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THE ONTOGENY OF ULTRAVIOLET SENSITIVITY, CONE DISAPPEARANCE AND REGENERATION IN THE SOCKEYE SALMON *ONCORHYNCHUS NERKA*

IÑIGO NOVALES FLAMARIQUE*

Department of Biology, University of Victoria, PO Box 3020, Victoria, British Columbia, Canada V8W 3N5

*Present address: Institute of Marine Research, Austevoll Aquaculture Research Station, N-5392 Storebø, Norway
(e-mail: inovales@hotmail.com)

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Summary

This study examines the spectral sensitivity and cone topography of the sockeye salmon *Oncorhynchus nerka* throughout its life history with special emphasis on ultraviolet sensitivity. Electrophysiological recordings from the optic nerve show that ultraviolet sensitivity is greatly diminished at the smolt stage but reappears in adult fish weighing about 201 g. Concomitantly, light microscopy observations of the retina show that ultraviolet cones disappear from the dorsal and temporal retina at the smolt stage but reappear at the adult stage. These changes occur for sockeye salmon raised in fresh water or salt water after smoltification. In contrast to this ultraviolet cycle, the other cone mechanisms (short-, middle- and long-wavelength-sensitive) and the rod mechanism remain present throughout ontogeny. The natural appearance and disappearance of ultraviolet cones in salmonid retinas follows surges in blood thyroxine at critical developmental periods. Their presence coincides with times of prominent feeding on zooplankton and/or small fish that may be more visible under ultraviolet light. It is proposed that the primary function of ultraviolet cones in salmonids is to improve prey contrast.

Key words: vision, retina, photoreceptor mechanism, ultraviolet-A light, ganglion cell, sockeye salmon, *Oncorhynchus nerka*.

Introduction

Since the first evidence of an ultraviolet visual pigment in the retina of a vertebrate, the Japanese dace *Tribolodon hakonensis* (Hárosi and Hashimoto, 1983), there have been an increasing number of studies reporting ultraviolet sensitivity in other species of fish (see Table 1 in Beaudet, 1997). Work with salmonids has shown that ultraviolet sensitivity in this group of fishes is a dynamic sensory modality that changes with life history stage. The number of ultraviolet cones and ultraviolet sensitivity diminish when the fish transform from parr to smolts (Lyall, 1957; Ahlbert, 1976; Bowmaker and Kunz, 1987; Kunz, 1987; Kusmic et al., 1993; Beaudet et al., 1993; Novales Flamarique and Hawryshyn, 1996). In contrast, mature reproductive salmon appear to have a higher number of ultraviolet cones than the smolts (Beaudet et al., 1997). These findings raise the possibility that ultraviolet cones may reappear in adult salmonids some time prior to or during spawning (Beaudet et al., 1997). Since both smoltification and sexual maturation are accompanied by a rise in blood thyroxine (Hoar, 1988; Alexander et al., 1994), and since thyroxine has been used to reversibly induce the appearance and disappearance of ultraviolet sensitivity in rainbow trout (*Oncorhynchus mykiss*) (Browman and Hawryshyn, 1992, 1994a), I hypothesised that wild sockeye salmon (*O. nerka*) would lose most of their ultraviolet sensitivity and regain it during the normal course of ontogeny.

To test this hypothesis, electrophysiological and histological measurements on the visual system of sockeye salmon were carried out at different life stages. The study used salmon raised in both fresh water and salt water after smoltification to determine whether the ontogeny of ultraviolet sensitivity was different for fish from the same stock living in different osmotic conditions. The histological results were also compared with those from coho salmon (*O. kisutch*) caught in the ocean during homeward migration to natal streams.

Materials and methods

Animals

Sockeye salmon (*Oncorhynchus mykiss*) alevins were obtained from Weaver Creek, near Harrison, British Columbia, Canada. The fish were raised for 4 years in 1000 l recirculating water tanks exposed to normal daylight in the aquatic facility at the University of Victoria, British Columbia, Canada. Fish were fed a daily ration of commercial trout pellets (Warrengton, Oregon, USA). Water temperature and oxygen concentration in the tanks were 13±1.5 °C and 11.02±0.63 p.p.m., respectively, during the 4 years.

When the fish started to show signs of smoltification (i.e. loss of parr marks and silvering of body), 20 fish were transferred to a 1000 l saltwater tank. Twenty other fish were

kept in a freshwater tank of equal dimensions. Individuals were removed from these two tanks at the alevin, smolt, juvenile and adult stages and tested for ultraviolet sensitivity. The term smoltification, strictly speaking, refers to the physiological transformation that occurs for anadromous salmonids only. However, many aspects of this transformation are similar for freshwater, landlocked species.

Two coho salmon, *Oncorhynchus kisutch*, were caught near Saltspring Island, British Columbia, Canada, on their homeward migration. The fish were killed by a blow to the head, after which the spinal cord was severed and the animal decerebrated. The eyes were marked for orientation, extracted, and put into cold (+4 °C) phosphate buffer (1.58×10^{-2} mol l^{-1} $NaH_2PO_4\cdot H_2O$, 8.72×10^{-2} mol l^{-1} Na_2HPO_4, pH 7.3). Once in the laboratory, the lens and cornea were removed, and the remaining eyecup was immersed in primary fixative. The ensuing histological procedure was as described below for sockeye salmon eyes. The two coho salmon were 6 and 8 years old from otolith and scale ring counts.

All animal handling and experimental procedures used in this study were approved by the University of Victoria Animal Care Committee, which complies with the guidelines set by the Canadian Council on Animal Care and NIH publication no. 86-23 *Principles of Animal Care*.

Absorption of coho lens and cornea

Each combination of lens and cornea from the coho salmon was placed on a specialized mount that fitted into the specimen chamber of a spectrophotometer. The lens/cornea complex was positioned near the sensor to avoid loss of light by scatter and refraction after passage through the sample. The amount of light absorbed through the central 2 mm^2 of cornea, lens plus some eye fluid was measured in this fashion.

Electrophysiology

To assess the spectral sensitivity of salmon, compound action potentials were recorded from the optic nerve of anaesthetised live animals. This technique involved surgical exposure of the fish's optic tectum and the placement of a Teflon-coated recording electrode with exposed silver tip through the optic tectum into the optic nerve. The background illumination was controlled by two optical channels equipped with tungsten–halogen sources whose intensity and spectral outputs were controlled by neutral and band-pass filters (Oriel). The outputs from the light guides at the ends of these channels were pointed to converge onto the fish's eye. The stimulus flash (750 ms in duration) was administered through a central optical channel equipped with a 300 W xenon arc source, a monochromator and a neutral-density wedge that controlled the wavelength and intensity of the output respectively. These three channels overlapped as much as possible onto the fish's eye and illuminated, primarily, the central retina (for a diagram of the optical and electrophysiological arrangement, see Novales Flamarique and Hawryshyn, 1997b).

