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The Developmental Trajectory of Ultraviolet Photosensitivity in Rainbow Trout is Altered by Thyroxine

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Small (<30 g) juvenile rainbow trout (*Oncorhynchus mykiss*) possess retinal photoreceptor mechanisms sensitive to ultraviolet (UV), short (S), middle (M), and long (L) wavelengths. During normal development, UV photosensitivity is lost progressively until, by approx. 60 g, individuals are no longer sensitive in the UV. This shift in spectral sensitivity is associated with the disappearance of small accessory corner cones (ACCs) from the retinal photoreceptor cell mosaic: the UV cone mechanism is lost. Exposing small (<16 g) rainbow trout to the thyroid hormone thyroxine (T_4) for a period of 6 weeks induced a precocial loss of the UV cone mechanism that was indistinguishable from the events that occur during normal development. Six weeks after termination of hormone treatment, the same individuals that had lost their UV photosensitivity after exposure to T_4 once again possessed a peak in spectral sensitivity at 360 nm. ACCs had reappeared in the retinae of these fish. After 6 weeks of exposure to thyroxine, large (>90 g) juvenile rainbow trout, which had lost their UV photoreceptor mechanism during normal development, were once again UV photosensitive and ACCs were found in their retinae. These results imply that the UV photoreceptor mechanism, although lost at one point during development, can reappear at another time during the life history of the same individual. Thyroid hormones appear to be involved in both the loss and reappearance of UV photosensitivity.

Retina Ultraviolet cones Fish vision Spectral sensitivity Heart-rate conditioning Ontogeny Regeneration Rod precursors *Oncorhynchus mykiss* Teleostei

INTRODUCTION

In at least five species of fishes, small juveniles possess an independent cone photoreceptor mechanism sensitive in the UV, but, during normal development, the UV cone mechanism is lost (Bowmaker & Kunz, 1987 for brown trout, *Salmo trutta*; Kunz, 1987 for Atlantic salmon, *S. salar*; Hawryshyn, Arnold, Chaisson & Martin, 1989 for rainbow trout, *Oncorhynchus mykiss*; Whitmore & Bowmaker, 1989 for rudd, *Scardinius erythrophthalmus*; Loew & Wahl, 1991 for yellow perch, *Perca flavescens*). In all of these species, loss of UV photosensitivity is associated with the almost complete disappearance of small accessory corner cones (ACCs) from the retinal photoreceptor cell mosaic (e.g. Bowmaker & Kunz, 1987; Kunz, 1987; Loew & Wahl, 1991; Browman & Hawryshyn, 1992; Beaudet, Browman & Hawryshyn, 1993). Further, in rainbow trout, the disappearance of ACCs is associated with the loss of UV-sensitive ganglion cell fibres from the optic nerve and of UV-sensitive single units from the optic tectum and torus semicircularis (Beaudet *et al.*, 1993; Coughlin & Hawryshyn, 1994a, b). Recent evidence indicates that rainbow trout ACCs contain a photopigment sensitive in the UV (Beaudet, *et al.*, 1993; Hawryshyn & Harosi, 1994). Thus, the loss of UV photosensitivity in rainbow trout apparently results from the disappearance of UV-sensitive cones from the retina and of UV-sensitive units from the optic centres of the brain, i.e. a loss of the UV cone mechanism.

In a previous study, we took up the question of what triggers the developmental loss of the UV cone mechanism in rainbow trout (Browman & Hawryshyn, 1992). We found that the thyroid hormone thyroxine (T_4) induces a precocial loss of UV photosensitivity, and a loss of ACCs from the retina, indistinguishable from the events that occur during normal development. Loss of the UV photoreceptor mechanism appears to be associated with a major life history event in salmonid fishes, the parr–smolt transformation, and is a size- and not age-dependent phenomenon (Browman & Hawryshyn, 1992).

In this paper, we report on experiments designed to further evaluate the developmental trajectory of UV photoreception in fishes, and the role of thyroxine in

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these processes. Specifically, we tested the hypothesis that the UV cone mechanism, although lost at one point during development, may reappear later in the same animal's life history.

MATERIALS AND METHODS

Animals, hormone treatment and control groups

An undomesticated, non-anadromous population of rainbow trout (Badger Lake, British Columbia), raised in outdoor ponds at the Fraser Valley Trout Hatchery in Abbotsford, British Columbia, was used in these experiments. After transport to our holding facility, fish were maintained at 15°C on a 12:12 hr light:dark photoperiod for at least 8 weeks prior to the first experiments. Fish were fed daily with BioDiet Grower pellets (BioProducts Inc., Warrenton, Ore.) containing fixed proportions of vitamins, nutrients and pigments. All experiments were initiated at least 1 hr after lights on in the holding facility and were terminated at least 1 hr before lights off. Illumination in the holding facility was provided by fluorescent bulbs at an intensity of $33.54 \pm 14.39\ \mu W/cm^2$ (integrated irradiance, 200–1100 nm, measured with a Photodyne Inc. radiometer).

