

**CORNER CONE TOPOGRAPHY IN THE RETINA OF THE ATLANTIC
SALMON: SPATIO-TEMPORAL DIFFERENCES WITH CORNER
CONE DISTRIBUTIONS IN THE RETINA OF RAINBOW TROUT**

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Abstract

The distribution of corner (ultraviolet-sensitive) cones in the retina of Atlantic salmon was examined from the small juvenile (parr) stage to the adult stage (approaching sexual maturation). Small parr weighing ~ 5 g lacked corner cones everywhere except near the dorsal periphery. Large fish (~ 5 Kg) approaching sexual maturation showed corner cones in other areas of the dorsal retina besides the periphery. These areas had similar corner cone densities to analogous areas in the smolt retina, suggesting that corner cones are formed in the periphery and re-incorporated into the dorsal retina of the Atlantic salmon some time during the smolt stage. This incorporation is partial both in numbers of cones and in location (only the dorsal retina is affected). These findings contrast with the situation in rainbow trout where corner cones from existing mosaics are only partially lost from the ventral retina, if at all, and where production and incorporation of these cones into the dorsal retina occurs throughout life. Thus, in salmonids, there are at least two different ontogenetic pathways that determine retinal corner cone distributions.

Introduction

The retinas of salmonid fishes possess specialized cones that are maximally sensitive to ultraviolet light (λ_{\max} : 360-390 nm, Bowmaker & Kunz, 1987; Kusmic et al., 1993; Hawryshyn & Hárosi, 1994). These cones occupy the so-called corner position in the square mosaic that characterizes the nuclear photoreceptor layer of salmonid retinas, facing the partitioning membranes that appose adjacent double cones (Lyll, 1957; Engström, 1963; Ahlbert, 1976;

Bowmaker & Kunz, 1987). Ultraviolet (UV) cones are therefore also known as corner or accessory corner cones (Bowmaker & Kunz, 1987; Beaudet et al., 1997). In Atlantic salmon, UV cones are present throughout the entire retina of newly-hatched fish (Kunz, 1987; Kunz et al., 1994; Forsell et al., 2001), but are progressively lost as the animal grows (Kunz, 1987; Kunz, et al., 1994). Indeed, previous studies have shown that the retinas of large Atlantic salmon juveniles (termed smolts because they have undergone a physiological transformation known as smoltification that readies them for life in saltwater) lack UV cones everywhere except along the peripheral growth zone (Kunz, 1987, and personal observations of corner cones labelled with a UV riboprobe). Two apoptotic events appear responsible for this loss of UV cones (Kunz et al., 1994). First, established UV cones in the main retina are removed as the animal grows. Second, newly-produced UV cones in the peripheral retina fail to incorporate into the main retina. The almost complete loss of UV cones observed in the retina of Atlantic salmon smolts (Kunz, 1987; Kunz et al. 1994) is different from that occurring in the rainbow trout where corner cones are only lost from the lower half of the retina, if at all (Martens, 2000; Novales Flamarique, 2001). In the rainbow trout, the distribution of corner (putative UV) cones in the sexually-mature adult is similar to that found in the retina of the smolt (Beaudet et al., 1997; Martens, 2000; Novales Flamarique, 2001), suggesting that regeneration of UV cones in the main retina is minor or inexistant. Whether corner cones are regenerated in the retina of the Atlantic salmon is unknown.

In this study, I generated topographic maps of corner cones in the retina of Atlantic salmon at different life stages. These maps were used to identify the juvenile stage when corner cones disappear from the retina and any regeneration that may occur as the animal approaches sexual maturation.

Materials and Methods

Atlantic salmon (*Salmo salar*) at the parr stage (total length \pm S.D. = 4.6 ± 0.5 cm, mass \pm S.D. = 5.2 ± 0.7 g, n=3) were obtained from Sea Springs Hatchery (Chemainus, British Columbia, Canada), while smolts (total length \pm S.D. = 24.7 ± 1.3 cm, mass \pm S.D. = 157 ± 33 g, n=3) and large fish approaching sexual maturation (total length \pm S.D. = 73 ± 2 cm, mass \pm S.D. = 5.2 ± 0.5 Kg, n=3) were obtained from Heritage Aquaculture (Campbell River, British Columbia, Canada). The large fish were gathered for processing in the aquaculture plant prior to extensive gonad development. It is assumed from their size, and external morphology (one of them), that maturation had begun. These fish are referred to as adults in later parts of the manuscript.