To obtain a spectral sensitivity curve, the fish was adapted to a given background light for 1 h, after which its sensitivity was assessed at various wavelengths in the range 350–720 nm. For a given wavelength, the light intensity was increased in a stepwise fashion and the optic nerve response recorded. This response consisted of two components: the ON compound action potential, which appeared at the onset of the light stimulus, and the OFF component, which appeared 750 ms later, at the stimulus offset. The two components underlie two different neural pathways that transmit visual information to the brain (Wheeler, 1979). The response voltages were plotted against stimulus light intensity and fitted with a third-order polynomial. A criterion voltage of 20 μV was chosen to calculate sensitivity, defined as the reciprocal of the light intensity required to elicit the criterion voltage response. The criterion met two conditions: (i) it fell within the linear part of the curve fit for all wavelengths, and (ii) it was the closest possible response to the initial response for most wavelengths (the initial response was the first compound action potential detected for a given wavelength trial). This analysis was performed for various wavelengths to obtain a spectral sensitivity curve.

The spectral sensitivity function measured at the level of the optic nerve is a product of photoreceptor absorbance and neural processing by associated retinal elements. However, when good isolation backgrounds are achieved, optic nerve recordings have been shown to give spectral sensitivity curves that accurately mimic photoreceptor absorbance α bands measured by microspectrophotometry (see, for example, for the green sunfish (*Lepomis cyanellus*), Dearry and Barlow, 1987; Novales Flamarique and Hawryshyn, 1997a; and, for the common white sucker *Catostomus commersoni*, Novales Flamarique and Hárosi, 1997; Novales Flamarique and Hawryshyn, 1998). In addition, optic nerve recordings have the advantage of assessing the total response from the retina and how it changes with background and stimulus conditions.

Although the primary aim of this study was to assess ultraviolet sensitivity, a variety of other light backgrounds were also used to reveal the other cone mechanisms present in the animals studied (for background spectral outputs, see Novales Flamarique and Hawryshyn, 1998). For the purpose of this study, a cone mechanism encompasses a given class of cone (e.g. the ultraviolet cones) and the associated retinal circuitry that results in the optic nerve response measured. To estimate the inputs from the various cone mechanisms to a spectral sensitivity curve, the Simplex algorithm (Caceci and Cacheris, 1984) was used to fit combinations of an eighth-order polynomial template (Palacios et al., 1996) with different maximum absorbances following the equation in Sirovich and Abramov (1977):

$$R = [\sum k_i A_i^p(\lambda)]^{1/p}. \quad (1)$$

The wavelengths of maximum absorbance (λ_{max}) used in the polynomial template were those from parr rainbow trout cones because similar measurements are not available for sockeye salmon. The cones of rainbow trout have λ_{max} values at 365 nm (ultraviolet), 434 nm (short, S, or blue), 531 nm (middle, M, or green) and 576 nm (long, L, or red) (Hawryshyn and Hárosi, 1994). In equation 1, R is the spectral sensitivity response at

each wavelength, $A_i(\lambda)$ is the absorbance of cone pigment *i* for light of wavelength λ (determined from the polynomial template given a certain λ_{max}), and *p* is an exponent resulting from the mathematical requirement in the derivation of the formula that the function to be fitted be differentiatable at the origin (see Sirovich and Abramov, 1977). Such a requirement applies to the analysis of spectral sensitivity curves because there are no infinite spikes (or poles) within the data. The parameter *p* accounts for non-linear interactions in the retina. The coefficients k_i are coupling constants derived from the best fit of the model to the data. They are the weighted inputs from each cone mechanism to the spectral sensitivity response.

Histology

The eyes from various fish were prepared for histological analysis. After removing the cornea, lens and some eye fluid, the remaining eyecup was immersed in primary fixative (2.5 % glutaraldehyde, 1 % paraformaldehyde in 0.06 mol l^{-1} phosphate buffer, pH 7.2) and stored at 4 °C overnight. The retina was then removed in cold phosphate buffer and divided into four sectors using radial incisions. The resulting retina was mapped onto a piece of paper by projecting the image using an overhead projector. Following this, the tissue was divided into 18–25 topographical locations (depending on the size of the retina) and retraced onto paper. This procedure allowed for accurate determination of the position of each piece in the original retina (see Beaudet et al., 1997). The pieces were then incubated in secondary fixative (1 % osmium tetroxide) for 1 h at 4 °C, dehydrated through a series of solutions of increasing percentage ethanol content and embedded in Epon resin. Tissue shrinkage was estimated at 25–30 % but was not corrected for in the area calculations. The Epon blocks were cut into thick (0.5–1 μm) tangential sections to reveal the cone mosaic at each location. Cone densities were determined for each cone type at each location by counting the number of cones in an area of 26 000 μm^2 using a Zeiss Universal R microscope equipped with a 40× objective. The values were then converted into numbers of cones per mm^2. To compute double cone packing (i.e. the percentage of the area occupied by double cones), a computerized image-analysis system was used (Optimas Corp.) to measure the ellipsoid area of 12 double cones and eight single cones of each type per retinal sector (in general, single cone areas approached half the double cone mean area). The average double cone area multiplied by the double cone density gave the double cone packing. Cone packing by single cones was computed similarly. The type of single cone [corner (ultraviolet) cone or centre (blue) cone] was determined from the position of these cones in the unit mosaic. In salmonids, the ultraviolet cones face the partitions of the adjacent double cones (see Bowmaker and Kunz, 1987; Beaudet et al., 1993).

Results

Electrophysiology

The ontogeny of ultraviolet sensitivity in sockeye salmon is illustrated in Fig. 1 (see also Table 1 for Simplex-derived parameters). Young alevins (i.e. fish that have absorbed their yolk sacs and are ready to begin first feeding) possess an ultraviolet-sensitive mechanism for the ON component of the optic nerve response that peaks at 380 nm and an S mechanism with λ_{max} around 430 nm (Fig. 1A). Ultraviolet sensitivity diminishes over a period of days as the fish approach smoltification (Novales Flamarique and Hawryshyn, 1996) so

Table 1. *Simplex-derived coefficients and least sum of squares statistic for the ON and OFF responses of the various cone mechanisms presented in this study*

Stage	M. response	k_1, UV	k_2, S	k_3, M	k_4, L	*p*	SS
Alevin	UV-ON	0.75	0.84	0	0.029	2.08	0.05
	UV-OFF	0	0.30	0.89	0.070	1.07	0.005
	M,L-ON	0.002	0.003	1.55	0.25	9.04	0.022
Smolt	UV-ON	0.20	0.47	0.01	0.45	0.38	0.18
	UV-OFF	0.004	0	0.84	0.27	1.16	0.04
Large 1	UV-ON	0.54	0.66	0.21	0.001	2.20	0.11
	UV-OFF	0	0.02	1.03	0.003	0.87	0.18
Large 2	UV-ON	1.011	0.70	0	0.11	5.14	0.64
	UV-OFF	0.001	0.009	0.76	0.39	1.09	0.031
Large	M-ON	0.19	0.08	0.94	0.49	3.32	0.11
	M-OFF	0.01	0.04	0.95	0	0.04	0.02
	L-ON	0.008	0.003	0.005	0.91	0.42	0.39
	L-OFF	0	0.07	0.76	0.49	1.28	0.034

SS is the sum of squares residual derived from the sum of the differences between mean spectral sensitivity points and those predicted by the Simplex model; M. response denotes the cone mechanism response; Large 1, Large 2 and Large are large salmon (type 1), large salmon (type 2) and large salmon (types 1 and 2); k_1–k_4, coupling constants for ultraviolet (UV), short (S), middle (M) and long (L) cones, respectively; *p*, exponent in equation 1 (see text for further details).

