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FUNCTIONAL MAPPING OF ULTRAVIOLET PHOTOSENSITIVITY DURING METAMORPHIC TRANSITIONS IN A SALMONID FISH, *ONCORHYNCHUS MYKISS*

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Summary

Ultraviolet visual sensitivity appears to be reduced and, possibly, lost during smoltification in anadromous populations of salmonid fishes. Similar changes occur in non-anadromous salmonids over a mass range that is associated with smoltification in their anadromous conspecifics. However, in sexually mature adult salmonids, ultraviolet-sensitive cones are present in the dorso-temporal retina, suggesting that ultraviolet sensitivity (i) may be regained with sexual maturity or (ii) might never be completely lost. Both smoltification and the transition to sexual maturity are regulated, in part, by the hormone thyroxine. Thyroxine treatment of juvenile *Oncorhynchus mykiss* results in precocial developmental changes that mimic smoltification, including a reduction of ultraviolet sensitivity. However, whether loss of ultraviolet sensitivity in *O. mykiss* or in other species of salmonids is complete during normal development (or in response to thyroxine treatment) is unclear. In the present study, we have 'mapped' topographically ultraviolet photosensitivity during natural and hormone-induced smoltification. Thyroxine-treated *O. mykiss* juveniles and anadromous steelhead *O. mykiss* smolts were examined for ultraviolet visual sensitivity by recording compound action potentials from the optic nerve. By selectively illuminating either the dorsal or the ventral retina, we have shown that the reduction of ultraviolet sensitivity occurs primarily in the ventral retina in both groups of fish. Ultraviolet sensitivity remains intact in the dorsal retina.

Key words: spectral sensitivity, vision, fish, ultraviolet sensitivity, developmental change, smoltification, thyroxine, *Oncorhynchus mykiss*, electrophysiology, optic nerve.

Introduction

Salmonid fishes are characterized by a three-stage life history separated by two metamorphic transitions (Groot and Margolis, 1991). Shortly after hatching and absorption of the yolk sac, juvenile salmonids are known as parr. Parr are invariably found in freshwater lakes or streams, and the length of the parr period varies dramatically depending on species. Through a combination of endogenous timing, environmental factors and size-related factors, salmonid parr enter a metamorphic transition called smoltification and thereafter are called smolts (Groot, 1982; Groot and Margolis, 1991). Smoltification prepares the salmonid for the transition to the salt water environment and is characterized by several physiological, morphological and behavioural changes in osmoregulation, activity patterns, feeding habits, and body coloration and silvering (Groot, 1982; Groot and Margolis, 1991). Only anadromous, or migratory, populations of salmonids actually make the journey to salt water, and therefore the term 'smoltification' is typically used only when referring to anadromous salmonids. However, within single species, there are often not only populations that migrate to sea water but also non-migratory, or non-anadromous, populations that remain in fresh water (e.g. in *Oncorhynchus mykiss*, these are known as steelhead and rainbow trout, respectively). Non-anadromous salmonids exhibit some, although not all, of the developmental changes associated with smoltification (e.g. non-anadromous fish exhibit similar changes in diet and vision, but do not show pronounced changes in osmoregulatory ability). In particular, non-anadromous salmonids exhibit similar developmental changes in visual sensitivity to ultraviolet light when they attain a size associated with smoltification in their anadromous conspecifics (see below; Hawryshyn et al., 1989; Browman and Hawryshyn, 1992; Beaudet et al., 1993). Therefore, in this paper, we will use the terms 'smoltification' and 'smolt' to refer both to anadromous fish that actually smolt completely and enter sea water and, more loosely, to refer to non-anadromous fish that exhibit similar changes in the visual system during corresponding developmental transitions. Regardless of whether individuals are anadromous or not, the third and final phase of every salmonid's life, sexual maturity, is characterized by gonadal

maturation and the development of secondary sexual traits (i.e. bright body colour, morphological changes in the jaw). Sexual reproduction occurs in fresh water. Therefore at sexual maturity, migratory populations must return to fresh water to breed.

In fish, ontogenetic changes in retinal photoreceptor content, chromophore ratio and retinal ganglion cell distribution occur in conjunction with changes in habitat and/or feeding behaviour (e.g. Beatty, 1966; Bowmaker and Kunz, 1987; Whitmore and Bowmaker, 1989; Hawryshyn et al., 1989; Loew and Wahl, 1991; Loew et al., 1993; Munz and Beatty, 1965; Shand et al., 1999; Shand et al., 2000). Age-related loss, or reduction, of ultraviolet photoreception appears to occur in many species of fish, including salmonids (Bowmaker and Kunz, 1987; Whitmore and Bowmaker, 1989; Hawryshyn et al., 1989; Loew and Wahl, 1991). The developmental trajectory of ultraviolet visual sensitivity in salmonid fishes appears to be strongly linked to the three stages of the salmonid life history. Parr are ultraviolet-sensitive, and possess ultraviolet-sensitive cones in the retinal mosaic (Bowmaker and Kunz, 1987; Browman and Hawryshyn, 1992; Browman and Hawryshyn, 1994; Beaudet et al., 1993; Novales Flamarique and Hawryshyn, 1996; Novales Flamarique, 2000; Parkyn and Hawryshyn, 2000). The ultraviolet-sensitive cone is identifiable histologically by its position in the retinal mosaic, which is square-shaped with ultraviolet-sensitive cones positioned at the four corners of a square formed by four double cones surrounding a central single cone (Bowmaker and Kunz, 1987). Because of their position in the square mosaic, ultraviolet-sensitive cones are also called accessory corner cones. As parr undergo smoltification, ultraviolet sensitivity appears to decrease and may disappear altogether in some species. In conjunction with this loss of ultraviolet sensitivity, ultraviolet-sensitive cones are no longer present in at least some portions of the retina of salmonid smolts (Bowmaker and Kunz, 1987; Hawryshyn et al., 1989; Beaudet et al., 1993; Novales Flamarique and Hawryshyn, 1996; Novales Flamarique, 2000; and see below). Examination of the retina of four species of sexually mature salmonids, however, has revealed the presence of accessory corner (ultraviolet-sensitive) cones, in the dorso-temporal retina (Beaudet et al., 1997). These findings led to the hypothesis that ultraviolet-sensitive cones may regenerate into the retinal mosaic of adult salmonid fish (Beaudet et al., 1997) and, hence, that sexually mature fish may be ultraviolet-sensitive. Because the teleost retina grows throughout life and new photoreceptors are continually added to the retinal mosaic (Lyall, 1957; Johns and Fernald, 1981; Raymond and Hitchcock, 1997), this is certainly a plausible hypothesis. However, it is also possible that the accessory corner cones found in the retina of sexually mature fish are never completely lost. That is, loss of ultraviolet-sensitive cones at smoltification may not occur over the whole retina, and ultraviolet-sensitive cells may be retained in the dorsal retina throughout the life of the fish.