Experimental animals were held in 5 or 10 l tubs (for small vs large fish respectively) containing 300 μg/l L-thyroxine sodium salt (Sigma Chemical Laboratories, T-2376) dissolved in 0.1 M NaOH. Half of the treatment solution was replaced daily. This manner of hormone treatment has been used to induce photopigment changes, and metamorphosis, in fishes (e.g. Cristy, 1974; Inui & Miwa, 1985; Miwa, Tagawa, Inui & Hirano, 1988), and produces a significant elevation in the serum titer of T_4 in rainbow trout (Allen, 1977). Control fish were handled in an identical manner, but no T_4 was added to their water. In order to minimize weight gain during the experiment, fish were fed on a maintenance diet.

Handling and maintenance of animals was in accordance with the guidelines set out by the Canadian Council on Animal Care.

Spectral sensitivity experiments

Spectral sensitivity curves were obtained using heart-rate conditioning. Animals were conditioned by pairing a 300 msec, 2–3 mA shock (delivered to the caudal peduncle) with monochromatic visual stimuli (Hawryshyn & Beauchamp, 1985). The methods and equipment used to obtain the spectral sensitivity data reported here were identical to those described in a previous publication (Browman & Hawryshyn, 1992). Details of the immobilization procedure, fish set-up, optical system, conditioning protocol, and threshold determination can be found therein.

Chromatic adaptation was used to isolate the spectral sensitivity of the UV cone mechanism. The illumination used to achieve this effect consisted of a yellow background (550 nm LP cut-off filter, Corion), which light adapted the M- and L-wavelength sensitive cone mechanisms, and a narrow-band blue background (460 nm narrow band interference filter, Corion) which light adapted the S-wavelength sensitive mechanism. The same background conditions were used during all training sessions and experiments. Fish were allowed a minimum of 60 min to adapt to the background conditions before initiation of a training session or experiment.

Since fish generally survive the heart-rate conditioning experiments, spectral sensitivity curves were obtained from the same individuals at the intervals defined below.

Small fish experiments

Spectral sensitivity curves were obtained from 14 small (< 16 g) rainbow trout, all of similar chronological age (±20 days), prior to the initiation of a control or treatment group experiment. Five of these fish were allocated to the control group, and their spectral sensitivity was measured again after 6 and 12 weeks. Another five individuals were exposed to T_4 for 6 weeks, after which their spectral sensitivity was measured again. T_4 exposure for these five individuals was then discontinued and their spectral sensitivity was measured again 6 weeks later. In the text that follows, these fish are referred to as "reverts". The remaining four small rainbow trout were sacrificed for histological examination of pre-treatment retinae.

In order to evaluate whether the UV sensitivity points exhibited by revert fish were generated by an independent cone mechanism, we continued the spectral sensitivity experiments by adding UV illumination to the background [using a 250 W tungsten bulb projected through a UG-11 filter (Corion) and superimposed over the yellow background used in all of the experiments]. Spectral sensitivity at 360, 440, 560 and 640 nm was remeasured for two of the five revert fish after 1 hr of adaptation to the new background conditions.

A cross-sectional experiment, in which spectral sensitivity curves were obtained for an additional 10 rainbow trout (of the same chronological age and size as the previous group), was also performed. Since we already had a robust pre-treatment sample size ($N = 14$, above), spectral sensitivity curves were not obtained for these individuals prior to the initiation of control or treatment experiments. Five of these fish were exposed to T_4 for 6 weeks and their spectral sensitivity was then measured. These five fish were then sacrificed for histological examination of their retinae. The other five fish were exposed to T_4 for 6 weeks after which they were removed from the treatment; their spectral sensitivity was measured 6 weeks after discontinuation of T_4 exposure. These five fish were then sacrificed for histological examination of their retinae.

Large fish experiments

Spectral sensitivity curves were obtained from eight large (> 90 g) juvenile rainbow trout, all of similar chronological age (±20 days, and of the same chronological age as the small fish described above), prior to the initiation of a control or hormone treatment experiment. Four of these fish were allocated to the control group.

and their spectral sensitivity was measured again after 6 weeks. These fish were then sacrificed for histological examination of their retinae. The other four individuals were exposed to T_4 for 6 weeks, after which their spectral sensitivity was measured again. These four fish were then sacrificed for histological examination of their retinae.

In order to evaluate whether the UV sensitivity points exhibited by large T_4-treated fish were generated by an independent cone mechanism, we continued the spectral sensitivity experiments by adding UV illumination to the background (as above). Spectral sensitivity at 360, 440, 560 and 640 nm was remeasured for three of the four revert fish after 1 hr of adaptation to the new background conditions.

Histological procedures

A total of 22 fish were sacrificed for histological examination of the retina. Four were small pre-treatment fish, five small T_4-treated fish, five small revert fish, four large control fish, and four large T_4-treated fish. We did not sacrifice any small control fish because we have already carried out such controls for other experiments (see Fig. 4 in Browman & Hawryshyn, 1992).