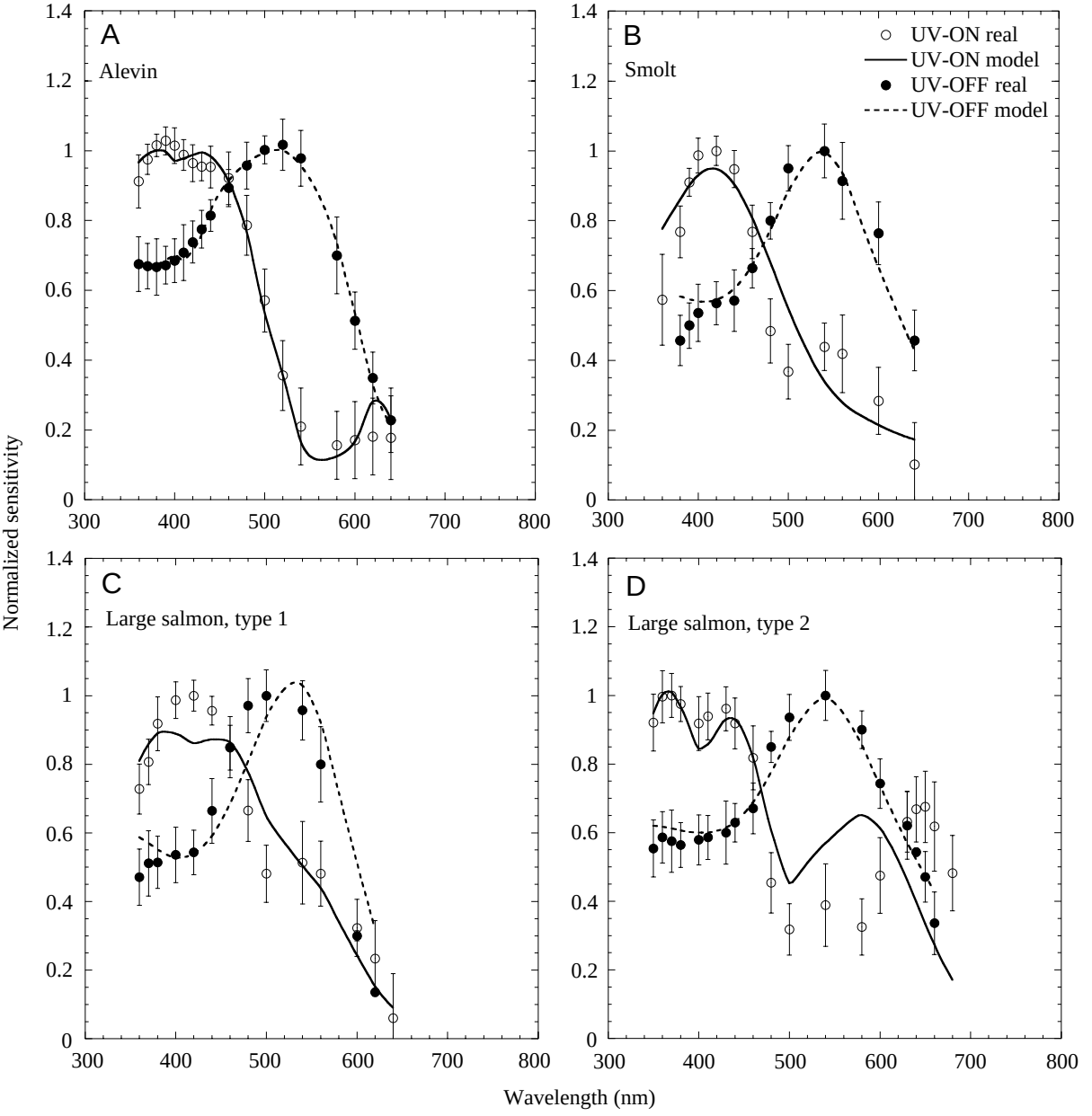

Fig. 1. Spectral sensitivity of ON and OFF responses under ultraviolet (UV) isolating conditions for sockeye salmon at different life history stages (N=6 per stage). The 'real' values are the experimental data points ± S.E.M.), while the model curves are the best fits generated by the Simplex algorithm using equation 1. (A) Alevin, mass 1.1±0.2 g, total length 4.7±0.3 cm. (B) Smolt, mass 4.5±0.14 g, total length 7.8±0.2 cm. (C) Large salmon (type 1), mass 201.4±13.6 g, total length 27.6±1.3 cm. (D) Large salmon (type 2), mass 281.75±49.7 g, total length 30.5±1.4 cm (means ± S.D., N=6). In B–D, the curves represent the average sensitivities of three salmon raised in fresh water and three in salt water after smoltification; the sensitivity curves were similar irrespective of the osmotic conditions in which the salmon were raised. For a given mean curve, the spectral sensitivity data were normalized by dividing each value by that at λ_{max} and inverting the result (Bernard, 1987; Palacios et al., 1996). The highest sensitivity values (given as the negative logarithm of the sensitivity measured in units of $cm^2\,s\,photons^{-1}$) and the wavelengths (in nm) at which they occurred for the various cone mechanisms were as follows: alevin, ultraviolet-ON (380 nm), −12.76; ultraviolet-OFF (520 nm), −13.26; smolt, ultraviolet-ON (420 nm), −12.48; ultraviolet-OFF (540 nm), −13.74; adult type 1, ultraviolet-ON (420 nm), −12.15; ultraviolet-OFF (500 nm), −13.00; adult type 2, ultraviolet-ON (370 nm), −13.49; ultraviolet-OFF (540 nm), −13.21.

that, by the time parr become smolts and are ready to enter the estuaries on their open-ocean migration, only a predominant S mechanism is present under ultraviolet isolation (λ_{max}=420 nm, Fig. 1B; the parr stage represents the most advanced alevin stage). Four-year-old fish tested weighing on average 201 g, whether kept in fresh water or salt water after smoltification, showed broad sensitivity responses in the ultraviolet–S region of the spectrum, and the average curve was best fitted by a model with similar inputs from both the ultraviolet and S mechanisms (Fig. 1C; Table 1). Larger fish