Non-anadromous *Oncorhynchus mykiss* have been one of the model species for studies on ultraviolet vision in salmonids. Using a heart rate conditioning paradigm, Hawryshyn et al. (Hawryshyn et al., 1989) have shown that ultraviolet sensitivity is reduced in non-anadromous *O. mykiss* as the fish attain a size associated with smoltification in anadromous conspecifics (40–60 g). These behavioural findings were confirmed using electrophysiological recordings from the optic nerve and histological examination of the retina (Beaudet et al., 1993). In addition, the hormone thyroxine plays a significant role in the reduction of ultraviolet sensitivity in *O. mykiss* (Browman and Hawryshyn, 1992; Browman and Hawryshyn, 1994). Thyroxine and its derivatives are the main hormonal agents driving smoltification in anadromous salmonids (for a review, see Hoar, 1988). Exposure to exogenous thyroxine can (i) induce smoltification and photopigment changes in salmonids, including rainbow and brook trout (Allen, 1977; McFarland and Allen, 1977; Alexander et al., 1994; Alexander et al., 1998) and (ii) cause a precocial loss, or reduction, of ultraviolet photosensitivity and the number of ultraviolet-sensitive cones in the retina of rainbow trout parr (Browman and Hawryshyn, 1992; Browman and Hawryshyn, 1994). However, whether loss of ultraviolet sensitivity is complete during smoltification (or in response to thyroxine treatment) in *O. mykiss* has not been addressed. The studies of Browman and Hawryshyn (Browman and Hawryshyn, 1992; Browman and Hawryshyn, 1994) and Beaudet et al. (Beaudet et al., 1993) used stimulus presentations that may have resulted in the illumination of primarily the ventral retina. Beaudet et al. (Beaudet et al., 1993) state that illumination of the retina was on the ventro-temporal quadrant of the retina. However, a liquid light pipe with an exit aperture of 68 ° was used to present the stimulus. Because of the large exit aperature of the light pipe and the diameter of the light pipe relative to the diameter of the eye of the fish, it is possible that the stimulus was not focused solely on the ventro-temporal retina, and other retinal areas may have been illuminated. Similarly, Browman and Hawryshyn (Browman and Hawryshyn, 1992; Browman and Hawryshyn, 1994) positioned the fish so that the pupillary plane of the eye was perpendicular to the stimulus at a roll of 20 °, which would cause the stimulus to be presented disproportionately on the ventral retina. However, the light was unlikely to be precisely focused on the ventral retina with this stimulus design, and the whole retina may have been illuminated (H. Browman, personal communication). Furthermore, in both these studies on *O. mykiss*, only the ventral retina was examined for the presence of ultraviolet-sensitive cones. The authors did not report data for the dorso-temporal retina, the area where ultraviolet-sensitive cones are present in sexually mature *O. mykiss* (Beaudet et al., 1993), but unpublished data suggests that at least some corner cones may have been present in the dorso-temporal retina (H. I. Browman, personal communication).

In this study, we have made the first attempt to map ultraviolet sensitivity topographically during smoltification in a salmonid using *O. mykiss* as our model species. On the basis of the findings described above, we hypothesized that ultraviolet sensitivity is not completely lost during

smoltification in *O. mykiss*. Rather, a population of ultraviolet-sensitive cones may remain in the dorso-temporal retina throughout the life of *O. mykiss*, or at least during smoltification. We used a thyroxine treatment paradigm similar to that used by Browman and Hawryshyn (Browman and Hawryshyn, 1992; Browman and Hawryshyn, 1994) to induce smoltification in non-anadromous *O. mykiss* parr. By treating the fish with exogenous thyroxine, we were able to examine both the topographic and temporal aspects of the reduction in ultraviolet sensitivity during smoltification. In addition, we examined a small number of anadromous steelhead *O. mykiss* smolts to determine whether thyroxine treatment of non-anadromous *O. mykiss* parr results in a similar pattern of loss of ultraviolet sensitivity as in naturally smolted anadromous fish.

Materials and methods

Non-domesticated rainbow trout parr and steelhead smolts (*Oncorhynchus mykiss*) were obtained from the Fraser Valley Hatchery, Abbottsford, British Columbia, Canada. The fish were maintained at a mean water temperature of 15±1 °C and under a 12 h:12 h L:D photoperiod in the Aquatic Facility at the University of Victoria, Canada. Light was provided by broad-spectrum fluorescent lights (Growlux) containing wavelengths from 350 to 750 nm (spectral emission provided in Parkyn and Hawryshyn, 2000). Fish were fed three times a week (Trout ABFW feed; Moore-Clark Inc.) prior to the start of the study. During treatment fish were fed a maintenance diet twice per week, so that they did **not** gain any appreciable mass during the study. The parr were of similar age and weighed between 3 and 15 g, well under the normal mass at which salmonids undergo smoltification (see Introduction). They were allocated to two groups, which were held in separate 30 l aquaria. Each group contained fish representing the full range of mass of the whole study group (that is, 3–15 g) One group was exposed to thyroxine added to the water (300 $\mu g\,l^{-1}$ L-thyroxine sodium salt (Sigma) dissolved in $0.1\,mol\,l^{-1}$ NaOH). The second group of parr, the control group, were held in the same conditions as the thyroxine-treated group, but received only the same volume of $0.1\,mol\,l^{-1}$ NaOH without hormone. Both the experimental and control fish were moved daily to a second pair of 30 l aquaria containing freshly treated or fresh untreated water, respectively. No more than 30 fish were maintained in one 30 l tank simultaneously. Spectral sensitivity measurements were collected after 2, 4 and 6 weeks of treatment.

Steelhead smolts were housed in a 350 l flow-through tank and were assessed for ultraviolet sensitivity without any prior hormone treatment. Spectral sensitivity was determined for four smolts (two for ventral retina and two for dorsal retina), which weighed between 60 and 85 g. By collecting spectral sensitivity data from these fish, we were able to determine whether naturally smolted anadromous *O. mykiss* exhibit similar changes in ultraviolet sensitivity to those found in non-anadromous *O. mykiss* treated with exogenous thyroxine. This comparison allowed us to determine the efficacy of thyroxine treatment on ultraviolet visual sensitivity. All procedures and care of the fish in this study were in accordance with the guidelines set by the University of Victoria Animal Care Committee under the auspices of the Canadian Council for Animal Care.

Surgical procedure

All experiments were conducted between 09:00 and 19:00 h during the light phase of the photocycle. Each fish was anaesthetized by immersion in MS-222 ($100\,mg\,l^{-1}$; CDate-222™, Crescent Research Chemicals, USA) until sedate enough to handle, and then paralyzed with an intramuscular injection of muscle relaxant ($0.01\,mg\,g^{-1}$ body mass, gallamine triethiodide or Flaxedil; Rhône-Poulenc Rorer Canada Inc.). The fish was placed in a moistened foam cradle within a Plexiglas holder. Moistened cheesecloth was placed over the animal to prevent dehydration. A mouthpiece inserted into the buccal cavity was connected to a fresh, aerated water supply containing MS-222 (approximately $50\,mg\,l^{-1}$) to irrigate the gills with a weak anaesthetic during the surgical procedure ($400\,ml\,min^{-1}$, 15 °C). The tissues and bone over the entire right optic tectum were removed to provide access to the recording area. The optic tectum was left intact. A local anaesthetic ointment (0.5 % Tetracaine and 0.5 % menthol or Pontocaine; Winthrop Laboratories, Canada) was applied to the cut edges of the surgical area, but not to the optic tectum. The fish was then moved within the Plexiglas holder to a Faraday cage where the gills were continuously irrigated with aerated water lacking MS-222. The fish were kept anaesthetized during the experiment with an intramuscular injection of a general anaesthetic (metomidate hydrochloride or Marinil, $0.003\,mg\,g^{-1}$ body mass; Wildlife Laboratories, Inc., USA). After the collection of a spectral sensitivity curve (see below), the fish were killed by double pithing.

In parr, blood samples were taken immediately after death. Serum was separated from cellular components by centrifugation and subsequently frozen. Because of the extremely small volume of serum obtained from each fish, individual samples from each group (controls and T_4-treated) were pooled for each week of treatment (2, 4 and 6 weeks). Pooled samples were analyzed by MDS Labs (Victoria, BC, Canada) to measure the thyroxine (T_4) and triiodothyronine (T_3) levels at 2, 4 and 6 weeks for both controls and thyroxine-treated fish (see Table 1). Because samples were pooled, the values presented in Table 1 are single point values. That is, the values are not averages of several samples, and therefore no mean or standard error can be calculated. No serum was collected from the smolts used in this study.