All individuals were fully light-adapted when sacrificed by spinal section and the eyes were immediately enucleated. Retinae were prepared for histological examination, by embedding in Epon. Full details of the protocol used here have been published elsewhere (Browman & Hawryshyn, 1992).

Tissue from the central ventral retina, the area to which stimuli were presented in the spectral sensitivity experiments, was sectioned tangentially (1 μm thick) to the base of the cone outer segments. Sections were stained with Richardson's stain for light microscope examination. To ensure that there was no T_4-induced displacement of cone cells within the photoreceptor layer, the central ventral retinae of at least one specimen from each experimental treatment were serially sectioned (1 μm thick sections) from the tips of the rod outer segments through to the cone pedicles.

RESULTS

Small fish spectral sensitivity

All small pre-treatment and control fish in the current experiments exhibited sensitivity peaks at UV and S wavelengths (Fig. 1). M and L mechanisms were also present, although their sensitivity was depressed by the adapting background (Fig. 1). Since the same pattern was observed for all individual control fish, the data are presented as mean spectral sensitivity curves. Before calculating these mean curves, and those described

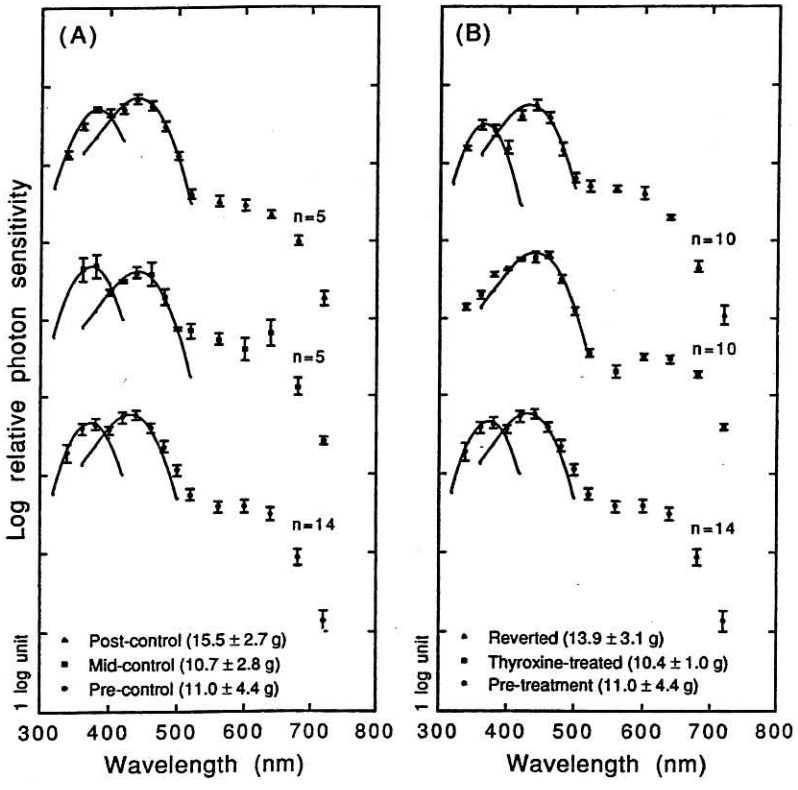

FIGURE 1. (A) Mean spectral sensitivity curves for small rainbow trout used as controls obtained, from the same individuals, before, and after 6 and 12 weeks of treatment. (B) Mean spectral sensitivity curves for small thyroxine-treated rainbow trout, and for reverts, obtained from five of the same individuals and five in cross-section, after 6 weeks of exposure to T_4 and again 6 weeks after T_4 exposure was discontinued. A yellow background was used to "isolate" the UV sensitive cone mechanism in all experiments. The 360 and 430 nm λ_{max} visual pigment absorption curves were compared with the appropriate spectral peaks for all fish. Note: (i) visual pigment absorption curves (corrected for ocular media absorption) are represented by solid lines, (ii) spectral-sensitivity curves were arbitrarily arranged on the ordinate, (iii) one major division on the ordinate equals 1 log unit.

below, absolute values of log photon sensitivity were normalized for inter-individual variability.

The UV sensitivity points were most effectively fitted with a 360 nm λ_{max} visual pigment absorption curve (Fig. 1). This is consistent with microspectrophotometric estimates of a 360–370 nm λ_{max} for the UV-sensitive cone pigment in rainbow trout (Hawryshyn & Harosi, 1994), and with estimates for UV-sensitive cones in other fishes (e.g. Hawryshyn & Beauchamp, 1985; Whitmore & Bowmaker, 1989; Hawryshyn & Harosi, 1991; Robinson, Schmitt, Harosi, Reece & Dowling, 1993). The visual pigment absorption curves fitted to our data were generated by an eighth-order polynomial template for vertebrate cone visual pigments, corrected for ocular media absorption (Browman & Hawryshyn, 1992).