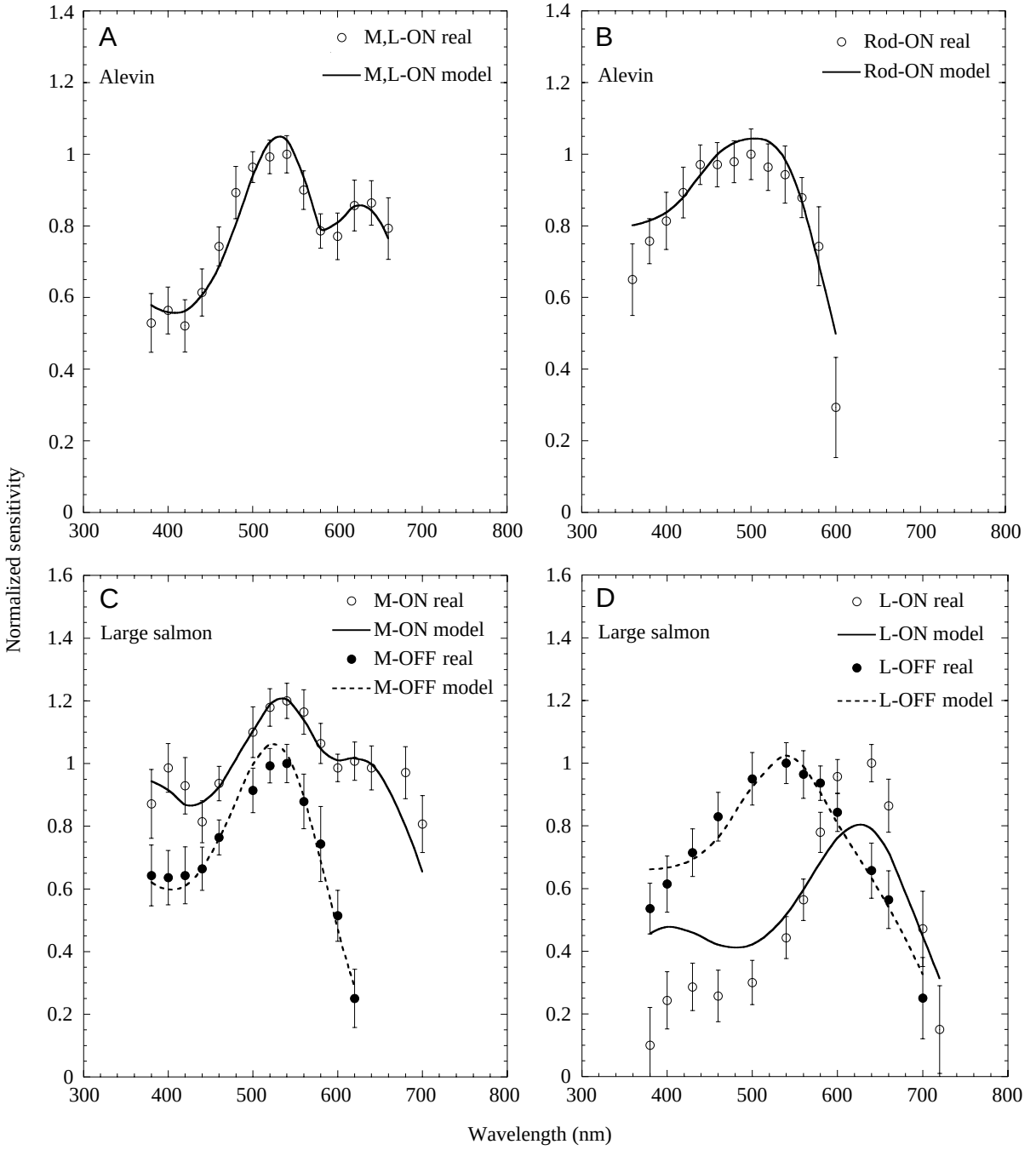

Fig. 2. Spectral sensitivity of ON and OFF responses under light backgrounds that isolated the middle- (M) and long- (L) wavelength mechanisms of sockeye salmon at different life history stages (A,C,D) and the rod mechanism at the alevin stage (B) (*N*=6 per stage). The response curves were obtained from the same animals as in Fig. 1, and the treatment and presentation of data are the same. In C and D, each curve is the mean sensitivity of three type 1 and three type 2 large salmon because all exhibited similar sensitivities. The highest measured sensitivity values (given as the negative logarithm of the sensitivity measured in units of $cm^2\,s\,photons^{-1}$) and the wavelengths (in nm) at which they occurred for the various cone mechanisms were as follows: alevin, M,L-ON (540 nm), −13.22; adult type 1, M-ON (540 nm), −13.29; M-OFF (540 nm), −13.20; adult type 2, L-ON (640 nm), −12.61; L-OFF (540 nm), −12.91. The rod curve was fitted with an eighth-order polynomial; the highest sensitivity occurred at 500 nm and was −14.87.

weighing on average 282 g, some of which possessed sexual maturation traits (adults), had distinct ultraviolet and S mechanisms (Fig. 1D). In all cases, the OFF response under ultraviolet isolation conditions was dominated by the M mechanism with maximum sensitivity wavelengths in the range 500–540 nm (Fig. 1).

Using light backgrounds that adapted the ultraviolet and S mechanisms, spectral sensitivity curves were obtained that

revealed similar M and L mechanisms at all life history stages (Fig. 2A,C,D). The maximum sensitivities for these mechanisms occurred at around 540 nm (M mechanism) and in the range 630–640 nm (L mechanism) for the ON component of the response. The L mechanism peaked at longer wavelengths (640 nm) as the fish reached sexual maturity; this was probably due to an increase in porphyropsin content in the retina. The OFF responses under these adapting backgrounds were again dominated by the M mechanism (Fig. 2C,D). Under complete darkness, the alevins showed a prominent rod mechanism (λ_{max}=500 nm, Fig. 2B). Unlike cone mechanisms, the rod mechanism was more sensitive to light and exhibited a broader response curve. A similar rod response curve was obtained for parr fish (Novales Flamarique and Hawryshyn, 1996) and for a few adults tested in this study (data not shown).

Histology

The retina of sockeye salmon has a photoreceptor layer with rods and single and double cones. In tangential view, at the level of the ellipsoids of the cones, double cones are arranged in rows or square formations termed mosaics (Fig. 3; for additional diagrams of typical cone mosaics in salmonid