Following euthanasia of a given fish, the left eye was marked for orientation by small incisions in the ventral and temporal iris, extracted and immersed in primary fixative (2.5% glutaraldehyde, 1% paraformaldehyde in 0.06 M phosphate buffer, pH 7.3). After overnight fixation at 4° C, the retina was removed from the eyecup in cold phosphate buffer and flattened underneath a transparent grid by making small radial incisions. The retina was placed with the optic nerve head in the middle of the grid and the ventral side downwards. This procedure permitted the analysis of similar retinal areas between fish of the same developmental stage. The retina was then cut into 12-19 pieces (depending on fish size) that corresponded to specific sectors on the grid. These pieces were incubated in secondary fixative (1% osmium tetroxide) for 1 hour at 4° C, dehydrated through a series of increasing concentration of ethanol solutions and embedded in Epon plastic. Thick (1 μm) tangential sections were stained with Richardson's solution and a quantitative analysis was then performed. In Atlantic salmon, as in other salmonids, the cones are arranged in a repeating square-like mosaic formation at the nuclear level (Novales Flamarique, 2001). Each square mosaic unit consists of four double cones (whose elliptical cross sections form the "sides" of the square), a center cone in the middle, and corner cones at the extremities of the square. Cone densities were determined for each cone type at each location by counting the number of cones in a 25000 μm^2 (for the parr fish) and in a 64000 μm^2 area (for the smolt and large, adult, fish) using a Zeiss Universal R Microscope equipped with a 40X objective (60-80 X total magnification). The numbers were then converted into numbers of cones per mm^2 . To compute cone packing (i.e. the percentage of the area occupied by a given cone type), a computerized image analysis system (Optimas Corp.) was used to measure the ellipsoid area of 10 cones of each type per retinal sector (see Beaudet et al., 1997). Ellipsoid areas were measured at the level of largest cross-sectional area for each cone type. Cone packing was calculated as the product of cone density and average cone area.

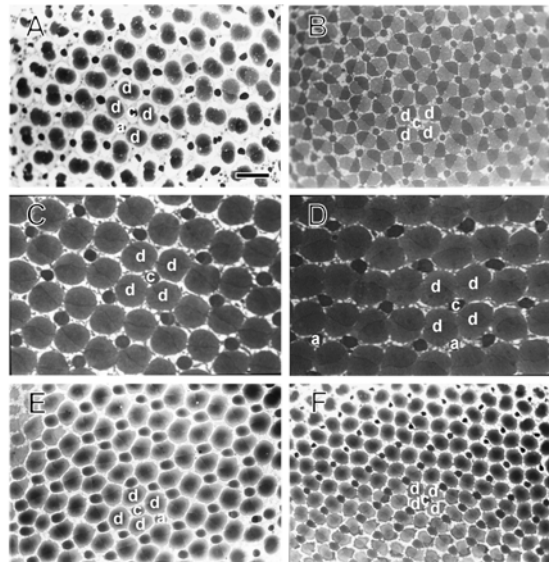
All animal handling procedures were approved by the Animal Care Committee at Simon Fraser University, which follows guidelines set by the Canadian Council for Animal Care.

Results

Previous studies have indicated that the recently-hatched Atlantic salmon possesses corner (UV-sensitive) cones throughout the retina (mass \approx 1g, Forsell et al., 2001; Kunz et al., 1994). By the time the fish weighs \sim 5 g, all the corner

cones have disappeared from the retina except along the dorsal periphery (Figs. 1A,B). At this stage, the fish retains vertical "parr" marks along its body and has not undergone the process of smoltification yet. Corner cones remain absent almost completely from the main (non-peripheral growth zone) part of the retina at the smolt stage (Fig. 1C); the only exceptions are isolated instances of single corner cones near the periphery (Fig. 1D), and around the central retina (embryonic fissure, Kunz, 1987; Kunz et al., 1994). As the animal approaches sexual maturation, however, corner cones are found in a larger proportion of the dorsal retina, though they are not ubiquitous in the mosaic (Fig. 1E). The majority of the retina does not show corner cones at this stage either (Fig. 1F).