Spectral sensitivity points in the neighbouring S-wavelength region were most effectively fitted with a 430 nm λ_{max} visual pigment absorption curve (Fig. 1). This is consistent with microspectrophotometric estimates of a 430–440 nm λ_{max} for the S-sensitive cone pigment in rainbow trout (Hawryshyn & Harosi, 1994), and with estimates for the S-sensitive cones of brown trout (Bowmaker & Kunz, 1987).

There was no significant change in the mean nor within-individual spectral sensitivity curves of the small control fish when measured again after 6 and 12 weeks [Fig. 1(A)]. Both UV- and S-wavelength sensitive cone mechanisms remained as described above.

Small fish exposed to T_4 for 6 weeks no longer exhibited a UV sensitivity peak [Fig. 1(B)]. These individuals possessed only S-, M-, and L-sensitive cone mechanisms. The S-sensitive cone mechanism points for these fish were most effectively fitted with a 430 nm λ_{max} visual pigment absorption curve [Fig. 1(B)]. Since all within-individual spectral sensitivity curves exhibited the same trend, whether for fish allocated to the longitudinal or cross-sectional experiments, these data were combined and mean curves for all 10 individuals are presented.

Six weeks after discontinuation of T_4 exposure, these same individuals once again exhibited a UV sensitivity peak [Fig. 1(B)]. The UV sensitivity points for these fish were most effectively fitted with a 360 nm λ_{max} visual pigment absorption curve [Fig. 1(B)]. The S-sensitivity points were most effectively fitted with a 430 nm λ_{max} visual pigment absorption curve [Fig. 1(B)]. Since all within-individual spectral sensitivity curves for revert fish exhibited the same trend, whether for fish allocated to the longitudinal or cross-sectional experiments, these data were combined and mean curves for all ten individuals are presented.

For two small revert fish, spectral sensitivity at 360, 440, 540 and 640 nm was remeasured after the addition of UV illumination to the background. For these individuals, the sensitivity of the 360 nm point was depressed by 1.20 ± 0.65 ($\bar{x} \pm$ SE) log units. There was little or no change in the sensitivity of the 440 (0.22 ± 0.11 log unit), 560 (0.21 log unit), and 640 (0 log unit) nm points.

Changes in the external appearance of small T_4-treated fish were also observed. Fish exposed to T_4 for 6 weeks no longer possessed the vertical pigmented bars typical of juvenile rainbow trout, but had become silvered. Such silvering is one of the external morphological changes associated with the parr-smolt transformation in salmonids (Hoar, 1988). Revert fish, which had become silvered after 6 weeks of exposure to T_4, had regained their parr markings six weeks after treatment was discontinued.

Histology of the ventral retina in small fish

The central ventral retina of small rainbow trout normally contains a regular square mosaic of cones, consisting of a single cone surrounded by four double cones, with small accessory single cones at each corner [see Fig. 4(A) in Browman & Hawryshyn, 1992]. The cone photoreceptor cell mosaic in the retina of small rainbow trout exhibits the same mosaic pattern over the size and age range of fish used in these experiments [see Fig. 4(C) in Browman & Hawryshyn, 1992].

ACCs were no longer present, at any level within the photoreceptor cell layer, in the central ventral retinae of fish exposed to T_4 for 6 weeks [Fig. 3(A)]. The cone mosaic in these retinae consisted of a single cone surrounded by four doubles. This arrangement is identical to that observed in larger ($> \approx 70$ g) individuals, albeit of the same chronological age, which have undergone normal growth and development [see Fig. 4(B) in Browman & Hawryshyn, 1992)].

The central ventral retinae in revert fish contained a regular square mosaic which included ACCs [Fig. 3(B)]. In some areas, there appeared to be multiple single cones in the corners [Fig. 3(B) arrows]. Some areas of the retina in these same fish did not possess full complements of ACCs.

Large fish spectral sensitivity

There was no significant change in the mean nor within-individual spectral sensitivity curves of the large control fish during these experiments [Fig. 2(A)]. S-, M- and L-sensitive cone mechanisms were present in all of these individuals, and there was no evidence of a sensitivity peak in the UV [Fig. 2(A)]. Spectral sensitivity points in the S-wavelength region were most effectively fitted with a 430 nm λ_{max} visual pigment absorption curve (Fig. 2). M and L mechanisms were also present, although their sensitivity was depressed by the adapting background (Fig. 2). Since the same pattern was observed for all individual fish in both control and treatment groups, the data are presented as mean spectral sensitivity curves.

Large fish exposed to T_4 for 6 weeks exhibited spectral sensitivity peaks at UV and S wavelengths [Fig. 2(B)]. The UV sensitivity points for these individuals were most effectively fitted with a 360 nm λ_{max} visual pigment absorption curve [Fig. 2(B)]. The S-sensitivity points were most effectively fitted with a 430 nm λ_{max} visual pigment absorption curve [Fig. 2(B)]. M and L mechanisms were also present although, as before, their sensitivity was depressed by the adapting background (Fig. 2).