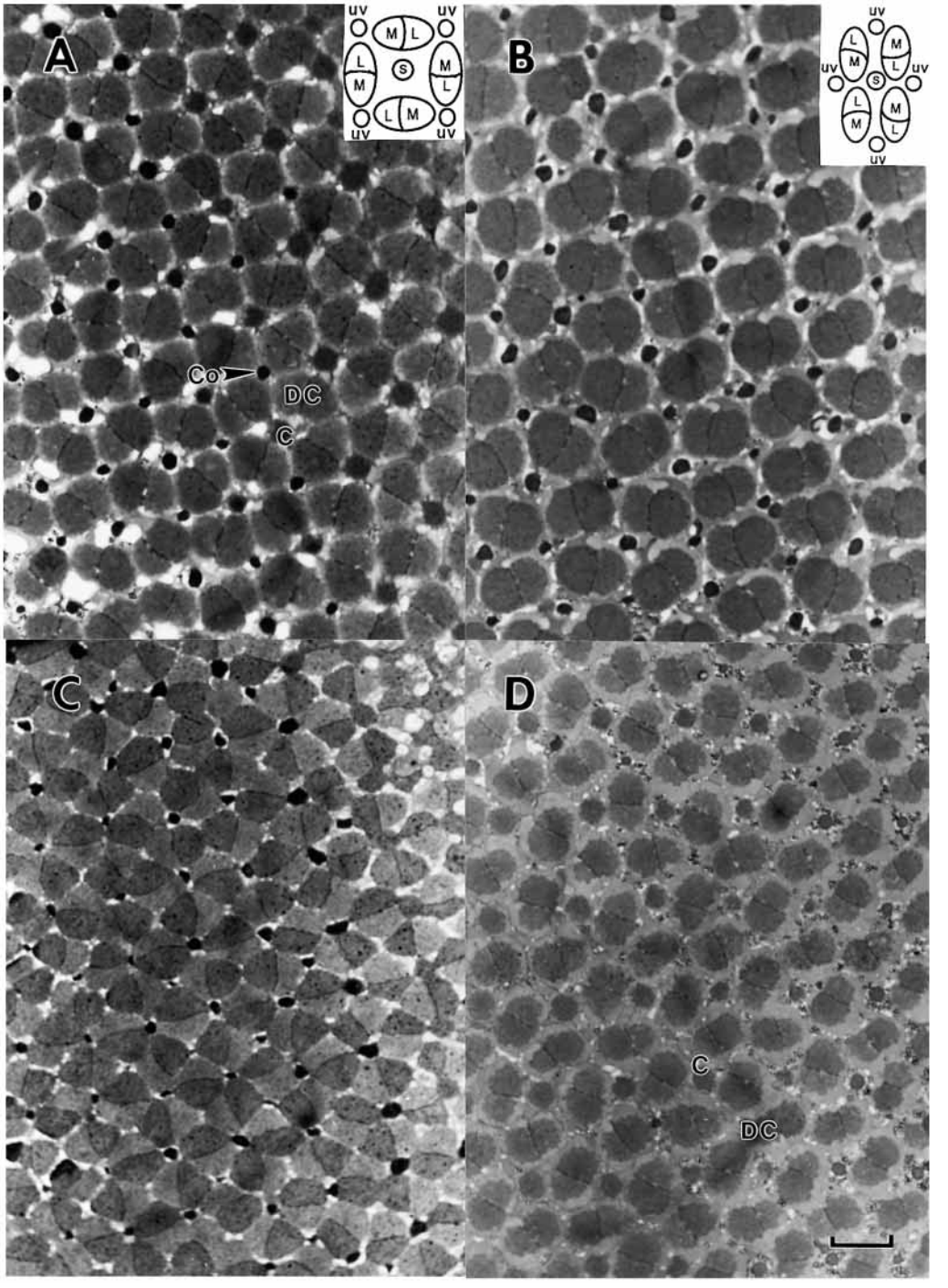

Fig. 3. Cone mosaics in alevin sockeye salmon. (A) Square mosaic with ultraviolet cones (dorsal retina); (B) row mosaic with ultraviolet cones (temporal retina); (C) square mosaic lacking most ultraviolet cones (ventral retina); (D) square to row mosaic lacking ultraviolet cones (ventro-nasal retina). In A and B, insets are idealized diagrams of a square mosaic with ultraviolet cones and a row mosaic with ultraviolet cones, respectively. Similar mosaics exist without ultraviolet cones. DC, double cone; Co, corner cone; C, centre cone; L, long; M, middle; S, short; UV, ultraviolet. Scale bar, 10 μm (in D; applies to all photomicrographs).

retinas, see Beaudet et al., 1997). These mosaics may vary in the presence and number of accessory corner (ultraviolet) cones which, in turn, determine the magnitude of ultraviolet sensitivity for a given retinal location.

The retinas of alevin fish show ultraviolet cones in every location examined except for the ventral periphery (Fig. 4A). Most of the locations have square mosaics, but there are rows in the centro-temporal area and in the nasal periphery (Fig. 4A). In contrast, when the fish become smolts, the ultraviolet cones are mainly concentrated in the central retina (Fig. 4B). In addition, row mosaics are more prevalent along the temporal retina in the smolt.

The number of single ultraviolet and blue cones, separately, in any given retinal location is usually less than half that of double cones (Fig. 5). For both alevin and smolt fish, the highest cone densities occur in the centro-ventral retina and in the ventral periphery (Fig. 5A,C). Double cone packing is highest in central and temporo-dorsal locations in the parr retina, and in the centro-ventral retina of smolts (Fig. 5B,D). In smolts, ultraviolet cone packing is highest in the nasal retina and along the embryonic fissure (Fig. 5D).

Large (type 1) salmon (mean mass 203 g) possessed retinas with ultraviolet cones in various dorsal and temporal locations (Fig. 6A). These locations were previously devoid of ultraviolet cones in the smolt (Fig. 4B). Larger salmon (type 2, mean mass 270 g) possessed ultraviolet cones throughout the dorso-temporal retina (Fig. 6B,C). In the case of the largest salmon examined (type 2, mean mass 347 g) (Fig. 6D), the only locations with ultraviolet cones missing were towards the ventral and nasal periphery, a situation that resembles that in the alevin retina (Fig. 4A). The increase in the number of ultraviolet cones among adult fish was independent of sex or the osmotic conditions in which the animals were raised after smoltification (Fig. 6).

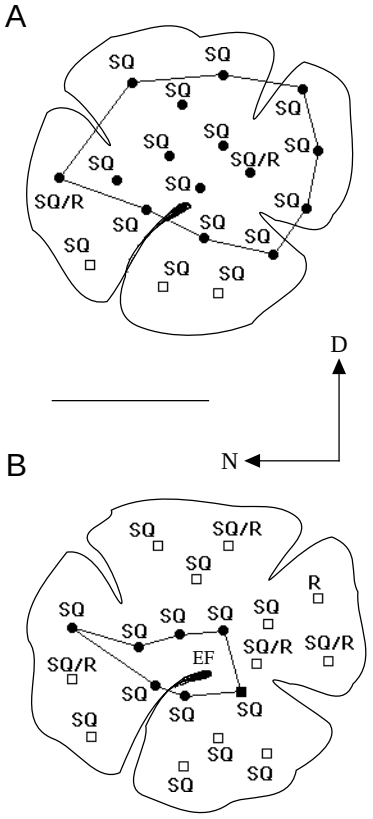

Fig. 4. Distribution of ultraviolet cones and mosaic types in (A) alevin and (B) smolt salmon (*N*=3 per stage). The retina contours are from one animal in each group representative of the group. Filled circles represent locations with ultraviolet cones, open squares locations without ultraviolet cones, and the filled square is a location with ultraviolet cones in only some animals. The polygons encompass the retinal surface with ultraviolet cones. SQ, square mosaic; R, row mosaic; N, nasal retina; D, dorsal retina. The black strip in each retina represents the embryonic fissure (EF, e.g. Fig. 4B); the optic nerve is located at the central 'head' region of the EF. The scale bar is 1.5±0.07 mm for the alevin retina and 1.9±0.08 mm for the smolts (mean ± S.D.).

