. Spectra of photopigments from salmonid retinas. All these fish were yearlings, about 7–8 inches long. The total extracts in 4 cc. of digitonin solution were measured in a layer 1 cm. thick. I. Eight chinook salmon retinas. II. Six rainbow trout retinas. III. *a*, six brook trout retinas. This solution was exposed to bright light for ½ minute and its spectrum thereafter re-measured in darkness, *b* at 1.7 minutes, *c* at 4.5, *d* at 13.7, and *e* at 60.5 minutes (26.7°C.). *f* is the control spectrum of the solvent alone.

It is clear that the eel, in accord with its catadromous nature, possesses a mixture of both the rhodopsin and porphyropsin systems, with a great predominance of the former.

The killifish, *Fundulus heteroclitus*, is remarkable in being euryhaline both as embryo and adult. Indeed its embryo is reported to develop and hatch normally even in distilled water (Loeb and Cattell, 1915). Conceivably, therefore, this fish might spawn in either salt or fresh water, and so cannot be classed strictly as either anadromous or catadromous.[1] Actually it inclines markedly throughout its life cycle toward the sea. It is commonly regarded

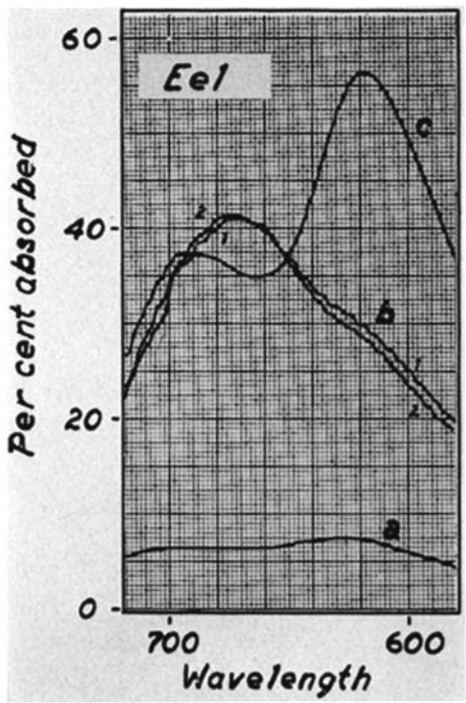

FIG. 4. Spectra of the antimony chloride reaction with extracts of seven eel retinas. Otherwise as in Fig. 1. Due to fluctuation in the spectrophotometer record, curve *b* was re-drawn.

as marine, and only rarely penetrates to fresh water. I know of no record of its spawning in fresh water. Ordinarily it maintains a stable if equivocal position close to shore and in tidal parts of streams; and this appears to be due as much to a predilection for shallow as for brackish water (*cf.* Bigelow and Welsh, 1924). In fresh water in the laboratory, it usually survives only a matter of several weeks, but this circumstance is complicated by its great sus-

[1] Dr. E. N. Warner of Ohio State University informs me, however, that he has attempted to fertilize *Fundulus* eggs in tap water without success, apparently because this medium rapidly inactivates the sperm.

ceptibility while in this medium to fungal attack, notably by *Saprolegnia* (Sumner, 1905). Among euryhaline fishes it is certainly much closer to the catadromous than to the anadromous way of life.

The visual system of *Fundulus* closely resembles that of the eel. A spectrum of its photopigment was published among those of permanently marine fishes before its peculiar status had been appreciated; significantly, in this collection