Determining spectral sensitivity

Spectral sensitivity was determined in a manner similar to that outlined by others (Beaudet et al., 1993; Novales Flamarique and Hawryshyn, 1996; Parkyn and Hawryshyn, 2000). Here, we provide a brief overview of the procedure. Compound action potentials (CAPs) were recorded from

Table 1. *Serum thyroxine (T_4) and triiodothyronine (T_3) levels in control and T_4-treated parr*

Treatment group	Total T_4 (nmol l^{-1})	Total T_3 (nmol l^{-1})
Week 2		
Control	28	1.73
T_4-treated	59	0.61
Week 4		
Control	34	1.05
T_4-treated	59	0.66
Week 6		
Control	42	1.43
T_4-treated	43	0.69

See text for details of how measurements were taken.

ganglion cell fibres in the optic nerve. An enamel-coated, chlorided-silver electrode (diameter 200 μm, A-M Systems, USA) was inserted through the rostral/medial portion of the right optic tectum (an area where ganglion cell fibres are known to project; Beaudet, 1997), and through the optic chiasm at a trajectory that resulted in the insertion of the electrode into the optic nerve fibres from the left eye (as in Parkyn and Hawryshyn, 2000). A reference electrode was placed into the nasal epithelium. The location of the recording electrode was confirmed in early experiments by gross dissection of the optic tectum and chiasm after determining spectral sensitivity. Positioning of the recording electrode in later experiments was aided by a stereotactic device and a micromanipulator. The location of the electrode was also confirmed during the experiment by the waveform of the CAP, which is a simple biphasic wave characterized by a positive deflection sometimes followed by a negative deflection (Beaudet et al., 1993; DeMarco and Powers, 1991; Parkyn and Hawryshyn, 2000). For analysis, we measured the amplitude of the peak of the positive deflection of the CAP in response to the stimulus onset (i.e. the ON response of the optic nerve). OFF responses were not analyzed in this study because previous results have shown that OFF responses are mediated either by the middle- or the long-wavelength cone mechanisms in salmonids, with little or no input from the ultraviolet and short-wavelength cone mechanisms (Beaudet et al., 1993; Parkyn and Hawryshyn, 2000).

Spectral sensitivity was determined by recording the CAP response to an incrementing intensity series of monochromatic light flashes. This was repeated for 12 wavelengths (350, 360, 380, 400, 420, 440, 460, 480, 500, 550, 600 and 650 nm); presented in quasi-random order to minimize overstimulation of individual cone mechanisms (Beaudet et al., 1993). The flashes were presented approximately 20 s apart, increasing in intensity with each step by 0.2 log units of intensity. At any given wavelength, 2 log units of intensity were covered to obtain one intensity–response function. The first few flashes were always subthreshold to establish the baseline noise in the preparation. The resulting intensity–response data for each wavelength was fitted with a Naka-Rushton equation (Naka and Rushton, 1966a; Naka and Rushton, 1966b) using a non-linear, least-squares fit.

Spectral sensitivity was calculated by determining the quantal flux at each wavelength necessary to produce a criterion response. The criterion response was chosen such that it was at the lower end of the linear portion of the response *versus* log intensity curve near the threshold for the CAP response (Beaudet et al., 1993; Parkyn and Hawryshyn, 2000). A low-amplitude criterion was chosen because at low intensities the CAP response is assumed to represent the mass response of individual ganglion-cell receptive field centers (Beaudet et al., 1993; Parkyn and Hawryshyn, 2000). Receptive field centers of individual ganglion cells in fish dominate over the surrounds at low intensities, but at higher intensities the surround can inhibit the center (Spekreijse et al., 1972). Therefore, at higher intensities, the receptive field surrounds may have inhibited the center and caused inhibition of the CAP response (see more detailed discussion by Beaudet et al., 1993). Using the Naka-Rushton fit (Naka and Rushton, 1966a; Naka and Rushton, 1966b), the amount of light (or threshold intensity) needed to produce the criterion response was interpolated for each wavelength. Sensitivity was defined as the inverse of threshold intensity (DeVoe et al., 1997).

The light stimulus was produced by a 300 W xenon arc lamp and power supply (Oriel Corporation, USA, models 66011 and 68811, respectively). The wavelength and intensity of the stimulus were controlled by a holographic-grating monochromator (ISA, USA) with a 10 nm bandwidth and an Inconel-coated, quartz neutral density wedge (4.0 maximum optical density; Melles-Griot). The monochromator and neutral density wedge were controlled by computer-driven stepper motors (Pontech, USA). Stimulus timing (500 ms duration, square-wave) was controlled by a computer interface with a shutter and shutter controller (UniBlitz SD-10; Vincent Associates, USA). The stimulus light was focused onto one leg of a trifurcated, quartz-core, fibre optic cable (Fiber Optic systems, Inc. USA), which transmitted the light into the Faraday cage, where the common end of the fibre cable (5.2 mm diameter) terminated approximately 8 mm from a quartz diffusing screen (12 mm in diameter). The quartz diffusing screen was positioned within 5 mm of the cornea of the fish's left eye. The fibre optic and diffusing screen were oriented at an angle to illuminate preferentially either the dorsal or the ventral portion of the retina. In addition, an opaque patch was placed over the cornea to decrease further the amount of light reaching half the retina (Fig. 1).

The irradiance of the light stimulus was calibrated using a Photodyne radiometer (Optikon model # 88XLA), which was positioned at a distance of 5 mm from the quartz diffusing screen. With the neutral density (ND) wedge set to the lowest amount of attenuation (0 ND), the maximum irradiance was measured directly every 10 nm from 340 nm to 710 nm. The neutral density wedge was calibrated at 540 nm for each 0.1 log unit from 0 ND attenuation to the maximum attenuation of the wedge (approximately 4.0 ND). At all other wavelengths

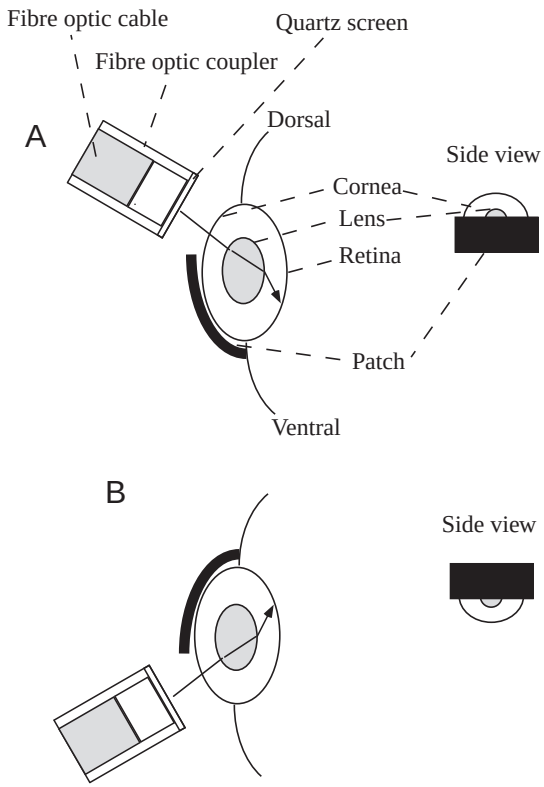

Fig. 1. Illumination of the ventral (A) and dorsal (B) retina. (A) A patch made of black, opaque, vinyl tape was affixed to the ventral cornea using cyanoacrylate glue. The patch was placed over more than half the retina (see side view), to reduce stimulation of the central retina, and the embryonic fissure where ultraviolet corner cones remain resident in all stages of salmonid development (see text). The fibre optic and quartz screen coupler were angled from above the eye, so that the light was incident on the dorsal cornea. The incident light ray shown is not necessarily representative of all light rays emerging from the fibre optic coupler; it is positioned for illustration purposes only. Light rays incident on the lens from above are refracted by the lens such that light will illuminate an area on the ventral retina. Because the stimulus was larger than the pupil of the fish, the configuration shown would result in illumination of the entire ventral retina. (B) A complementary arrangement of the patch and stimulus allowed for illumination of the dorsal retina.

used in our study, the neutral density filter was calibrated in 0.5 log unit increments from 0–4.0 ND, and linear interpolation was used to calculate the irradiance for intermediary neutral densities.

The CAP signal was amplified (Grass Instruments, P-5 AC preamplifier, 3–300 Hz band-pass filter settings) and simultaneously displayed on an oscilloscope and acquired by an A/D board (National Instruments, Inc.) for analysis. A custom-designed computer software package (TVH Systems Inc. and Racca Scientific Consulting) was used for data collection, on-line analysis and to control the wavelength, intensity and duration of the stimulus light. The sensitivity of the fish to a reference wavelength (420 nm) was measured periodically to check for any drift in the sensitivity of the preparation during recording. If the sensitivity at the reference wavelength drifted by 0.2 log units or more, the sensitivities of the wavelengths sampled since the reference was previously checked were remeasured. In stable preparations, however, sensitivity at the reference wavelength rarely drifted by more than ±0.1 log unit during the course of the experiment.