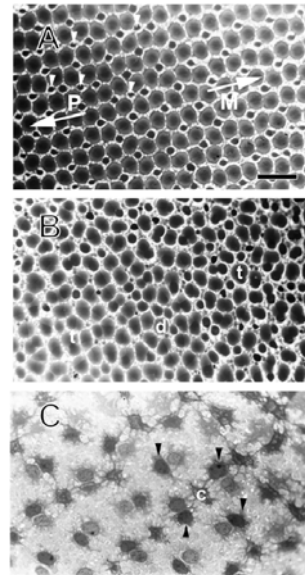
Figure 1. Retinal mosaics in Atlantic salmon at different developmental stages. (A) In the dorsal periphery of the parr fish, the square mosaic consists of double cones (d) forming the sides of the "square", with a centre (c) cone in the middle, and accessory corner (UV) cones (a) at the corner positions, when present. (B) Most of the parr retina shows a square mosaic without corner cones. (C) Square mosaic without corner cones in the retina of the smolt. (D) Isolated corner cones from the peripheral retina; corner cones occur infrequently in the smolt retina. (E) Square mosaic with corner cones in the dorsal retina of fish approaching sexual maturation (adults). (F) Most of the adult retina shows a square mosaic without corner cones. The magnification bar in (A) equals 15 μm (A-D) and 25 μm (E,F).



As in the parr and smolt retinas, corner cones produced along most of the peripheral retina in the fish approaching sexual maturation are not incorporated into the main retina (Fig. 2A). On two occasions, near the nasal periphery, triple cones occupied a large area of the retinal surface (Fig. 2B). In these instances,

the mosaic was random and unusually large single cones were found scattered among normal size cones (Fig. 2B). The square mosaic present in most of the retina shows double cones with nuclei that stain differently between members (Fig. 2C), as observed for the rainbow trout (Novales Flamarique, 2001). Double cone members with similar stain alternate around the unit square mosaic (Fig. 2C).

Figure 2. (A) Corner cones (white arrowheads) produced in the temporal periphery (P) are not incorporated into the main retina (M), as shown by the absence of these cone types towards the main retina. (B) Random mosaic with triple cones (t) and large single cones in the nasal periphery. (C) the nuclei of double cone members stain differently from each other; the stain alternates around the unit square mosaic. Abbreviations as in Figure 1. Magnification bar in (A) equals 25 μm (A, B) and 16 μm (C).



Total cone densities are always higher in the centro-ventro-temporal quadrant of the retina and along the periphery (Figs. 3A-C). On average, there is a progressive decline in cone densities as the animal grows [the average double cone densities \pm SD were 9104 \pm 1875 (parr), 5574 \pm 2030 (smolt), and 2183 \pm 740 (adult); one-way ANOVA: $F_{2,44} = 73.42$, $P < 0.0001$]. Corner cone densities are low compared to those of other cone types and the highest numbers occur near the dorsal periphery (Figs. 3A-C). Cone packing trends do not necessarily follow those of cone densities; the highest cone packing numbers are often found near the central retina and around the ventro-temporal and naso-dorsal peripheries (Figs. 3D-F). The average cone packing is statistically the same regardless of developmental stage [double cone packing means \pm S.D. were 48 % \pm 6 % (parr), 54 % \pm 13 % (smolt), and 53 % \pm 12 % (adult); one-way ANOVA: $F_{2,44} = 0.95$, $P = 0.394$].

Corner cone densities for locations of similar eccentricity show statistical differences between stages (one-way ANOVA, $F_{3,19} = 48.18$, $P < 0.001$, $n = 5$ per location (I-IV), Fig. 3). A Student-Newman-Keuls grouping test performed on the ANOVA results (with $\alpha = 0.05$) revealed that location I is statistically

different from all others, II is similar to III, III is similar to IV, but II and IV are different from each other (Fig. 3). Thus, dorsal corner cone densities ~ 6 mm away from the retinal center are similar in the smolt and adult retinas. This location represents the lower tip of the corner cone distribution in the smolt and adult retinas (Fig. 4).