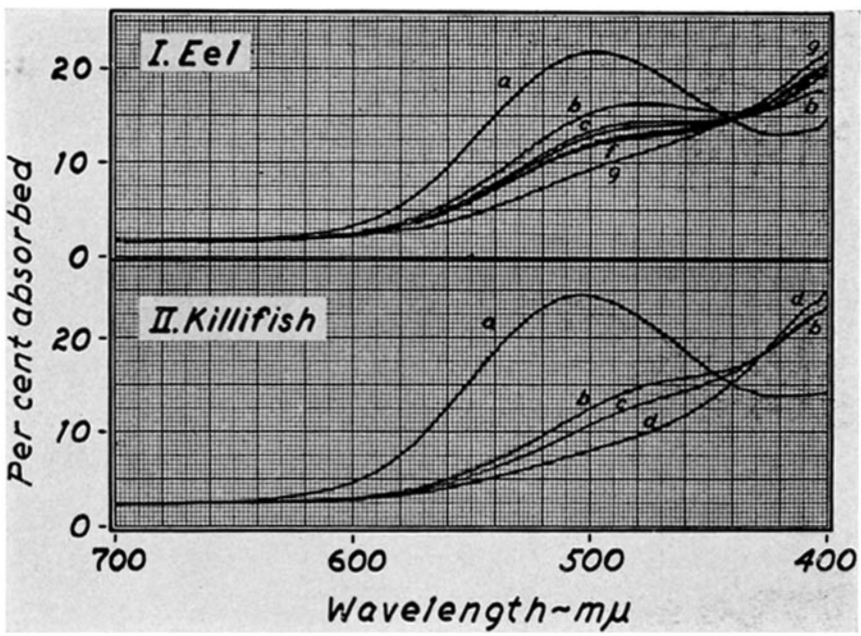

FIG. 5. I. *a*, Spectrum of photopigment from the retinas of a single eel, 26 inches long, in 4 cc. of digitonin solution, measured in a 1 cm. layer. Following 20 seconds exposure to bright light, the spectrum was re-measured in darkness, *b* at 1.5 minutes, *c* at 4.0, *d* at 6.3, *e* at 17.7, and *f* at 37 minutes. The preparation was re-irradiated or 20 seconds, and at 39.5 minutes yielded curve *g* (pH 6.3; 27°C.). II. *a*, Spectrum of photopigment from ten killifish. This preparation was irradiated for 30 seconds, and spectra re-drawn in darkness, *b* at 1.7, *c* at 4.0, and *d* at 31 minutes (pH 7.0).

of otherwise pure rhodopsin spectra that of *Fundulus* occupies the extreme long wavelength position, with a maximum at 502 mμ (Wald, 1937–38, Fig. 1). The spectrum of another preparation, maximal at about 505 mμ, is shown in Fig. 5. *Fundulus* spectra in general vary within about the same range as those of the eel (Fig. 7). That they represent mixtures of both rhodopsin and porphyropsin is shown by examination of the retinal vitamins A. An extract of bleached and faded retinas tested with antimony chloride displays the bands of both vitamins A_1 and A_2 with the former greatly predominant.

IV. White Perch and Alewife

The white perch (*Morone americana*) and the alewife (*Pomolobus pseudoharengus*) are typically anadromous. Unlike the salmonids each of them possesses close relatives among permanently marine forms. The visual systems of two of the latter have already been described: the black sea bass, a serranid like the white perch; and the common herring, a clupeid like the alewife (Wald, 1936–37; 1938–39*a*). Both possess pure rhodopsin systems. It is all the more remarkable that their anadromous kin display precisely the opposite situation.

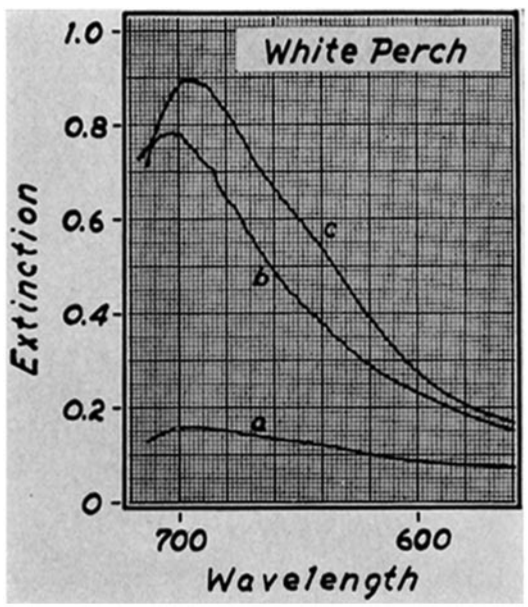

FIG. 6. Spectra of the antimony chloride reaction with extracts of six white perch retinas. Otherwise as in Fig. 1.

The photopigment of the white perch retina has already been examined (Wald, 1938–39*b*). It possesses the usual properties of porphyropsin (Fig. 7). That it includes no admixture of rhodopsin is confirmed by examination of the carotenoid cycle (Fig. 6). This reveals bands due only to vitamin A_2 and retinene$_2$, with no trace of their analogues in the rhodopsin system.

In the alewife only extracts of light adapted retinas to which the pigment epithelia were attached have been examined. They show the presence of vitamin A_2 alone, with no trace of A_1 (Wald, 1938–39*a*). This observation has been confirmed repeatedly with fish taken from brackish water at the beginning of their spawning migration from the sea, and therefore in a condition which might maximally favor the presence of vitamin A_1.

V. DISCUSSION

It may be concluded that many euryhaline fishes give retinal evidence of their euryhalinity in possessing mixtures of the rhodopsin and porphyropsin systems; and of their anadromous or catadromous natures in having predominantly the system ordinarily associated with the spawning environment. The latter tendency is pursued to completion in fishes like the white perch and alewife, which possess the spawning-type of visual system alone. A remarkable parallelism obtains, therefore, between a graded series of salinity relations and of retinal components. This is illustrated in Fig. 7. Beginning with rhodopsin from a permanently marine elasmobranch, the spectrum of retinal photopigment precesses regularly toward longer wavelengths in preparations from the eel, a marine fish which migrates into fresh water; the killifish, a stable brackish water form; the brook trout, a fresh water fish which enters the sea; and the white perch, an anadromous fish which has completed the transition to porphyropsin.