In addition to the stimulus light, the left eye was continuously illuminated with a constant-intensity, 500 nm long-pass background light during the experiment. This light was produced by a tungsten–halogen source. A 500 nm long-pass filter and a number of Inconel-coated neutral density filters (Corion, Inc.) allowed for control over the wavelength and intensity, respectively, of the background light, which was kept constant for all fish (log irradiance = 18.58, where irradiance is measured in photons $m^{-2}\,s^{-1}$). The trifurcated fibre optic cable (third leg unused) was used to combine the light from the stimulus and background channels. Because the common end of the fibre optic was well mixed and covered with a quartz diffusing screen, the final result was a constant background light with a stimulus light flash seen by the fish on the diffusing screen.

The fish were exposed to the long-wavelength-adapting background light for approximately 1 h prior to beginning the experiment. This coloured background was used to chromatically adapt the middle- and long-wavelength-sensitive cone mechanisms of *O. mykiss*, to isolate the ultraviolet- and short-wavelength-sensitive cone mechanisms. Salmonids possess four independent cone mechanisms: long-wavelength (red), middle-wavelength (green), short-wavelength (blue) and ultraviolet-sensitive (Bowmaker and Kunz, 1987; Hawryshyn et al., 1989). The photopigments underlying these cone mechanisms absorb optimally at a characteristic wavelength (λ_{max} = 576, 531, 434 and 365, respectively, for *O. mykiss*; Hawryshyn and Harosi, 1994), called the alpha-band (for which they are usually named), and secondarily at a shorter wavelength, the beta-band. The beta-band for the middle- and long-wavelength pigments is located in the ultraviolet area of the spectrum (Hawryshyn and Harosi, 1994). Therefore, because the absorption of energy at any wavelength to which the pigment is sensitive gives qualitatively indiscriminate signals (i.e. the principle of univariance), a visual response to ultraviolet light could be due to input from the beta-band of the middle- or long-wavelength cone mechanisms. Long-wavelength adaptation of these cone mechanisms causes a proportional reduction in both the primary (i.e. alpha) peak and in the beta peak absorption of the photopigment. Previous experiments have shown that this treatment is effective in spectrally isolating the contribution of ultraviolet-sensitive receptors to the spectral sensitivity function in birds, fish and amphibians by decreasing the contribution of the longer-wavelength cones (Chen et al., 1984; Hawryshyn and Beauchamp, 1985; Beaudet et al., 1993; Deutschlander and Phillips, 1995; Parkyn and Hawryshyn, 2000). Furthermore, theoretical models suggest that selective adaptation of the visual system can reveal the presence of an ultraviolet-sensitive cone population even if the ultraviolet-sensitive cones are only

a small proportion of the total receptor population (Goldsmith, 1986).

Analyzing spectral sensitivity data

For the parr/thyroxine-treatment study, all fish were grouped by treatment week (2, 4 and 6 weeks) and by retinal stimulation (dorsal and ventral). The spectral sensitivity curves from individuals in these groups were normalized to 420 nm (i.e. the sensitivity at each wavelength was determined by subtracting the absolute sensitivity obtained for 420 nm, hence 420 nm always had a relative value of zero) and averaged. Normalization to 420 nm allowed us to factor out any differences in absolute sensitivity between fish and recording preparations. A *t*-test (conducted using SPSS version 10) was used to test for significant differences in relative sensitivity between groups at specific wavelengths (e.g. comparison of ventral sensitivity to 360 nm in thyroxine-treated and control fish at 6 weeks). Because we predicted a reduction in ultraviolet sensitivity with thyroxine treatment, we examined differences in the relative sensitivity in the ultraviolet using a one-tailed *t*-test. 360 and 380 nm were chosen for this comparison because they represent the two wavelengths around the peak of the absorptance curve, 370 nm, for the ultraviolet cone mechanism. *Post-hoc* examination of the data showed a strong difference in the relative sensitivity in the green portion of the spectrum. Therefore, we also analyzed the mid-portion of the spectrum for differences in sensitivity using 500 and 550 nm wavelengths. For this comparison a two-tailed *t*-test was used since we had no prior prediction about a direction in change. The data from four steelhead smolts (two exposed to dorsal illumination and two exposed to ventral illumination) were analyzed individually, and statistical tests were not performed due to the small sample size.

To assess qualitatively the contribution of the ultraviolet-sensitive cone mechanisms to the spectral sensitivity, absorptance curves of the ultraviolet- and blue-sensitive photopigments were plotted over the spectral sensitivity curves. Using methods described (Parkyn and Hawryshyn, 2000), rainbow trout cone absorbance curves (from Hawryshyn and Harosi, 1994) were converted to absorptance values, and then corrected for wavelength-dependent losses in transmission for ocular media (Hawryshyn et al., 1989; who reported no difference in the transmission of ocular media of small and large rainbow trout at wavelengths above 320 nm). The resulting absorptance curves were fitted by eye to the experimental data to determine the presence or absence of the ultraviolet-sensitive cone mechanism in the spectral sensitivity curves. The absorptance curves were fitted to the spectral sensitivity data by vertically adjusting the absorptance curves for the best visible fit. This method of examining the data and pigment-curve-fitting provides a good indication of which cone mechanisms contribute to the spectral sensitivity function (Browman and Hawryshyn, 1992; Browman and Hawryshyn, 1994; Beaudet et al., 1993; Hughes et al., 1998; Parkyn and Hawryshyn, 2000).

In addition, a more quantitative analysis was performed by fitting a linear-additive model of the cone mechanisms to the experimental data (Coughlin and Hawryshyn, 1994; DeMarco and Powers, 1991; Hughes et al., 1998). The model takes the form:

$$S_w = (K_U A_U) + (K_S A_S) + (K_M A_M) + (K_L A_L), \quad (1)$$

where S_w is the spectral sensitivity at any given wavelength, K_X is the weight coefficient of cone type X (where X is L, long; S, short; M, medium; U, ultraviolet) and A_X is the relative absorptance of cone type X at wavelength W. The weights of the cone mechanisms were determined using a non-linear least-squares fit to the spectral sensitivity data (using Interactive Data Language, Research Systems, Inc.), and the presence, absence or reduction of the ultraviolet-sensitive cone mechanism was assessed. Because we used chromatic adaptation to reduce the sensitivity of the middle- and long-wavelength cone mechanism, and because we were interested mainly in the short-wavelength end of the spectral sensitivity function, we set K_M and K_L to zero and fitted the data for the ultraviolet- and short-wavelength mechanisms only (i.e. K_U and K_S). When we compared these results with the weights when K_M and K_L were allowed to be non-zero numbers, we found little difference in the relative weights of the ultraviolet and short-wavelength mechanisms. Therefore, only the results of the former analysis are presented here.

Results

T_4 treatment resulted in an elevated level of serum T_4 compared with controls, but only after 2 and 4 weeks of treatment (Table 1). T_3 levels of treated fish were reduced relative to controls, which is consistent with the effect of exogenous T_4 on the enzymatic pathway leading to T_3 production (MacLatchy and Eales, 1993). Because the values presented in Table 1 are only single point measurements (see Materials and Methods), one must be careful in any interpretation of these data. However, the data suggest that the T_4 treatment used in our study does have an effect on serum thyroxine levels, as we would expect. Further evidence of a physiological effect of our T_4 treatment was apparent as a significant silvering of the body in fish treated with thyroxine for 2 weeks. After 2 weeks of treatment, body-silvering persisted throughout the experiment in thyroxine-treated fish, but did not occur in any of the control fish (M. E. Deutschlander, D. K. Greaves, T. J. Haimberger, C. W. Hawryshyn, personal observation). Body silvering is a trait that is often used as a positive indicator of smoltification status (Groot and Margolis, 1991).