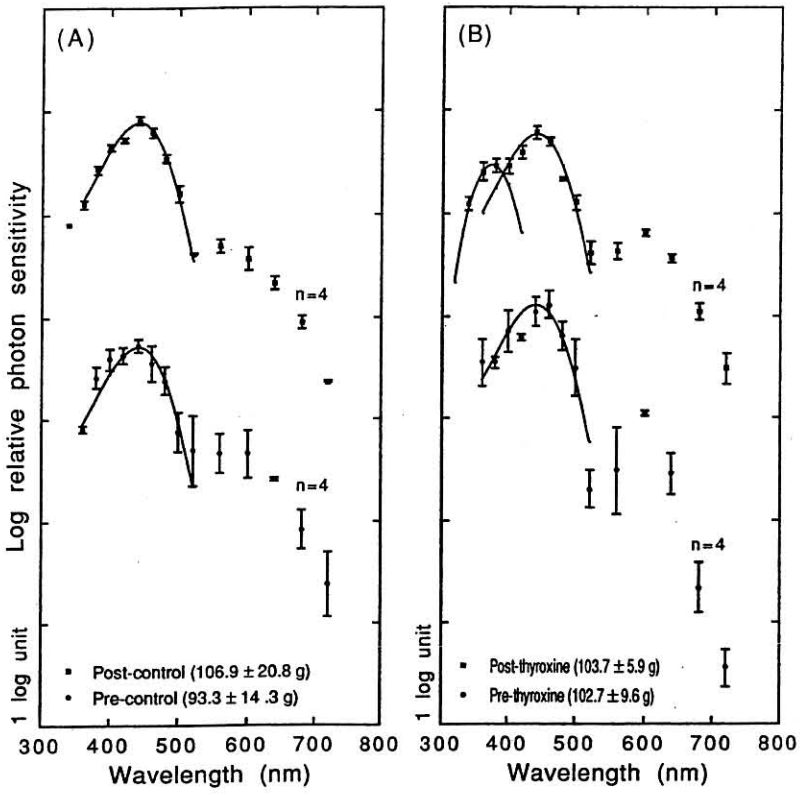

FIGURE 2. (A) Mean spectral sensitivity curves for large rainbow trout used as controls obtained, from the same individuals, before, and after 6 weeks of treatment. (B) Mean spectral sensitivity curves for large thyroxine-treated rainbow trout obtained, from the same individuals, before, and after 6 weeks of exposure to T_4. A yellow background was used to "isolate" the UV sensitive cone mechanism in all experiments. The 360 and 430 nm λ_{max} visual pigment absorption curves were compared with the appropriate spectral peaks for all fish. Note: (i) visual pigment absorption curves (corrected for ocular media absorption) are represented by solid lines, (ii) spectral-sensitivity curves were arbitrarily arranged on the ordinate, (iii) one major division on the ordinate equals 1 log unit.

For three large T_4-treated fish, spectral sensitivity at 360, 440, 540 and 640 nm was remeasured after the addition of UV illumination to the background. For these individuals, the sensitivity of the 360 nm point was depressed by 1.38 ± 0.25 ($\bar{x} \pm$ SE) log units. There was some depression of the 440 nm point (0.55 ± 0.09 log unit) and little change in the sensitivity of the 560 (0.20 ± 0.08 log unit) and 640 (0.06 ± 0.09 log unit) nm points.

Histology of the ventral retina in large fish

The central ventral retina of large rainbow trout normally contains a regular square mosaic of cones, consisting of a single cone surrounded by four double cones [see Fig. 4(B) in Browman & Hawryshyn, 1992]. ACCs, which are present at each corner of the square in small rainbow trout, are not present in the central ventral retina of larger individuals. However, a small population of ACCs is apparently present near the optic nerve head and along the embryonic fissure even in large (80–914 g) fish (see Fig. 5 in Beaudet *et al.*, 1993).

The central ventral retinae of all large control fish in these experiments (and the same individuals from which the spectral sensitivity curves were obtained) possessed a cone photoreceptor mosaic consisting of a single cone surrounded by four double cones [Fig. 3(C)]. No ACCs were found, at any level within the photoreceptor cell layer.

Extensive areas of the central ventral retinae of large T_4-treated fish possessed ACCs [Fig. 3(D)], and all of these individuals were UV photosensitive. However, ACCs were not found in all areas of the central ventral retinae of large T_4-treated fish.

DISCUSSION

Loss and reappearance of the UV cone mechanism in T_4-treated rainbow trout

Our results imply that (1) the developmental loss of the UV cone mechanism is reversible; (2) ACCs apparently reappear in areas of the retina from which they had previously disappeared, and away from the circumferential growth zone and/or embryonic fissure, and (3) T_4 is either directly or indirectly involved in both the loss and regeneration of UV photosensitivity.