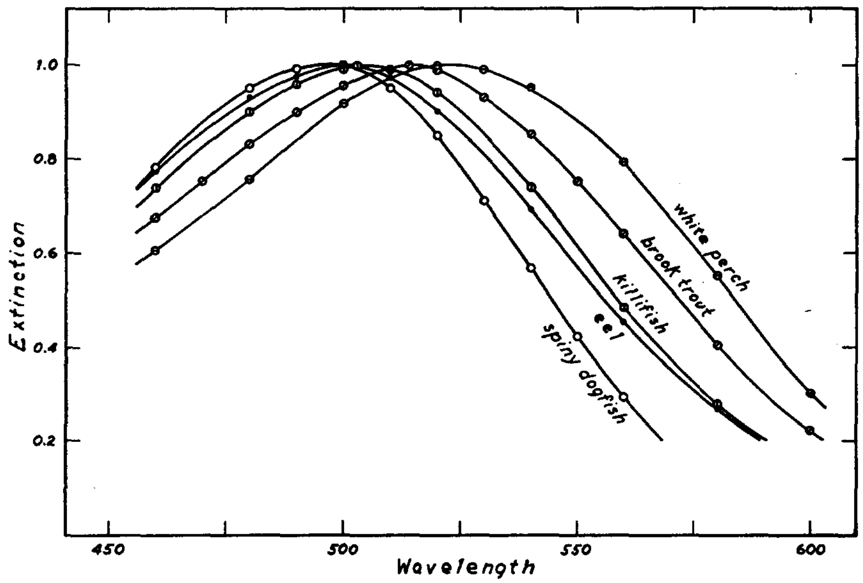

FIG. 7. Spectra of photopigments from various fishes, illustrating the transition from an exclusively rhodopsin to an exclusively porphyropsin system. The permanently marine dogfish possesses rhodopsin alone, the catadromous eel and approximately catadromous killifish predominantly rhodopsin, the anadromous brook trout predominantly porphyropsin, and the anadromous white perch porphyropsin alone.

It is important to recall that the Labridae, all permanently marine, constitute one already known exception in this distribution. The retinas of the tautog

(*Tautoga onitis*) and cunner (*Tautogolabrus adspersus*), like those of anadromous forms, contain a great predominance of vitamin A_2 (Wald, 1938–39*a*; unpublished observations). Yet both are obligate marine fish. I have confirmed Sumner's observation (1905) that both species die within several hours in fresh water. The linkage between osmotic habit and type of vitamin A metabolism, therefore, while remarkably complete, is not absolute.

These patterns are genetic, and to a first approximation independent of the environment (Wald, 1938–39*a*). The duplexity of visual systems of salmonids raised exclusively in fresh water; the great predominance of rhodopsin in the eel after years of fresh water existence, and of porphyropsin in the permanently marine Labridae; the directly opposed systems of the closely related herring and alewife after long periods in the sea; and other similar observations all lead to this conclusion. Whatever genetic factors govern the salinity relations of these animals simultaneously determine the composition of their visual systems.

The latter have therefore a phylogenetic rather than an ontogenetic significance. In this sense, it is possible that the euryhaline fishes represent transitional stages in evolutionary migrations between fresh water and the sea. This view, if valid at all, furnishes no hint of the direction of migration. Disregarding other types of information, the eel for example might equally well be regarded as a marine fish which has become euryhaline preparatory to its transition to fresh water; or as a fish which, having all but completed migration to the sea, retains euryhalinity as a vestige of previous fresh water existence.

Finally the euryhaline fishes provide a retinal situation of peculiar theoretical interest for color vision. Rhodopsin and porphyropsin, both rod pigments, probably play no direct role in this function; yet they provide an exact model of the kind of variation in visual systems which should meet the peripheral requirements of hue discrimination (Wald, 1938–39*b*). The model is completed in those euryhaline fishes which possess mixtures of both systems. In this respect, though possibly in no other, these animals have satisfied one basic requirement of dichromatic vision.

SUMMARY

1. The retinas of all marine fishes so far examined except the Labridae, and of all terrestrial vertebrates contain the rhodopsin system alone; those of fresh water fishes the porphyropsin system alone. In the present paper the visual systems of a number of euryhaline fishes are examined—fishes capable of existence in a wide range of salinities, though usually restricted in spawning either to the sea (catadromous) or to fresh water (anadromous).

2. The retinas of the anadromous salmonids (brook trout, rainbow trout, and chinook salmon) contain mixtures of the rhodopsin and porphyropsin systems, predominantly the latter. The retinas of the catadromous eel and the killifish also contain mixtures of both systems, but in reverse proportions.

The retinas of the anadromous white perch and alewife contain the porphyropsin system alone.

3. There is therefore an extensive parallelism between the salinity relations of these animals and the composition of their visual systems. All of them possess predominantly or exclusively the visual system commonly associated with the environment in which the fish spawns.

4. These patterns are fixed genetically, and are to a first approximation independent of the history of the individual. They may represent transitional stages in the evolutionary migration of fishes to and from the sea. The presence of both types of visual system in the retinas of some euryhaline fishes incidentally satisfies one formal requirement of two-component color vision.

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