Comparative studies of vision can be used to examine how sensitivity to visual stimuli varies among taxa and within taxonomic groups. While comparative examinations of the visual systems of disparate fish taxa exist, studies focusing on variation within a single group of fishes are limited (e.g. Bridges and Yoshikami, 1970; Bowmaker et al., 1994; Lythgoe et al., 1994; Smit and Anker, 1997). Early comparative studies of visual sensitivity in fishes were conducted through examination of rod photopigment extracts (Clarke, 1936; Wald, 1938/39, 1941; Wald et al., 1957; Bridges and Yoshikami, 1970). More recently, microspectrophotometry has dominated comparative studies of vision (Partridge et al., 1988; Lythgoe and Partridge, 1989; Crescitelli, 1991; Bowmaker et al., 1994; Lythgoe et al., 1994). However, several comparative studies on other features of the fish visual system, such as ocular media transmission characteristics and visual acuity, have also been conducted (Tamura and Wisby, 1963; McCandless et al., 1969; Douglas and McGuigan, 1989; Thorpe and Douglas, 1993).

Salmonids are a diverse group with respect to number of species, life history strategies and habitats, making them an interesting group in which to examine how visual sensitivity varies among species. However, physiological data on the comparative photopic (daylight) spectral sensitivity of fishes are generally lacking and, as a group, salmonids are no exception. Similarly, the comparative aspects of polarisation sensitivity, regardless of methodological approach or taxa, have not been examined in vertebrates; this is still an emerging topic of research (Parkyn and Hawryshyn, 1993; Parkyn, 1998).

A few studies have explored aspects of the salmonid visual system on a comparative basis. McCandless et al. (1969) examined lens and ocular-media transmission in rainbow trout (Oncorhynchus mykiss), a shallow-water species, and lake char (Salvelinus namaycush), a deep-water species. In a notable study, Bridges and Yoshikami (1970) compared the rod photopigments in many Salmonidae species including not only the subfamily Salmoninae (char and trout) but also the...
Coregoninæ (whitefishes). Recently, Beaudet et al. (1997) quantified the various morphological types of cone photoreceptor in adults of several species in the salmonid genus *Oncorhynchus* during the return phase of their migration from the ocean to their natal rivers.

Morphological studies have shown that salmonids have a duplex retina containing both rods and cones (Ali and Ancil, 1976). A single class of cells (rods) contributes to the scotopic or night vision of salmonids (Allen and Munz, 1983). From studies using various methods, juvenile salmonides have been shown to have four spectrally distinct classes of cones for at least a portion of their life history (Bowmaker and Kunz, 1987; Douglas et al., 1989; Hawryshyn et al., 1989; Brownman and Hawryshyn, 1992; Kusmic et al., 1993; Hawryshyn and Hárosi, 1994; Novales Flamarique and Hawryshyn, 1996; Beaudet et al., 1997). In at least four species, rainbow trout, brown trout (*Salmo trutta*), Atlantic salmon (*S. salar*) and sockeye salmon (*O. nerka*), one of these cone photoreceptors, the accessory cone, has maximum sensitivity ($\lambda_{\text{max}}$) of the $\alpha$-absorption peak in the ultraviolet (Bowmaker and Kunz, 1987; Kunz, 1987; Douglas et al., 1989; Kunz and Callaghan, 1989; Hawryshyn et al., 1989; Brownman and Hawryshyn, 1992; Beaudet et al., 1993; Hawryshyn and Hárosi, 1994; Novales Flamarique and Hawryshyn, 1996). Photopic spectral sensitivity in the genus *Salvelinus*, however, has remained uncharacterised.