Fig. 2, Fig. 3, Fig. 4 show the results obtained for thyroxine-treated fish and control fish for 2, 4 and 6 weeks of exposure, respectively. The absorptance curves for the ultraviolet- and blue-cone mechanisms (purple and blue dotted lines, respectively) were fitted by eye **prior** to fitting the linear-additive model for the ultraviolet and blue mechanisms (black line) to the data. Our placement of the absorptance curves was congruent with the linear-additive model near the peaks of the

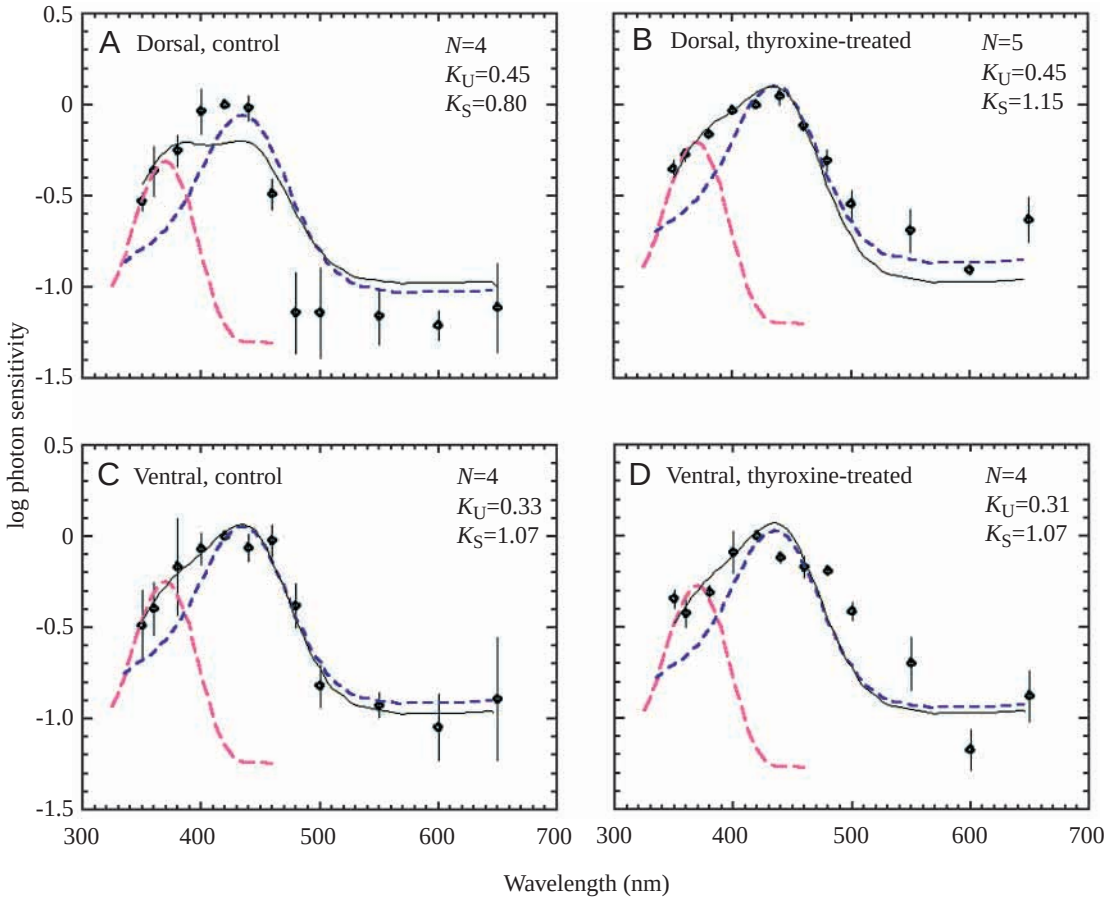

Fig. 2. Spectral sensitivity of fish after 2 weeks of thyroxine, or control, treatment. All spectral sensitivity curves were obtained under a long-wavelength background to isolate the short-wavelength and ultraviolet cone mechanisms (see text). Both control (A,C) and thyroxine-treated (B,D) fish were tested for dorsal and ventral retinal sensitivity (Fig. 1). (A,B) Results for dorsal stimulation, and (C,D) results for ventral stimulation. The values are means ± S.E.M. for all fish from each group (filled diamonds, with sample size (*N*) indicated on each graph). Data were first normalized to 420 nm (i.e. sensitivity at 420 nm was set to 0 in all fish). Pigment absorptance curves for the ultraviolet cone mechanism (purple dashed line) and the short-wavelength cone mechanism (blue dashed line) were fitted by eye to the averaged data. The resulting function of the linear-additive model for the ultraviolet and short-wavelength cone mechanisms is represented by the continuous black line. The weights determined by the linear-additive model for the ultraviolet (K_U) and short-wavelength (K_S) mechanisms (see text for further details) are presented on each graph.

two mechanisms, and for the short-wavelength tail of the ultraviolet mechanism and the long-wavelength tail of the blue mechanism. The absorptance curves fit poorly at wavelengths intermediate to the two peaks of the cone mechanisms (i.e. at 380 nm and 400 nm), where one would expect that summation between the blue and ultraviolet cone mechanisms would produce a sensitivity value higher than either mechanism could account for alone. Because the linear-additive model allows for summation, this method of analysis produced a much better fit to the sensitivity at the wavelengths intermediate to the ultraviolet and short-wavelength mechanisms. However, both types of analysis led to similar conclusions regarding ultraviolet sensitivity and the presence of the ultraviolet cone mechanism (see below).

The fit of the model and the absorptance curves to the sensitivity values at 550, 600 and 650 nm was often poor. This is to be expected because we did not allow the model to incorporate the middle and long-wavelength mechanism (i.e. K_M and K_L were forced to zero values), nor did we attempt to place green or red absorptance curves on the data. The primary reason for sampling at these longer wavelengths was to ensure that long-wavelength sensitivity was reduced below the level of ultraviolet and short-wavelength sensitivity to isolate the ultraviolet and short-wavelength cone mechanisms (see above). Therefore, the fit of the two analyses to these long-wavelength points is not critical to the interpretation of the results.

After 2 weeks of treatment, both thyroxine-treated fish and control fish exhibited ultraviolet sensitivity that could be accounted for only by the presence of an ultraviolet-sensitive cone mechanism in both the dorsal and ventral retina (Fig. 2). Similar results were obtained at week 4 (Fig. 3), and there were no significant differences in sensitivity at 360 or 380 nm for control versus treated fish (as determined by the *t*-test). However, there appeared to be a non-significant reduction in ultraviolet sensitivity in the ventral retina of thyroxine-treated

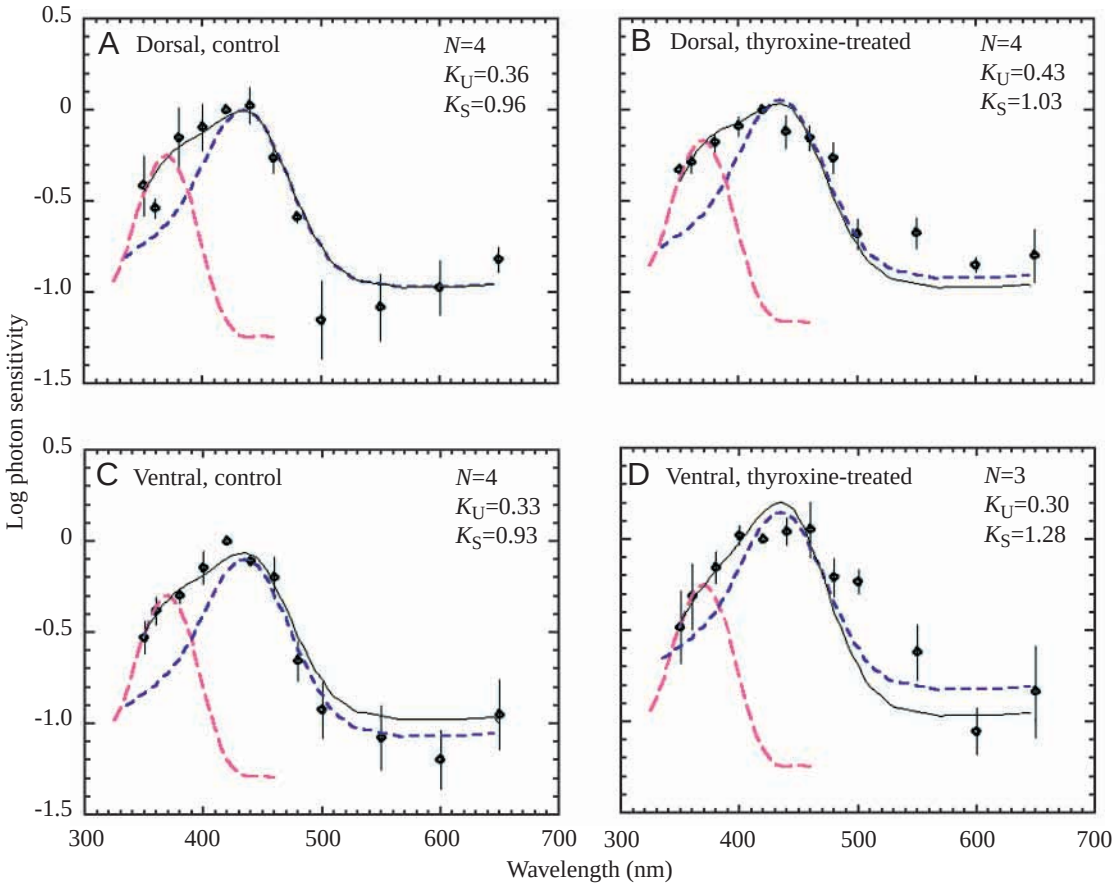

Fig. 3. Spectral sensitivity of fish after 4 weeks of thyroxine, or control, treatment. Data plotted as in Fig. 2.