Small, UV photosensitive rainbow trout exposed to T_4 for 6 weeks lost their UV photosensitivity, and their ACCs, despite being substantially smaller than the size at which the UV cone mechanism is normally lost (Browman & Hawryshyn, 1992; this study). Six weeks after exposure to T_4 was discontinued, these same fish were once again UV photosensitive (Fig. 1). ACCs were found in the central ventral quadrant of their retinae, a

location in which there were no ACCs in small fish exposed to T_4 for 6 weeks [Fig. 3(A, B)]. Large rainbow trout, which had lost their UV photosensitivity during normal development, regained their UV photosensitivity after 6 weeks of exposure to T_4 (Fig. 2). ACCs were found in the central ventral quadrant of their retinae, an area in which there are no ACCs in normal large fish [Fig. 3(C, D)]. Further, the UV sensitivity points in small and large revert fish were selectively depressed after addition of UV wavelengths to the background,

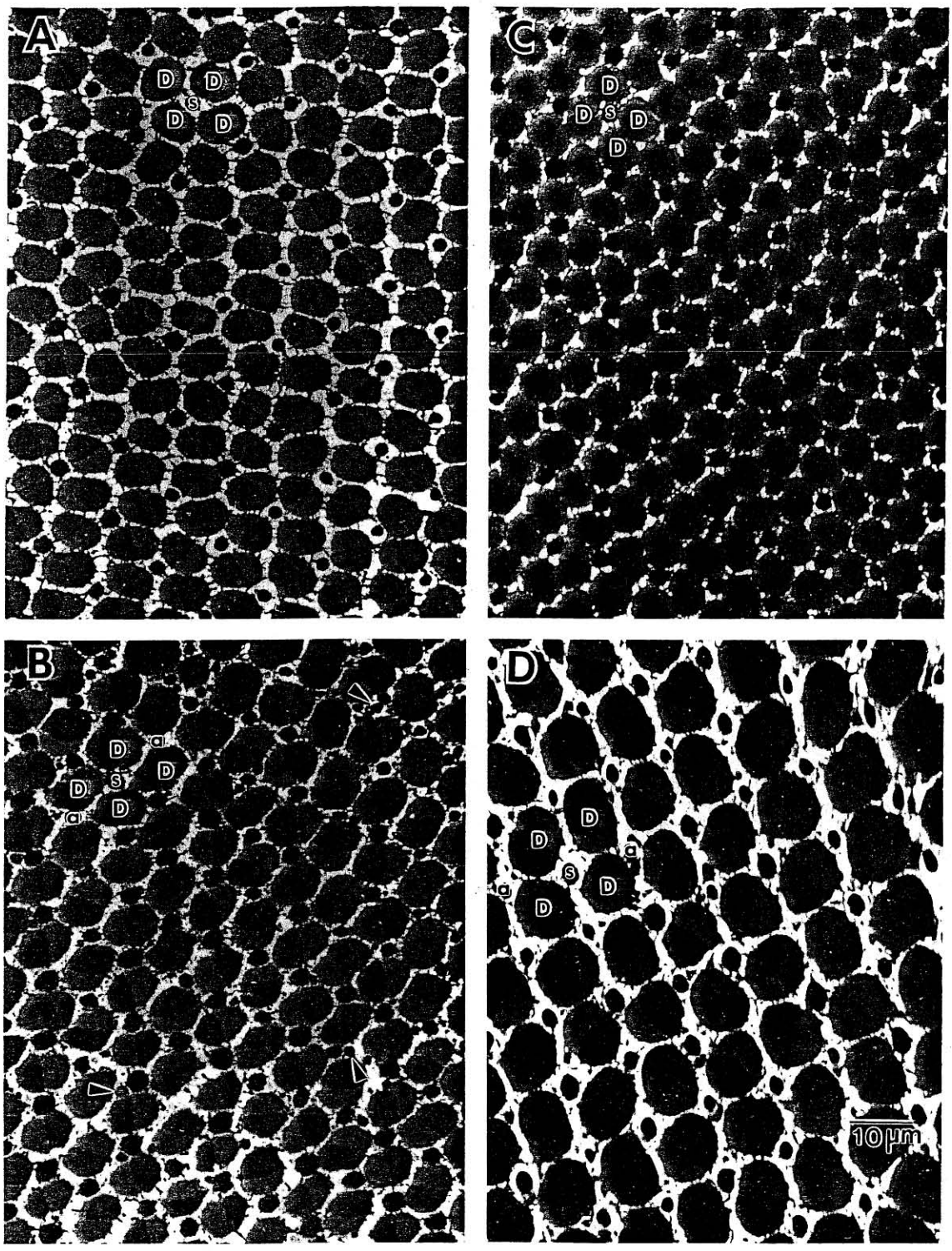

FIGURE 3. (A) Cone photoreceptor cell mosaic in the ventral retina of a small rainbow trout (11 g) exposed to T_4 for 6 weeks. (B) Cone photoreceptor cell mosaic in the ventral retina of a small rainbow trout (12 g) exposed to T_4 for 6 weeks and sacrificed 6 weeks after T_4 exposure was discontinued. (C) Cone photoreceptor cell mosaic in the ventral retina of a large rainbow trout (110 g) from the control group of the spectral sensitivity experiments. (D) Cone photoreceptor cell mosaic in the ventral retina of a large rainbow trout (112 g) exposed to T_4 for 6 weeks from the treatment group of the spectral sensitivity experiments. All micrographs are from tangential sections cut through the base of the cone cell outer segments. a, accessory corner cone (=small single cone); D, double cone; s, central single cone. Arrows highlight areas in which there appear to be multiple single cones.

indicating that these points were generated by an independent cone mechanism.

Examination of the central ventral retina of small and large revert fish revealed that the timing and extent of ACC regeneration to these areas is variable. Although the revert fish that we examined possessed tracts of ACCs in the central ventral retina, some areas contained few or no ACCs. Multiple single cones were occasionally observed in the corners of the mosaic [see arrows in Fig. 3(B)]. We interpret this latter observation as a possible artifact of the hormone treatment, i.e. an overproduction of new cone elements induced by T_4 exposure (see below). However, multiple single corner cones have been observed in the square mosaics of two freshwater fishes near the time when these cones are lost from the retina during normal development (Wahl, 1992; C. M. Wahl, personal communication).