Electrophysiological studies of salmonids have determined that, at the level of the optic nerve, responses to light can be further divided into two categories: ON-responses and OFF-responses (Allen and Munz, 1983; Beaudet et al., 1993; Parkyn and Hawryshyn, 1993, 1999; Coughlin and Hawryshyn, 1994a, b; Novales Flamarique and Hawryshyn, 1996). An ON-response in this study is defined as a transient voltage change at the level of the optic nerve following an increment of a light stimulus (e.g. the flash of reflected light from the side of another fish), while an OFF-response is a transient voltage change associated with the decrement of light (e.g. a passing shadow of an overhead predator). Visual sensitivity in teleost fishes is mediated primarily by cones under photopic conditions and by rods under scotopic conditions (Wheeler, 1982; Allen and Munz, 1983; Hawryshyn et al., 1989; Demarco and Powers, 1991; Beaudet et al., 1993; Parkyn and Hawryshyn, 1993; Coughlin and Hawryshyn, 1994a, 1995; Bilotta et al., 1995; Novales Flamarique and Hawryshyn, 1996, 1997; Parkyn, 1998). Both cones and rods also contribute to these ON- and OFF- responses in the teleost species that have been studied (Wheeler, 1982; Allen and Munz, 1983; Beaudet et al., 1993; Parkyn and Hawryshyn, 1993; Bilotta et al., 1995).

The primary objectives of this study were to characterise and compare photopic spectral and polarisation sensitivity in juvenile trout, salmon and char in the subfamily Salmoninæ. Rainbow trout and steelhead (both *Oncorhynchus mykiss*), coastal cutthroat trout (*O. clarki clarki*), kokanee (*O. nerka*) and brook char (*Salvelinus fontinalis*) were examined. Rainbow trout and steelhead represent, respectively, the freshwater and anadromous (ocean-going) individuals of the same species of fish. It has been hypothesised that the visual sensitivity of these two groups might differ because the habitat of anadromous juvenile and adult salmonids is vastly different from the small coastal lakes and streams of their non-anadromous counterpart. Similarly, the visual sensitivity of kokanee, the non-anadromous form of *O. nerka*, is characterised for the first time and compared quantitatively with that of other salmonids. The coastal cutthroat trout is a weakly migratory species that makes use of estuaries for a portion of the year only (Scott and Crossman, 1973). It is hypothesised that, because cutthroat trout occupy a similar stream habitat to that of rainbow trout, a very close relative, both species should have similar spectral sensitivities. Finally, brook char, a member of the genus *Salvelinus*, a group that is distantly related to *Oncorhynchus*, was examined for the first time to establish whether ultraviolet and polarisation sensitivity are present (as in other salmonines thus far examined). If this were the case, it would suggest that ultraviolet-cone photoreceptors are a ubiquitous feature of these fishes. This is of interest because near-ultraviolet-polarised stimuli are sufficient for accurate orientation of trained rainbow trout (Hawryshyn et al., 1990), and the ultraviolet-cone mechanism appears to be involved in the perception of one of two channels of polarised light information in these fish (Hawryshyn, 1992; Parkyn and Hawryshyn, 1993; Coughlin and Hawryshyn, 1995; Novales Flamarique et al., 1998).

**Materials and methods**

The juvenile salmonids used in this study were obtained as 1–5 g parr from British Columbia Ministry of Environment hatcheries, with stock origins as specified (Table 1). Fish were maintained on a 12 h:12 h dark:light photoperiod at a mean temperature of 14±1 °C for 10 weeks prior to experimentation. Recording of optic nerve responses was initiated 2 h after the onset of the day portion of the diel cycle and ended 2 h prior to dusk. The fish were then transferred to the dark and kept in the dark until recording was complete.

**Table 1. Species, geographic origins and migratory patterns of salmonids used in spectral and polarisation sensitivity experiments**

<table>
<thead>
<tr>
<th>Species</th>
<th>Common name</th>
<th>Origin*</th>
<th>Anadromous</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Oncorhynchus</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>clarki</em> clarki</td>
<td>Coastal cutthroat</td>
<td>Sooke Lake</td>
<td>Yes</td>
</tr>
<tr>
<td><em>O. mykiss</em></td>
<td>Coastal rainbow</td>
<td>Badger Lake</td>
<td>No</td>
</tr>
<tr>
<td><em>O. mykiss</em></td>
<td>Steelhead</td>
<td>Cowichan River</td>
<td>Yes</td>
</tr>
<tr>
<td><em>O. nerka</em></td>
<td>Kokanee</td>
<td>Kootenay River</td>
<td>No</td>
</tr>
<tr>
<td><em>Salvelinus</em></td>
<td>Brook char</td>
<td>Aylmer Lake</td>
<td>No</td>
</tr>
<tr>
<td><em>fontinalis</em></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*All species originated in British Columbia, Canada.