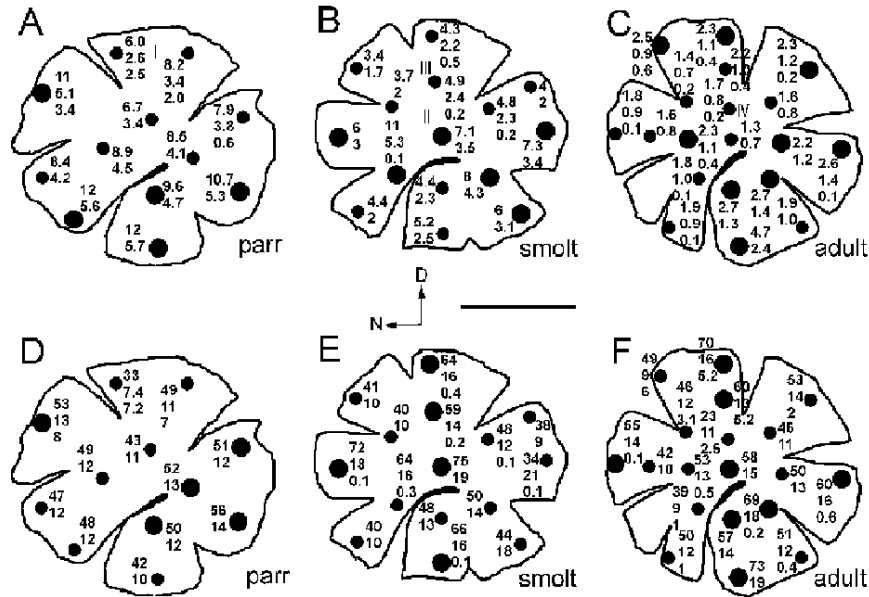


Figure 3. Topography of cone types in the retina of Atlantic salmon at different developmental stages ($n=3$ each). (A-C) densities of double cones (top number), centre cones (middle number) and corner cones (lower number, when present) for each retinal location examined in parr, smolt and adult fish. (D-F) associated cone packing numbers in parr, smolt and adult fish. Locations with larger circles (\bullet) have densities, or cone packing numbers, greater than the average from all locations. Locations I and II, and III and IV have the same approximate eccentricity, respectively. D, dorsal retina; N, nasal retina. Magnification bar equals 3 mm (A,D), 8 mm (B,E) and 14 mm (C,F).

The ontogenetic distribution of corner cones in Atlantic salmon is different from that in rainbow trout (Fig. 4). In Atlantic salmon, corner cones are lost almost entirely from the retina prior to smoltification (Fig. 4A), while in rainbow trout these cones become absent only from the ventral retina (Fig. 4D), and when the

animal is larger (Novales Flamarique, 2001). Corner cones reappear in the upper dorsal retina of the Atlantic salmon (Figs. 4B,C), whereas the distributions of corner cones in the smolt and adult rainbow trout are similar (Figs. 4E,F; Novales Flamarique, 2001).