fish at week 4. That is, the relative weight of the ultraviolet-sensitive cone mechanism was reduced relative to the blue-sensitive mechanism, and the placement of the ultraviolet absorptance curve was slightly lower than in controls, and in the dorsal retina of thyroxine-treated fish. Examination of the three individuals tested for ultraviolet sensitivity in the ventral retina at week 4 indicated that one fish had lost ultraviolet sensitivity completely (K_U=0.07), whereas the other two fish still had fairly high ultraviolet sensitivity (K_U=0.42 and 0.40; individual data not shown).

At week 6, ultraviolet sensitivity was significantly reduced in all thyroxine-treated fish tested for ventral sensitivity (Fig. 4). Ultraviolet sensitivity was significantly lower in the ventral retina of thyroxine-treated fish than in the ventral retina of control fish (t=2.44 at 380 nm, d.f.=6, P<0.05; t=1.11 at 360 nm, d.f.=4, P<0.17; one-tailed t-test). In addition, ultraviolet sensitivity was significantly lower in the ventral retina of thyroxine-treated fish than in the dorsal retina of thyroxine-treated fish (t=2.07 at 380 nm, d.f.=6, P<0.05; t=2.03 at 360 nm, d.f.=6, P<0.05; one-tailed t-test). When fitting absorptance curves to the thyroxine-treated, ventral week-6 data, we concluded that only the blue cone mechanism was necessary to explain the data. Similarly, the linear-additive fit produced a weight for the ultraviolet-sensitive mechanism that was reduced relative to controls. Both the dorsal and ventral retina of control fish still had significant input from the ultraviolet-sensitive cone mechanism at week 6. In addition, the dorsal retina of thyroxine-treated fish also exhibited sensitivity to ultraviolet light that was accounted for only by invoking the presence of an ultraviolet-sensitive cone mechanism in the dorsal retina. Dorsal sensitivity to ultraviolet light at 360 and 380 nm was not significantly different in controls and thyroxine-treated fish (as determined by the t-test).

One other notable finding was that in thyroxine-treated fish (regardless of retinal portion or treatment week), chromatic isolation of the short-wavelength and ultraviolet mechanisms was not as pronounced as in controls. In both dorsal and ventral control fish, the sensitivity at 500 and 550 nm was approximately 1 log unit lower than the peak sensitivity around 420–440 nm (average relative sensitivity values for 2, 4 and 6 weeks equal to –1.04, –1.08 and –1.08, respectively, at 550 nm, and –0.98, –1.04 and –1.10 at 500 nm). The trend was more variable in thyroxine-treated fish; however, the sensitivity at 500 and 550 nm was often only 0.4–0.7 log units lower than the peak sensitivity (average relative sensitivity values for 2, 4 and 6 weeks, equal to –0.69 –0.65 and –0.58, respectively, at 550 nm, and equal to –0.48, –0.49 and –0.38 at 500 nm). The difference in relative sensitivity between control fish and thyroxine-treated fish was significant for 2, 4 and 6 weeks at both 500 and 550 nm (P<0.02 for all comparisons; two-tailed t-test).

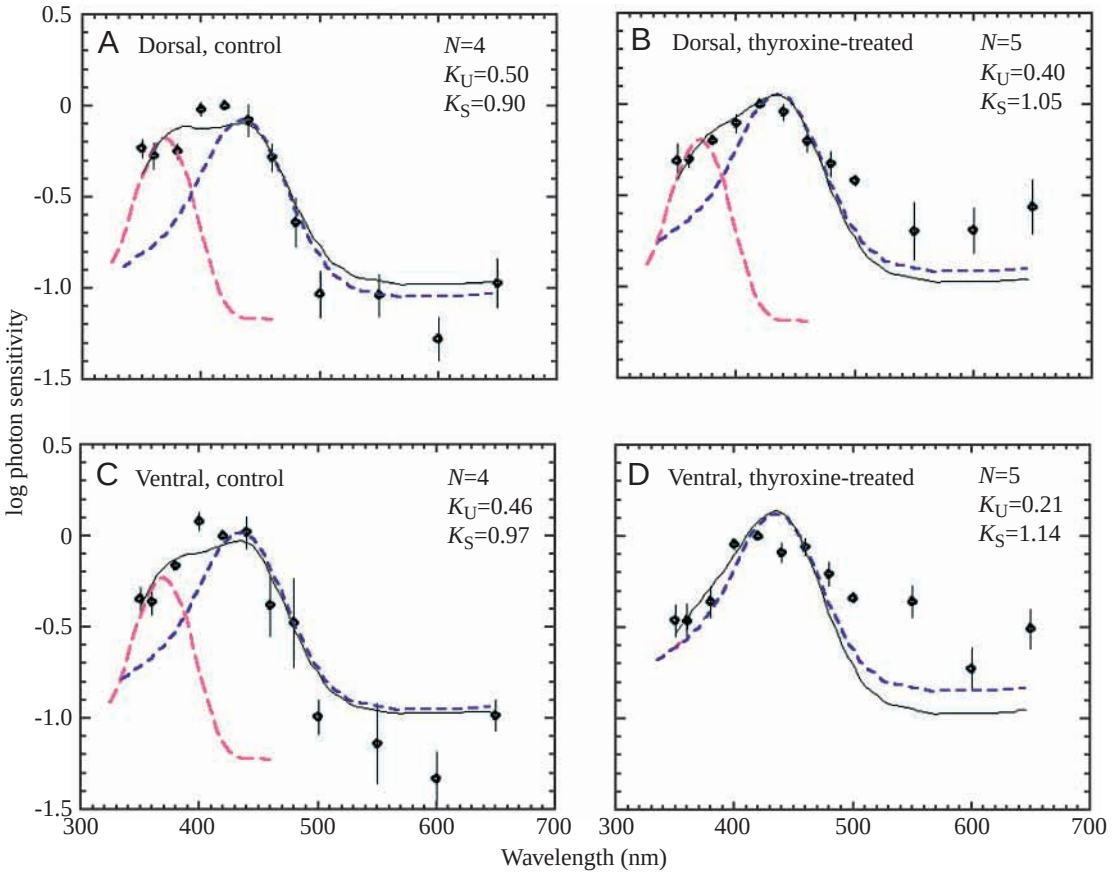

Fig. 4. Spectral sensitivity of fish after 6 weeks of thyroxine, or control, treatment. Data plotted as in Fig. 2.

In 'natural' (untreated) steelhead smolts, we found changes in ultraviolet sensitivity similar to those in the fish treated with thyroxine for 6 weeks. The dorsal retina of two steelhead (weighing 60 g and 65 g) exhibited ultraviolet sensitivity, indicating the presence of an ultraviolet-sensitive cone mechanism in dorsal retina (Fig. 5). In the two steelhead (weighing 70 g and 85 g) tested for ventral sensitivity, ultraviolet sensitivity was much reduced, and almost absent in one individual. Chromatic isolation appeared to be similar in all four fish, with a difference of approximately 1 log unit in sensitivity between 500 nm and 420 nm.