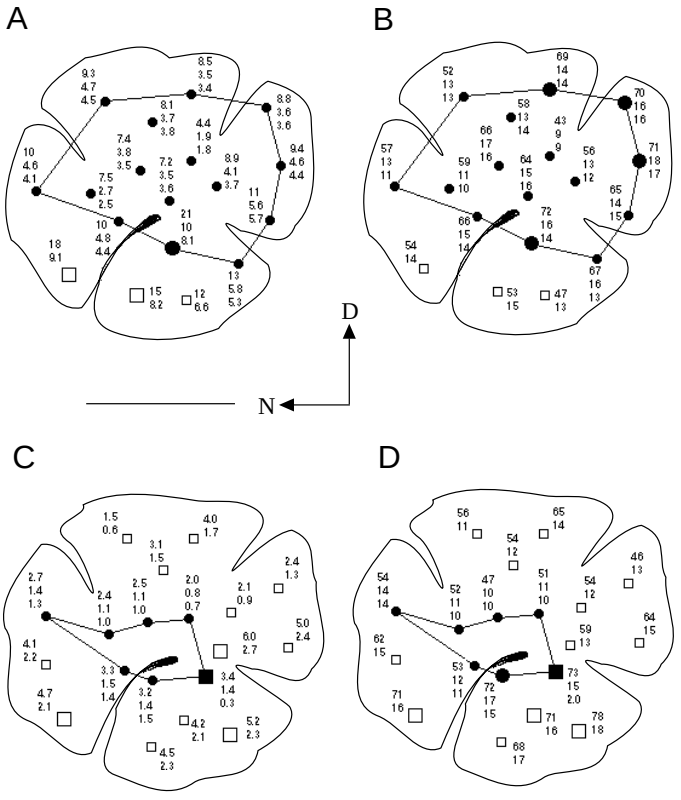

Fig. 5. (A) Average cone density and (B) cone packing for the alevin sockeye salmon, and (C) average cone density and (D) cone packing for the smolt (*N*=3 per stage). The density of cones is expressed in thousands per mm^2, and the cone packing is expressed as a percentage of the total area occupied by a given cone type (the standard deviations were less than 8.4 % of the means). For each location, the top number corresponds to the density or cone packing of double cones, the middle number to that of single short (S) cones and the bottom number, if present, to that of the single ultraviolet cones. Larger symbols denote locations where the density or cone packing is one standard deviation above the average from all locations. Other details are as in Fig. 4.

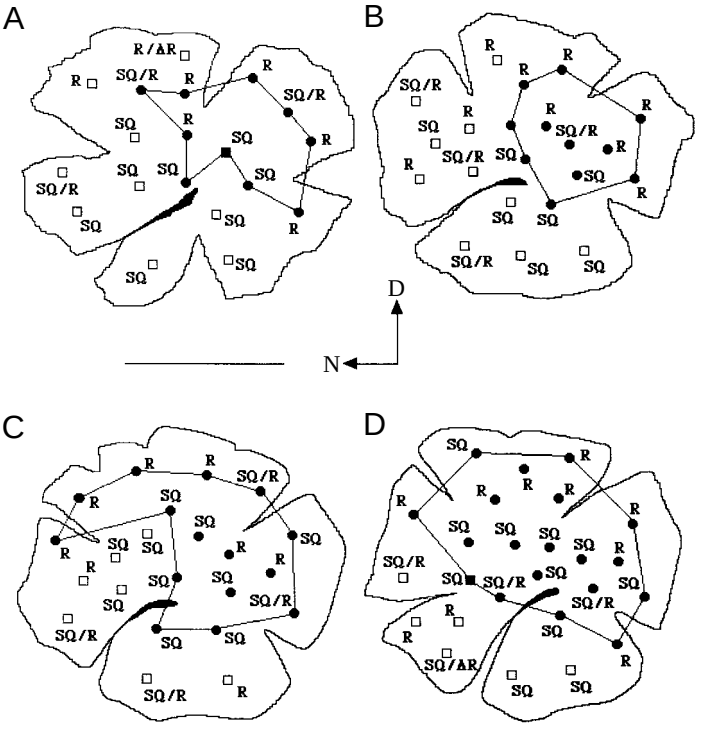

Fig. 6. Distribution of ultraviolet cones and mosaic types in (A) three large (type 1) freshwater sockeye salmon (mass 203±6.65 g, total length 26.1±3.24 cm), (B) two large males and one large female (type 2) saltwater salmon (mass 262±5.58 g, total length 29.8±2.17 cm), (C) a large male (type 2) freshwater salmon (mass 288 g, total length 30.2 cm), and (D) a large male and two large females adult (type 2) freshwater salmon (mass 347±9.26 g, 33.4±2.45 cm). The scale bar is 0.84±0.13 cm. Values are means ± S.D. AR, alternating row mosaic. Other details are as in Fig. 4.

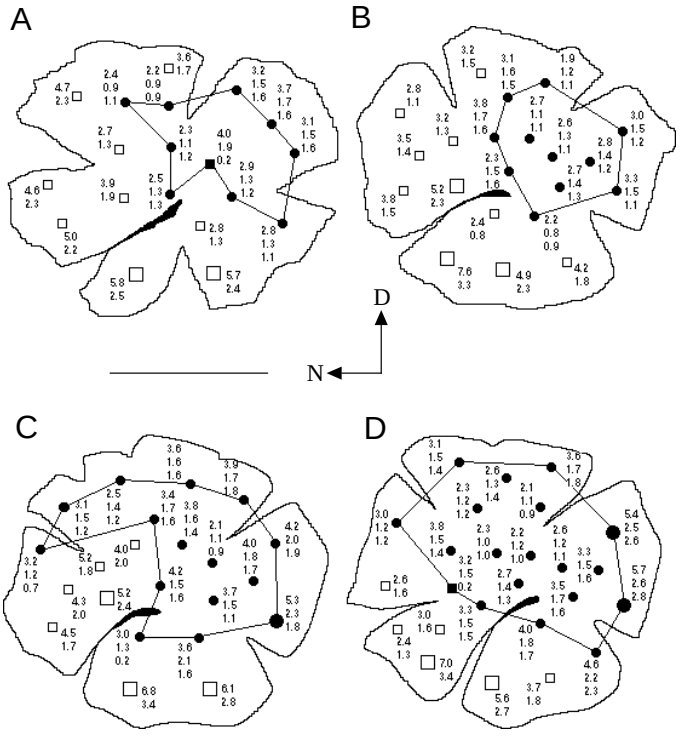

Fig. 7. Density of cones in the retinas of the fish from Fig. 6. Other details are as in Fig. 5.