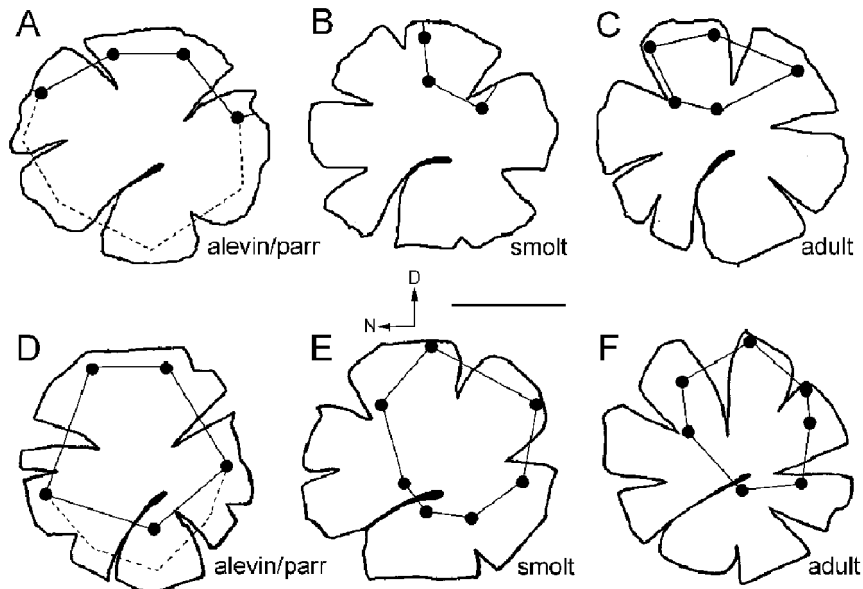


Figure 4. Ontogeny of corner cone distribution in the retina of Atlantic salmon (A-C) in comparison with that of rainbow trout (D-F). (A) The hatched Atlantic salmon (alevin) has corner cones throughout the retina (union of full line and dash line), while the parr lacks corner cones everywhere except along the dorsal periphery (union of full line and retinal perimeter). (B) The smolt has corner cones in the upper dorsal retina. (C) Fish that approach sexual maturation (adults) show corner cones in various dorsal retinal locations. (D) The rainbow trout alevin shows corner cones throughout the retina (union of full line polygon and dashed line), while the parr lacks corner cones in the lower half of the ventral retina (full line polygon, redrawn from Novales Flamarique, 2001). (E) The rainbow trout smolt shows corner cones throughout the dorsal and temporal areas of the retina (redrawn from Martens, 2000). (F) The reproductive adult has corner cones in the dorsal and temporal areas of the rainbow trout retina (redrawn from Beaudet et al., 1997). Abbreviations and magnification bar as in Figure 3.

Discussion

Two ontogenetic pathways for corner cone distributions in salmonid fishes

Hatched Atlantic salmon have corner cones throughout the retina (Forsell et al., 2001). Some time during the parr stage (characterized by small juveniles with “parr” marks along their bodies) the corner cone distribution becomes restricted to the periphery of the dorsal retina (Fig. 4A). This almost-complete loss of corner cones from the retina is also found in the retina of the anadromous (sea-going) brown trout (Lyall, 1957; Ahlbert, 1976; Kunz, 1987; Bowmaker & Kunz, 1987), but contrasts with the partial absence of corner cones reported in the rainbow trout (Martens, 2000; Novales Flamarique, 2001) and, to a lesser extent, in the sockeye salmon (Novales Flamarique, 2000). In the rainbow trout, corner cones persist throughout the dorso-temporal retina of the smolt (Martens, 2000), whereas in the sockeye salmon smolt, corner cones are concentrated along the centro-dorsal retina (Novales Flamarique, 2000).

By the time the Atlantic salmon attains a retinal size of ~ 6 mm in radius, my results indicate that small numbers of corner cones produced in the retinal periphery become incorporated into the main retina (locations III and IV are statistically similar, and more dorsal locations contain corner cones at both the smolt and adult stages; Figs. 3B,C). There is thus a switch from lack of incorporation (locations I and II are statistically different, and I comprises corner cones, Figs. 3A,B) to some incorporation at a later time during development. These results also suggest that regeneration of corner cones into existing mosaics should be minimal, if at all, since the cone densities observed in the dorsal retina of the adult can be ascribed to peripheral production and retention, at the young smolt stage, coupled to retinal stretching. Regeneration of corner cones at sexual maturation has been inferred from electrophysiological evidence in the sockeye salmon (Novales Flamarique, 2000), and such regeneration may also occur in the retina of anadromous brown trout (Ahlbert, 1976). In rainbow trout, regeneration of corner cones should be minor judging from the similar distributions of this cone type in the smolt and adult retinas (Figs. 4E,F; Novales Flamarique, 2001).

Together, the above results establish the existence of at least two ontogenetic pathways for corner (UV) cone distributions in salmonid retinas. The first, exemplified by the Atlantic salmon, involves an almost complete loss of corner cones from the juvenile retina followed by a partial re-incorporation (and, perhaps, some regeneration) in the dorsal retina of the adult. The second, exemplified by the rainbow trout, involves a gradual disappearance of corner

cones from the ventral retina of the juvenile and minor regeneration, if any, in the dorsal retina of the adult. In the Atlantic salmon, lack of incorporation of corner cones in the juvenile occurs throughout the retinal periphery (Kunz et al., 1994) while in the rainbow trout, this lack of incorporation is restricted to the lower retina (Novales Flamarique, 2001). In the Atlantic salmon, corner cones disappear from the main (non-peripheral) retina (present results, Kunz et al., 1994) while in the rainbow trout, the disappearance may be primarily due to retinal growth coupled to lack of corner cone incorporation, and not to apoptosis (cell death) of corner cones in the main retina *per se* (Novales Flamarique, 2001).