1 Behnke (1992) classifies coastal rainbow and steelhead as *O. mykiss irideus*. However, this subspecies designation has not been recognised formally.

2 Introduced from Lake Nipigon, Ontario, stock in the early 1900s.
to the night portion to reduce any potential effects of endogenous changes in sensitivity that might result from retinomotor movements or disk shedding (Parkyn and Hawryshyn, 1993). Fish were tested when they weighed between 8 and 12 g. Lighting in the rearing facility was provided by broad-spectrum fluorescent lights (Growlux) containing wavelengths from 350 to 750 nm (Fig. 1). Surgical procedures were similar to those described by Parkyn and Hawryshyn (1993, 1999) and will be summarized here. Prior to surgery, a fish was placed in a neutrally buffered solution of tricaine methanesulphonate (100 mg l⁻¹) until it reached Stage 4 anaesthesia (Jolly et al., 1972). The fish was then placed into a restraining cradle, a tight-fitting mouthpiece was inserted into the buccal cavity, and the gills were irrigated with a 40 mg l⁻¹ solution of tricaine in water. The restrained fish was then injected with 0.05 mg g⁻¹ body mass of the immobilising agent Pavulon (Organon Canada Ltd). The body of the fish was covered with wet gauze to prevent desiccation, and a drip irrigation system was also used to help keep the body of the fish moist and cool. The skin overlying the right frontal bone was removed using a scalpel, followed by the extraction of this bone with a fine dental burr. A local anaesthetic saline (0.4 % Tetracaine) was applied to the cut edges of the surgical area.

At the end of the experiment, the fish was killed. All procedures and care of the animals in this study were in accordance with the guidelines of the Canadian Council for Animal Care.

Experimental apparatus and protocol

The experimental recording apparatus has been described previously (Parkyn and Hawryshyn, 1993, 1999; Novales Flamarique and Hawryshyn, 1996). In brief, background illumination (described below) was provided by two 250 W tungsten/halogen lamps (Spectro) with voltage-regulated direct current sources and various long- and short-wave bandpass interference filters (Corion) described below. Inconel neutral-density filters (Corion) were used to control background-channel intensity. Ultraviolet/visible light-transmissive liquid guides (Oriel) were used to project light from the background channels onto the surface of the eye. A diffuser end-cap on each light guide (Albanene) was used to remove inherent polarisation. To ensure uniform illumination of the eye, the light from each of the two background channels was superimposed to form a single overlapping spot with a diameter twice the size of the pupil on the corneal surface. A third light pipe was used to superimpose the stimulus channel onto the pupil. Opening and closing of the stimulus channel were controlled by a computer-controlled shutter (Uniblitz), and the light was projected from a 300 W xenon lamp (Oriel). The wavelength of the stimulus beam was controlled by a holographic-grating monochrometer (SA Instruments), and stimulus intensity was controlled by a quartz neutral-density wedge (Optikon). The photon irradiance of the stimulus was measured during pre-experimental calibration for each test wavelength and intensity. The spectral sensitivity of the fish was determined in 20 nm increments between 340 and 660 nm.