In general, the adult retinas had a larger proportion of row mosaics than the alevin and smolt retinas. Square mosaics were most preponderant in the central and ventral retinas (Fig. 6). Some retinas also showed an alternating row mosaic at peripheral locations (Fig. 6A,D); this formation has been described previously by Beaudet et al. (1997). In addition to single mosaic types, some of the sections examined exhibited a mixture of square and row mosaics indicating zones of transition, as previously noticed in other salmonids (Lyall, 1957; Ahlbert, 1976; Beaudet et al., 1997).

The density of double cones in adult retinas was highest in the ventral, temporal and centro-nasal regions. Ultraviolet cone density was highest for dorso-temporal locations (Fig. 7). The highest cone packing numbers were generally found in the ventro-temporal retina, although high percentages were also found in the nasal retina (Fig. 8).

Retinas from reproductive coho salmon caught in the ocean during their homeward migration showed similar cone distributions to those from large (adult) sockeye salmon (Fig. 9). In particular, ultraviolet cones were again present along central, dorsal and temporal locations (Fig. 9A). Spectrophotometric measurements of light absorbance through the cornea and lens of these coho salmon showed that

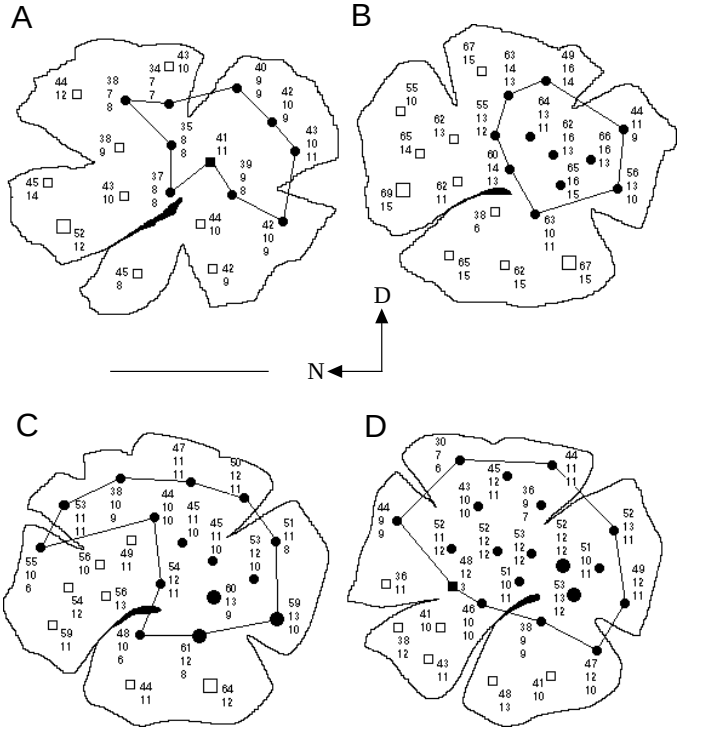

Fig. 8. Cone packing in the retinas of the fish from Fig. 6. Other details are as in Fig. 5.

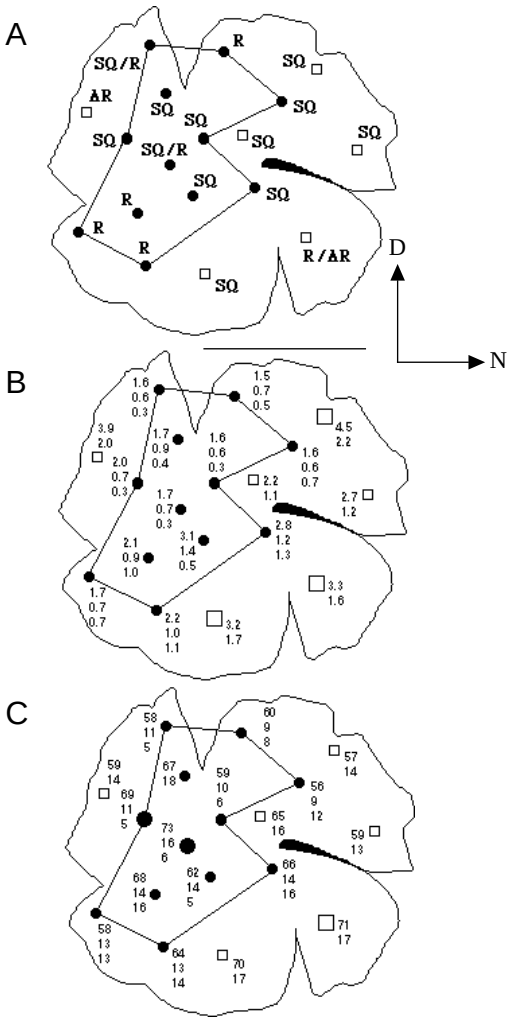

Fig. 9. Cone distributions in adult ocean-dwelling coho salmon (*Oncorhynchus kisutch*) (total length 54.2±2.71 cm, *N*=2). (A) Location of ultraviolet cones and mosaic types, (B) densities of various cone types and (C) cone packing densities. The scale bar is 0.91±0.08 cm (mean ± S.D). Other details are as in Figs 6–8.

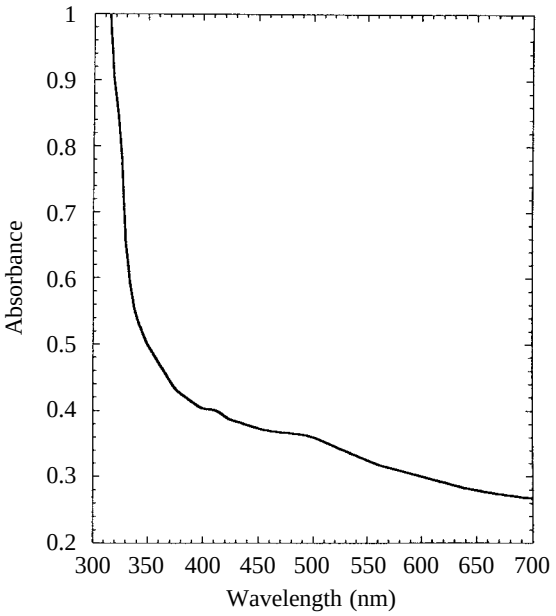

Fig. 10. Light absorbance by the cornea, lens and some eye fluid of adult coho salmon.

ultraviolet-A wavelengths as short as 320 nm could reach the retina for visual processing (Fig. 10). Such a result is consistent with transmission curves through the lens, cornea and vitreous fluid of the eye of juvenile rainbow trout (Hawryshyn et al., 1989) and with the minute concentrations of ultraviolet-absorbing compounds found in the rainbow trout lens (Thorpe et al., 1993).