Factors that may influence the timing of corner cone loss

The process of smoltification in salmonid fishes is controlled by several hormones including growth hormone, cortisol and thyroid hormone (Clarke et al., 1996). Thyroid hormone changes body colouration, erasing the vertical “parr” marks and transforming the fish into a silver-coloured smolt (Clarke et al., 1996). Previous studies have reported that the precursor form of this hormone (T_4) can induce the loss and regeneration of corner cones in rainbow trout (Browman & Hawryshyn, 1992, 1994a,b). However, recent studies on rainbow trout smolts (Martens, 2000) and our preliminary experiments with rainbow trout parr and Atlantic salmon parr and smolts show that T_4 does not affect corner cone distributions in these animals, though it does change body colouration to silver. The fact that corner cones are lost during the parr stage of Atlantic salmon (Fig. 4A), when no external signs of smoltification are visible, support the findings that thyroid hormone is not directly involved in the loss of corner cones (since peak plasma thyroid levels occur 2-3 weeks after the start of the smoltification process; Alexander et al., 1994). Previous studies (involving behavioural recordings and histology) on the effects of T_4 on corner cone distributions in rainbow trout focused on the ventral retina (Browman & Hawryshyn, 1992, 1994a,b). Because corner cone densities in this area of the retina vary naturally due to retinal growth and lack of corner cone incorporation from the periphery (Novales Flamarique, 2001), the effects of T_4 treatment could have been confounded with those from natural growth. This provides an alternative interpretation that reconciles results from previous studies.

The present results also suggest that corner cone disappearance need not be associated with the smoltification process, nor with the passage from freshwater to saltwater, at least in the Atlantic salmon. It is possible that other variables (e.g. size) that are independent of environmental changes (such as the increased light intensity and water temperature associated with smoltification, Clarke et

al., 1996) may trigger the hormonal signals that dictate the loss or re-incorporation of corner cones.

Trends in cone densities and cone packing

The high double cone densities observed in the centro-ventro-temporal quadrant and the peripheral parts of the retina are in accordance with trends observed in the retinas of Atlantic salmon (Ahlbert, 1976) and in those of several other salmonid species (Beaudet et al., 1997; Novales Flamarique, 2000, 2001). It is believed that the ventro-temporal retina, where high cone densities are found, is an area of high resolving power specialized for the capture of small prey located in front and above the fish (Ahlbert, 1976). The high cone densities found along the periphery, which also occur in other fishes besides the salmonids (Ahlbert, 1969), may serve in the early detection of predators (Lythgoe, 1979), which usually attack at oblique angles (personal SCUBA diving observations).

It is interesting to note that although the density of cones decreases with growth, the average area occupied by these photoreceptors remains similar (Figs. 3D-F). This implies that cone growth compensates for the stretching that occurs during retinal expansion. Such a phenomenon is very important if the fish is to maintain the same spectral photon catch on each sector of the retina throughout life. The progressive loss of corner cones in the retinas of salmonid fishes poses a challenge to maintain a constant photon catch. There are several ways that the fish may cope with this that include: (1) a change in the vitamin A₁/A₂ chromophore ratio of the remaining cones towards a preponderance of vitamin A₁ (which absorbs shorter wavelengths, Alexander et al., 1994; Hárosi, 1994), (2) a change towards a shorter-wavelength-absorbing opsin (Shand et al., 1988), (3) a redistribution of interneuron connections from different sectors of the retina, and (4) a change in swim behaviour to depths where UV light is not present (see Bowmaker & Kunz, 1987). Salmonids do tend to swim deeper as they grow, especially following smoltification (Ahlbert, 1976; Bowmaker & Kunz, 1987). However, since corner cones are lost before smoltification in the Atlantic salmon (Fig. 3A), and since they commence disappearing before this transformation in the rainbow trout (Fig. 4E, Novales Flamarique, 2001), it is likely that chromophore compensation (hypothesis 1) also takes place as part of the adaptation process. Microspectrophotometric analyses of visual pigment absorbance from the various cone types at different developmental stages should be carried out to test the first two hypotheses.

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