The timing and extent of ACC loss from the retinae of small T_4-treated rainbow trout is also variable (Browman & Hawryshyn, 1992). This is true for normally developing fishes as well (Lyall, 1957a, b; Ahlbert, 1976; Wahl, 1992; Kunz, Wildenburg, Goodrich & Callaghan, 1994). Further, and as we have reported elsewhere (Browman & Hawryshyn, 1992), the mosaic pattern itself exhibits topographic and inter-individual variability: in some areas the mosaic is square, in others the geometry of the double cone elements is not perfectly perpendicular, while in other areas the mosaic is a row (Fig. 3, also see Lyall, 1957a, b; Ahlbert, 1976; Kunz, 1980, 1987). Double cone elements in at least one fish species exhibit a diel pattern of twisting around their long axis which alters the geometry of the cone mosaic (Wahl, 1994). Thus, within-individual variability in the geometry of the square mosaic appears to be common in fishes. Clearly, variability in the developmental loss and reappearance of ACCs requires further study.

The fate of the ACCs that disappear from the retina of rainbow trout is still uncertain. It was recently suggested that, at least in Atlantic salmon (*Salmo salar*), they die, degenerate, and are removed from the photoreceptor cell layer by phagocytosis over a period of hours or days (Kunz *et al.*, 1994). However, in adult brown trout, Atlantic salmon, and rainbow trout, ACCs remain at the embryonic fissure and peripheral growth zones (Kunz, 1987; Bowmaker & Kunz, 1987; Beaudet *et al.*, 1993). It is not known whether these cells contain an UV photopigment. Further study is required to resolve these issues.

In the fish retina, which continues to grow throughout life, new cone cells are normally added at the circumferential germinal zone, and perhaps at the embryonic fissure (Raymond, 1985; Kunz, 1987; Kunz & Callaghan, 1989; Fernald, 1991; Kunz *et al.*, 1994). Thus, our observation that ACCs reappear in central areas of the retina is unusual, but not without precedent. In fishes, rod cells are constantly recruited throughout the retina as it grows (Raymond, 1985; Fernald, 1991). These new rods are inserted into the photoreceptor mosaic interstitially and then establish synaptic connec-

Fernald, 1991). The source of new rods is a specialized population of undifferentiated neuroepithelial cells termed "rod precursors" (Raymond, 1985). Rod precursors are scattered throughout the differentiated retina and are normally located in the outer nuclear layer (ONL) at the time of their differentiation into rods (Raymond & Rivlin, 1987). Recent evidence indicates that rod precursors, and other retinal precursor cells, are pluripotent and are not restricted to producing new rods (Raymond, Reifler & Rivlin, 1988; Raymond, 1991; Braisted & Raymond, 1992; Hitchcock & Raymond, 1992; Hitchcock, Myhr, Easter, Mangione-Smith & Dwyer Jones, 1992). Based upon these observations, we suggest that rod precursors are the source of new ACCs and that these cones are added to the photoreceptor cell mosaic in a manner analogous to that described for rods (Raymond, 1985; Fernald, 1991). Further, it seems likely that T_4, or one of its metabolic relatives, induces rod precursor cells to produce pluripotent neuroepithelial cells (which would go on to become cones) rather than differentiating into rods (see Raymond, 1991). In this regard, it is noteworthy that intercalary cell addition to the retina of metamorphosing amphibians is induced by T_4 (Hoskins, 1990).