Discussion

Ontogeny of ultraviolet sensitivity in salmonids

This study demonstrates that ultraviolet cones and ultraviolet sensitivity in sockeye salmon follow a natural cycle in which ultraviolet cones diminish in number during the process of smoltification and reappear in the retina at the late juvenile or adult stage (at approximately 201 g). The distribution of ultraviolet cones observed in adult sockeye salmon retinas is similar to that found for saltwater coho salmon migrating back to natal streams and for various species of salmon (coho, chinook, *O. tshawytsha*, and chum, *O. keta*) spawning in streams (Beaudet et al., 1997). Previous studies with rainbow trout have shown a loss of ultraviolet sensitivity when the animals transform to juveniles (>30 g; Beaudet et al., 1993), and an increase in the densities of ultraviolet cones in reproductive fish compared with the numbers found in the central retina of parr specimens (Beaudet et al., 1993, 1997). Together, these data suggest that the ontogeny of ultraviolet sensitivity found for sockeye salmon in the present study also applies to the other salmonid species whether anadromous or freshwater-dwelling.

The OFF response

The OFF response in this study was dominated by the M mechanism, as is also the case for rainbow trout under ultraviolet isolation backgrounds (Beaudet et al., 1993). Nonetheless, both young sockeye salmon (Novales Flamarique and Hawryshyn, 1996) and rainbow trout (Beaudet, 1997) can exhibit OFF responses that peak in the short-wavelength region of the spectrum. These responses are much less common and usually occur under ultraviolet/short- or long-wavelength isolation backgrounds. Because the technique used only records from a bundle of fibres in the optic nerve, the predominant M mechanism input to the OFF response measured during most experiments suggests that these are the most numerous OFF-type fibres in the visual system of salmonids.

Ultraviolet cones regenerate in selected areas of the retina

The retinas of adult sockeye salmon regain ultraviolet cones in the same areas where they previously disappeared during

smoltification, notably in the dorsal and temporal areas (Fig. 6). Such precise targeting is probably hardwired and dependent on the interaction of specific cellular messengers with the nuclear DNA of pluripotent cells such as the rod precursor cells of the outer nuclear layer (see Raymond and Rivlin, 1987; Raymond et al., 1995; Julian et al., 1998; Browman and Hawryshyn, 1994a,b).

At present, the cellular signals that control the loss and regeneration of ultraviolet cones and their precise targeting in the retinal mosaic are not known. However, both thyroxine and retinoic acid are thought to induce the loss and regeneration of ultraviolet cones in rainbow trout (Browman and Hawryshyn, 1994a,b; see related sampling discussion in Beaudet et al., 1997). Retinoic acid, in particular, can provoke differentiation of stem (precursor) cells into various retinal cell types (Kelley et al., 1994) and is a major determinant in the early development of vertebrate retinas (Marsh-Armstrong et al., 1994; Hyatt et al., 1996; Mey et al., 1997; Hoover et al., 1998). Thyroxine may bind to the heterodimers formed by thyroid and retinoic acid receptors, inducing similar changes to those observed after perturbation with retinoic acid (Glass and Rosenfeld, 1991; Marks et al., 1992; Browman and Hawryshyn, 1994b). The concentration of thyroxine in blood plasma increases some time prior to smoltification and during sexual maturation in wild salmon (Woodhead, 1975; Sower and Schreck, 1982; Biddiscombe and Idler, 1983; Youngson, 1989; Hoar, 1988; Youngson and Webb, 1993; Hamano et al., 1996). This hormone has been shown to affect body silvering, osmoregulation and growth (Higgs et al., 1982; Dickhoff and Sullivan, 1987), making it a likely key molecule in the mechanisms behind ultraviolet cone apoptosis and regeneration (Browman and Hawryshyn, 1994b; Beaudet et al., 1997).

Organization and abundance of cone types

The presence and distribution of mosaic types found for sockeye salmon are in accordance with previous observations on juvenile Atlantic salmon, rainbow trout and brown trout (*Salmo trutta*) (Ahlbert, 1976; Beaudet et al., 1993) and with findings from various species of Pacific salmon at the reproductive stage (Beaudet et al., 1997). In general, small fishes tend to have more square mosaics and these are usually concentrated in the central retina at all life stages (Figs 4, 6). The high cone densities found in the ventral, temporal and centro-nasal parts of the retina agree with results from previous studies (Ahlbert, 1976; Beaudet et al., 1997). The ventral area probably represents a region of high visual acuity specialized for the detection of small objects. In sockeye salmon, ultraviolet cones in centro-ventral locations may improve the contrast of zooplankton prey.

Cone packing did not follow trends in cone density for any of the stages examined. As the fish grows, however, differential growth in cone surface area in various parts of the retina seems to compensate to some extent for the differences in cone density between locations (Figs 7–9). This result is similar to that observed for other salmonid species at the reproductive stage (Beaudet et al., 1997) and may be a strategy to balance luminosity input across the retina.

Ecological significance of ultraviolet sensitivity in salmonids

Ultraviolet sensitivity has been shown to improve the foraging performance of small rainbow trout (Browman et al., 1994), and it is believed that such a function may be widespread among ultraviolet-sensitive fish species (e.g. Bowmaker and Kunz, 1987; Loew and Wahl, 1991; Loew et al., 1993). Zooplankton, on which small fish with ultraviolet sensitivity commonly feed in nature, have lipid saccules and carotenoid pigments that absorb ultraviolet wavelengths (Lee et al., 1970; I. Novales Flamarique, personal observation). These animals should therefore stand out to their predators against an ultraviolet background.

Large sockeye salmon often feed on euphausids and larger zooplankton (Scarsbrook et al., 1978) that also show high contrast against an ultraviolet background (I. Novales Flamarique, personal observation). In addition, many salmonids feed on juvenile herring and other small silvery fish (Groot and Margolis, 1991) which would appear more visible if they were to reflect more or less ultraviolet light than the water background (see Cronin et al., 1994). Since feeding is of major importance before the stream migration phase, it is likely that ultraviolet sensitivity plays an important role in the feeding activity of large salmon as well. Why then do smolts lose most of their ultraviolet sensitivity? Smolts also feed on zooplankton or small fish, a situation in which ultraviolet sensitivity would be advantageous. In addition, the ultraviolet cone in salmonids has been implicated in a polarization-detection mechanism that could be used, under ideal atmospheric and water conditions (Novales Flamarique and Hawryshyn, 1997b), in navigation during migratory episodes. Ocean-migrating smolts, however, would not be expected to detect the polarization of light judging from previous work with juvenile rainbow trout (Hawryshyn et al., 1990). These observations suggest the following conclusions: (i) that the loss of ultraviolet cones in smolts may be an accidental consequence of hormonal changes necessary for other processes (e.g. homeostasis, oxygen consumption, temperature tolerance) during smoltification; the reverse process as the fish matures would inadvertently bestow ultraviolet sensitivity on the animal again, which it could use to improve foraging performance; (ii) that ultraviolet sensitivity may be involved in other functions such as communication with conspecifics (see Hárosi, 1985), in mate choice (although see Foote, 1998) or in finding suitable locations for maturing (alevins) or spawning (adults); and (iii) that ultraviolet and polarization sensitivities in salmonids probably constitute accessory capabilities that complement the sensory performance of the animal when present, but are not crucial for its survival.

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