Discussion

Our results indicate that *O. mykiss* smolts have reduced ultraviolet sensitivity in the ventral retina, but retain significant ultraviolet sensitivity in the dorsal portion of the retina. This was the case for both artificially induced smolts (i.e. non-anadromous rainbow trout parr treated with thyroxine for 6 weeks) and naturally smolted anadromous steelhead (weighing 60–85 g). Our finding implies that a population of ultraviolet-sensitive cones reside in the dorsal retina of *O. mykiss* after smoltification. Ultraviolet-sensitive cones (i.e. accessory corner cones) in the ventral retina disappear from the retinal mosaic during smoltification in *O. mykiss* (Browman and Hawryshyn, 1992; Browman and Hawryshyn, 1994; Beaudet et al., 1993). However, accessory corner cones have been observed in dorsal regions of the retina (H. I. Browman, personal communication), which apparently impart the regional ultraviolet sensitivity demonstrated here. In addition, our results also indicate that the reduction in ultraviolet sensitivity in the ventral retina occurs between 4 and 6 weeks of thyroxine treatment for most fish. After 4 weeks of thyroxine treatment, one fish had reduced ultraviolet sensitivity in the ventral retina, but two others still had significant ventral ultraviolet input. By 6 weeks, all the fish examined for ventral ultraviolet sensitivity had reduced ultraviolet sensitivity. Browman and Hawryshyn (Browman and Hawryshyn, 1992) found a similar time for reduction of ultraviolet sensitivity in thyroxine-treated *O. mykiss* parr. In two fish treated for 5 weeks, ultraviolet sensitivity was not apparent. However, in the same fish treated only for 3 weeks, ultraviolet sensitivity was appreciable, implying the presence of the ultraviolet cone mechanism.

Comparison with other studies on the reduction of ultraviolet sensitivity in salmonids

Earlier studies on ultraviolet sensitivity in both artificially induced *O. mykiss* smolts (i.e. rainbow trout parr treated with thyroxine) and larger, more developed, juvenile rainbow trout showed a reduction of ultraviolet sensitivity using stimulus presentations that may have illuminated primarily ventral

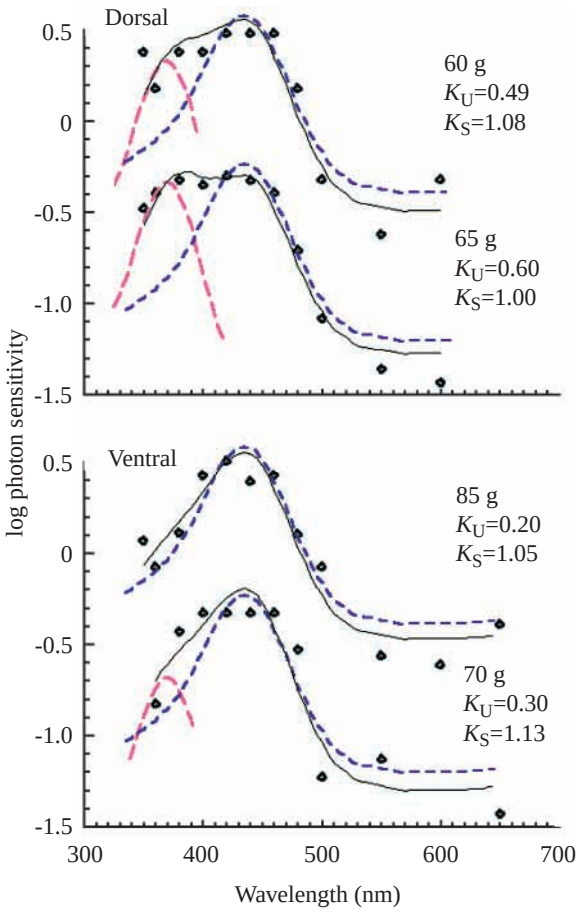

Fig. 5. Spectral sensitivity of natural, untreated steelhead smolts. The data for each of four individual smolts are shown separately (two in which dorsal sensitivity was measured and two in which ventral sensitivity was measured). The data have been displaced on the *y*-axis so that the data from each fish can be easily viewed. The mass of each fish is shown to the right of each data set. Pigment absorptance curves and the linear-additive model were plotted for each individual fish as described in Fig. 2, and the values for the ultraviolet (K_U) and short-wavelength (K_S) mechanisms are also shown for each data set.

retina (Browman and Hawryshyn, 1992; Browman and Hawryshyn, 1994; Beaudet et al., 1993). Given stimulus design considerations, however, it is unclear how well focused these stimuli were on the retina (see Introduction). Using a downwelling light stimulus, Beaudet et al. (Beaudet et al., 1993) found that large *O. mykiss* (60–835 g; a size at which fish would be smolts) had no apparent ultraviolet sensitivity in the optic nerve. However, small parr (<30 g) had significant ultraviolet sensitivity that was attributable to an ultraviolet-sensitive cone mechanism. The absence of any appreciable ultraviolet sensitivity in large fish suggests that it was primarily the ventral retina that was illuminated. If a significant portion of the dorsal retina was illuminated in these studies, we would have expected, on the basis of the findings presented here, ultraviolet sensitivity to have been higher because of the presence of ultraviolet-sensitive cones in the dorsal retina. Indeed, careful examination of the sensitivity function of thyroxine-treated *O. mykiss* parr in Browman and Hawryshyn's study (Browman and Hawryshyn, 1994) indicates a slight 'hump', or elevation, in ultraviolet sensitivity that is not explained completely by the short-wavelength cone mechanism. This suggests that some ultraviolet-sensitive cones remained in the retinal mosaic of thyroxine-treated fish, and may be explained by illumination of the dorsal retina. Although ultraviolet-sensitive cones were absent from most of the ventral retina of thyroxine-treated fish, some areas were found in the central and dorso-temporal retina that possessed accessory corner cones (H. I. Browman, personal communication). Therefore, consistent with our study, thyroxine treatment did not appear to result in a complete loss of ultraviolet sensitivity or of ultraviolet-sensitive cones in the retina of *O. mykiss*. Although ultraviolet sensitivity was dramatically reduced in the ventral retina after 6 weeks of thyroxine treatment, we also found a small 'hump' in sensitivity, indicating that even in the ventral retina ultraviolet-sensitive cone loss may not be complete (Fig. 4).

There is one study that is difficult to reconcile with the data presented here (Hawryshyn et al., 1989), in which a heart-rate conditioning technique was used to analyze spectral sensitivity in rainbow trout of different sizes. The illumination in this study was sidewelling and would have cast light on the entire retinal surface. Yet, no ultraviolet sensitivity was found in fish weighing 60 g or more. One possibility for this seemingly conflicting finding with our current study is that heart-rate conditioning may not yield the same spectral sensitivity functions as recording directly from the optic nerve. That is, ultraviolet-sensitive cones in the dorsal retina may not provide significant input to the neural circuitry involved in heart-rate conditioning. Differences in spectral sensitivity functions have been reported for the ultraviolet-sensitive cone mechanism in optic nerve recordings and heart-rate conditioning. In heart-rate conditioning, a polynomial expression for pigment shape with a peak at 360 nm best fits the spectral sensitivity function of parr (Hawryshyn et al., 1989; Browman and Hawryshyn, 1992; Browman and Hawryshyn, 1994). In contrast, a polynomial with a peak at 390 nm provided a better fit of the data obtained from the optic nerve (Beaudet et al., 1993). Similarly, had we used polynomial functions to fit our data rather than known absorptance curves and the linear-additive model, a polynomial with a peak at 360 nm would have fitted the data poorly, as can be seen by examining the fit of the 360 absorptance curve in our data.