Thyroid hormones and their role in the development of the vertebrate visual system

In fishes and amphibians, thyroid hormones are associated with life history transformations such as metamorphosis (Norris, 1983; Inui & Miwa, 1985; Lam, 1985; Hoar, 1988), and with significant morphological, physiological and biochemical changes in the visual system (Hara, Ueda & Gorbman, 1965; Beatty, 1972; Allen & Munz, 1983; Hoskins, 1986, 1990; Evans & Fernald, 1990). Further, T_4 can influence the morphology of nerve cells, and CNS connectivity, well after the embryonic and metamorphic stages of the life history (Hofmann, Michler & Meyer, 1989; Arnold, 1992; Porterfield & Hendrich, 1993). These studies demonstrate that thyroid hormones are involved in a broad spectrum of morphogenetic events occurring in embryonic, juvenile, and adult nervous systems. However, how T_4 acts to effect these changes, whether directly on structural genes or indirectly by induction of secondary autocrine or endocrine factors, remains unclear (Hoskins, 1990; Chatterjee & Tata, 1992).

Binding sites for thyroid hormones are present in the nuclei of a wide variety of cells (Nunez, 1988). On association with specific chromatin-bound receptors, thyroid hormones exert their effects by directly regulating gene expression, or via other hormone or growth factors (Nunez, 1988; Chatterjee & Tata, 1992). Thyroid hormone receptors (TR) have been located in several brain areas of fishes (White, Scholz, Baker & Liljgren, 1990) and in the retinae of metamorphosing *Xenopus* (Kawahara, Baker & Tata, 1991). A variant of TRβ, cTRβ2, has been identified in developing chick retina (Sjöberg, Vennström & Forrest, 1992). cTRβ2 is found predominantly in the outer nuclear layer of the retina

regulating genes important in chick eye development such as photopigment, or other rod and cone differentiation markers (Sjöberg *et al.*, 1992). It is interesting to note that the rod precursor cells discussed above are also located in the retina's outer nuclear layer.

Based upon these recent studies, we postulate that the death of ACCs may be controlled by TRs in their nuclei, and that their possible regeneration may be regulated by TRs in the nuclei of rod precursor-like cells. It is noteworthy that elevated levels of thyroid hormones are associated with the initiation of both the seaward migration in salmonid fishes (during which the UV cone mechanism is lost), and the freshwater migration (during which the UV cone mechanism may reappear) (Woodhead, 1975; Ueda, Hiroi, Hara, Yamuchi & Nagahama, 1984; Hoar, 1988; Youngson, 1989; Youngson & Webb, 1993).

Ecological and behavioural significance of developmental plasticity in the UV cone mechanism

Evaluations of the possible adaptive roles of UV photoreception in fishes have only recently been undertaken (Douglas & Hawryshyn, 1990; Hawryshyn, 1992). In rainbow trout, the UV cone mechanism is involved in the detection of, and orientation to, the e-vector of the polarized light field (Hawryshyn, 1992; Parkyn & Hawryshyn, 1993). In addition, the UV cone mechanism is apparently directly involved in colour vision and extends the range of wavelengths and intensities over which colour discriminations can be made (Neumeyer, 1992; Coughlin & Hawryshyn, 1994a). The UV cone mechanism also contributes to visually-guided foraging behaviour, perhaps as a contrast enhancer (Browman, Novales-Flamarique & Hawryshyn, 1992, 1994; Loew, McFarland, Mills & Hunter, 1993). It would not be surprising to find that the UV cone mechanism, like the other cone mechanisms, makes a multi-faceted contribution to the visual abilities of these animals. It is also likely that the nature of this contribution changes during an individual's life history. Despite the recent studies cited above, the adaptive significance of a developmental loss of UV photosensitivity, and of its possible return at a later time during the life history of the same individual, is unclear.

In all of the species for which a developmental loss of UV photoreception has been documented, the UV mechanism disappears near the time when these fishes move from shallow to deeper waters, and when they change from feeding upon small crustacean zooplankton to larger food items (see Hart, 1973; Scott & Crossman, 1979; Wootton, 1990; Groot & Margolis, 1991 for general discussions of habitat and diet shifts in salmonids, trouts and sunfishes). Similar changes occur in the retinae of other fishes when they move to deeper waters (e.g. Boehlert, 1979; Kitamura, 1990). Based upon these observations, we suggest that the loss of the UV cone mechanism in small juvenile rainbow trout, and perhaps in other species, is associated with a habitat shift to deeper waters (in which a UV photoreceptor would be useless), and with a change from a diet of small zooplanktonic crustaceans (the location of which would be improved by a contrast-enhancing UV receptor) to larger crustaceans and small fishes. Further, we propose that an ability to regenerate the UV mechanism may be associated with its role in orientation to the e-vector of polarized light fields and, therefore, with long distance migration (Hawryshyn, 1992; Parkyn & Hawryshyn, 1993). The next step will be to examine the spectral sensitivity and retinal structure of adult salmonids during their migration to freshwater.

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