Beaudet et al. (Beaudet et al., 1997) reported the presence of accessory corner, or ultraviolet-sensitive cones, in the dorso-temporal retina of sexually mature *O. mykiss*. These cones may be the source of ultraviolet sensitivity in our fish. That is, the dorsal retina may remain ultraviolet-sensitive throughout the life of the fish. This explanation deviates from earlier suggestions that dorso-temporal accessory corner cones are regenerated into the retinal mosaic of *O. mykiss* some time after smoltification, but before sexual maturity (Beaudet et al., 1993). However, our findings cannot preclude the possibility that dorsal ultraviolet sensitivity is completely lost at some

point after smoltification. Indeed, in brown trout *Salmo trutta*, accessory corner cones may disappear from the retinal mosaic in a ventral to dorsal direction over the course of 2 years (Bowmaker and Kunz, 1987). Juvenile brown trout possess accessory corner cones over the entire retina. 1-year-old brown trout possess accessory corner cones only in the dorsal portion of the retina. However, 2-year-old brown trout show an almost complete loss of accessory corner cones over the entire retina, with the exception of a small number remaining along the embryonic fissure of the retina (Kunz, 1987). In our study, it is entirely conceivable that we have only caught the first phase of reduction in ultraviolet sensitivity, and further examination of older fish may reveal a complete loss of ultraviolet sensitivity in *O. mykiss*. If this were the case, then regeneration of ultraviolet-sensitive cones into the dorsal retina at sexual maturity would have to occur to produce the adult pattern in the retinal mosaic (Beaudet et al., 1997).

In sockeye salmon *Oncorhynchus nerka*, both physiological and histological evidence provide support for regeneration of ultraviolet-sensitive cones in sexually mature fish (Novales Flamarique, 2000), but whether the developmental pattern of ultraviolet sensitivity found in *O. nerka* is indicative of other salmonids species is unknown. It is even uncertain whether the same developmental pattern of ultraviolet-sensitive cone loss is to be expected in different populations of the same species. Two different studies have produced conflicting results with respect to the loss of ultraviolet sensitivity at smoltification in *O. nerka*. Using a stimulus in which the whole eye was evenly illuminated, Novales Flamarique and Hawryshyn (Novales Flamarique and Hawryshyn, 1996) found that, although ultraviolet sensitivity was reduced in sockeye smolts, the fish retained some ultraviolet sensitivity (possibly due to retention of ultraviolet-sensitive cones in the dorsal retina). In a more recent study, however, Novales-Flamarique (Novales Flamarique, 2000) found that sockeye smolts had no ultraviolet sensitivity. The reason for these conflicting results is unclear. Like other species of salmonids, sockeye smolts do retain accessory corner cones along the optic fissure and also some near the periphery of the retina (Novales Flamarique, 2000). Whether these cells are photoactive or ultraviolet-sensitive is not known. Furthermore, if these cells are ultraviolet-sensitive, one would expect to see ultraviolet sensitivity reported in both studies on *O. nerka*. One possibility for the conflicting data may be that there are population differences in the pattern of ultraviolet loss in *O. nerka*. Sockeye salmon from different populations were used for the two studies cited above. Therefore, not all populations of *O. nerka* may show complete loss of ultraviolet sensitivity during smoltification. Similarly, other species of salmonids may exhibit population differences in loss of ultraviolet sensitivity, and this could explain some of the differences we have outlined in the various studies on *O. mykiss*. Careful comparative studies on the developmental trajectory of ultraviolet sensitivity in salmonids are needed both between and within species. In addition, other factors known to affect smoltification (such as environmental lighting, temperature, body condition, growth rate, etc.) may also affect the fate of ultraviolet sensitivity in salmonids and need to be rigorously assessed. At this point, we do not know enough about the factors controlling ultraviolet sensitivity in salmonids. All the parr examined to date (representing 4 species) appear to possess ultraviolet sensitivity (Parkyn and Hawryshyn, 2000), but only *O. mykiss* and *O. nerka* have been examined over parr, smolt and sexually mature stages.

Possible consequences of ultraviolet plasticity on behavior and ecology in salmonids

The reduction in ultraviolet sensitivity in *O. mykiss* occurs at a time when salmonids (i) shift habitats from shallower to deeper water and (ii) shift their diet from small zooplanktonic crustaceans to larger crustaceans and small fishes (see discussion by Browman and Hawryshyn, 1994). Ultraviolet photoreception is known to contribute to prey detection in zooplanktivorous fish (Loew et al., 1993; Browman et al., 1994), and the loss of ultraviolet photoreception from the ventral retina may reflect a change in diet and search strategy for prey. Alternatively, the remaining ultraviolet photosensitivity in the dorsal retina may represent a conservation in prey-search behavior utilizing dorsal ultraviolet photoreception. Not enough is currently known about topographic retinal specializations for behavioral tasks in salmonids to make any clear predictions, and the pattern of ultraviolet sensitivity loss may differ both between and within species (see discussion above). Interestingly, however, reduction in ventral ultraviolet sensitivity occurs at a time when rainbow trout lose the ability to orient to overhead polarized light stimuli (a task that requires ultraviolet light in salmonids; Hawryshyn et al., 1990), which suggests that the ventral retina may be specialized for detection of overhead polarized light cues used for orientation and navigation.

Other effects of thyroxine on visual processing in fish

In addition to the changes in ultraviolet sensitivity of *O. mykiss*, other changes in visual sensitivity were observed with thyroxine exposure. Thyroxine-treated fish exhibited a relative increase in middle-wavelength sensitivity compared with short-wavelength sensitivity. We found a difference of 0.5 log value in the relative sensitivity between the short-wavelength- and middle-wavelength-sensitive cone mechanisms in control and thyroxine-treated fish. This was an unexpected result since the spectral characteristics and intensity of the adapting background remained constant for all fish tested. Beaudet et al. (Beaudet et al., 1993) observed a similar, yet less pronounced, change in sensitivity between large and small *O. mykiss*. However, we found no difference in chromatic isolation in the four smolts we examined. One might expect a relative increase in middle-wavelength sensitivity as ultraviolet photoreceptors are lost from the ventral retina, simply as a result of reduced short-wavelength input. However, the relative increase in middle-wavelength sensitivity was also seen in the dorsal retina of thyroxine-treated fish, in which there was no loss of ultraviolet

sensitivity. Also, the relative increase in middle-wavelength sensitivity was apparent at 2 and 4 weeks of thyroxine treatment, prior to the loss of ultraviolet sensitivity in the ventral retina. Therefore, our findings suggest that, in addition to the loss of ventral ultraviolet-sensitive cones, there may be other changes to post-photoreceptor retinal circuitry or to intrinsic properties of photoreceptors after exposure to thyroxine. In goldfish *Carassius auratus*, exposure to thyroxine sensitizes light-evoked potentials in the optic tectum (Hara et al., 1965). In addition, exogenous thyroxine influences the ratio of porphyropsin (vitamin-A_2-based pigment) to rhodopsin (vitamin-A_1-based pigment) in the retina of salmonids (Beatty, 1969; McFarland and Allen, 1977; Allen and Munz, 1983; Alexander et al., 1994; Alexander et al., 1998). Vitamin-A_1-based photopigments have a higher molar extinction coefficient than vitamin-A_2-based pigments, which have a molar extinction coefficient that is 70 % that of rhodopsin (Dartnall, 1968). In measures of spectral sensitivity, an A_1-biased system can be approximately 0.5–1.0 log values higher in absolute sensitivity than an A_2-biased system (Allen and Munz, 1983; C. W. Hawryshyn, personal observation). Typically, thyroxine exposure for a time scale similar to that of our experiment causes an increase in porphyropsin levels in the retina of salmonids (Beatty, 1969; McFarland and Allen, 1977; Allen and Munz, 1983; Alexander et al., 1994; Alexander et al., 1998). These findings would lead us to expect an overall decrease in absolute sensitivity, but it is unclear what we might expect to see in relative sensitivities of different spectral mechanisms. Although changes in chromophore ratios occur in both rods and cones (Loew and Dartnall, 1976), a full examination of how these changes affect photopic and chromatically isolated sensitivity functions has not been conducted. Furthermore, whether changes in chromophore ratios are equal for all photoreceptor types in salmonids is unknown.

More studies are clearly needed on the effects of thyroxine on (i) chromophore ratios in cones and (ii) retinal processing to identify what physiological changes underlie the changes we observed in the relative sensitivity of the short-wavelength- and long-wavelength-sensitive cone mechanism. In addition to using short-wavelength-isolating background lighting, future electrophysiological studies should examine the changes that occur under more typical photopic conditions, as well as under background lighting that isolates the other cone mechanisms (as in Hawryshyn, 1991). A concerted approach combining electrophysiological studies, anatomical observation, and microspectrophotometry may help provide more insight into the ontogenetic effects of thyroxine on salmonid visual processing.

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