Stimuli were presented with restricted randomisation to prevent light adaptation to one region of the spectrum by the stimulus during presentation. For experiments involving polarised light, another diffuser (to remove any inherent polarisation of the stimulus channel) and a linear polariser (HNP'B, Polaroid) were attached to the exit aperture of the stimulus light guide. The polariser on the stimulus channel was rotated using restricted randomisation in 30° increments between 0 and 180° to determine the change in sensitivity with orientation of the e-vector (0°/180° was defined as vertical and 90°/270° as horizontal). Previous studies have indicated that plane-polarised ultraviolet light is sufficient for spatial orientation of rainbow trout, and these trout did not respond correctly when ultraviolet light was lacking (Hawryshyn et al., 1990). Subsequently, it was shown that, at the level of the optic nerve ganglion cells, the ultraviolet-, M- and L-cone mechanisms of *O. mykiss* are differentially sensitive to different angular orientations of plane-polarised near-ultraviolet light. However, the S-cone and rod mechanisms are not polarisation-sensitive (Parkyn and Hawryshyn, 1993; Coughlin and Hawryshyn, 1995; Parkyn, 1998). For the purposes of this paper, a cone mechanism is defined as the resultant sensitivity attributable to a single cone-photoreceptor class and its interneurons as measured at the level of the optic nerve. For the sake of comparison with previous behavioural studies and as a consequence of the sufficiency of ultraviolet as a cue for polarised light orientation, the present set of polarisation sensitivity experiments was restricted to a stimulus of 360 nm.

Testing condition one: white-light background conditions

With the white-light background condition, fish were adapted using the two quartz/halogen light channels. Each channel was fitted with both a 700 nm short-pass filter and a 2.0 neutral-density (ND) Inconel filter, to adapt the eye of the study fish to photopic white-light conditions. These filters provide transmission of violet and near-ultraviolet light (Fig. 1). This is relevant to the study not only because of the potential role of the S- (blue/violet) photoreceptors in the detection of veiling illumination (Douglas and Hawryshyn, 1990) but also because the visual system of the fish was in a photopic state and all classes of cones underwent some degree of adaptation. The background and stimulus channels were positioned to overlap completely at the pupil.

Testing condition two: ultraviolet-cone isolating background

The second background condition was used to test for the presence of an independent ultraviolet-sensitive mechanism. Ultraviolet in the context of this paper refers to the ultraviolet-A range of wavelengths between 360 and 400 nm, which are transmitted by the ocular media of the trout eye (Hawryshyn et al., 1989). Under this condition, fish were adapted to a tungsten background with a 450 nm long-pass interference filter (1.5 ND) filter in one background channel and a 550 nm long-pass interference filter in the second channel (2ND) (Fig. 1). This coloured background was used to chromatically
adapt the eye in a manner analogous to the technique used by Stiles (1959). Additional details of this background condition are provided in Parkyn and Hawryshyn (1993) and Parkyn (1998). When ultraviolet-cones are present, these isolating conditions allow the characterisation of ultraviolet sensitivity since the relative sensitivity of the ultraviolet-cone mechanism will be higher than that of more adapted cone mechanisms (Hawryshyn and Beauchamp, 1985; Hawryshyn, 1991; Beaudet et al., 1993; Parkyn and Hawryshyn, 1993; Coughlin and Hawryshyn, 1994a).

**Recording from the optic nerve**

Fish were retained in the cradle apparatus and placed into a Faraday cage. The buccal cavity was irrigated at 240 ml min\(^{-1}\) with water at 14 °C. Epoxy-coated NiCrO\(_4\) electrodes (0.5 mm diameter, 0.5 mm exposed tip) were inserted into the optic nerve of the left eye (Fig. 2) and into a reference site (right naris) following Parkyn and Hawryshyn (1993). The caudal peduncle of the fish was grounded via a metal alligator clip. Fish were then adapted to one of the two background conditions for 60 min prior to data collection.

Both anatomical and electrophysiological observations were used to ensure that recording was restricted to the optic nerve (Fig. 2). During the early stages of this recording technique, the brain was examined to determine the anatomical position of the electrode. First, the whole head was preserved in 10 % phosphate-buffered formalin (Hinton, 1990). Following a 24 h fixation period, the electrode was removed, and the opening in the brain was flooded with Methylene Blue for a minimum of 1 h. The brain was then excised from the cranium and coarsely sectioned at 0.5 mm intervals. The sections were examined to determine whether the electrode had been inserted into the optic nerve. A stereotaxic apparatus was used to provide an approximation of electrode position in subsequent experiments. Physiological comparisons of optic nerve and tectal responses were used to provide further confirmation of electrode placement in the optic nerve. This approach was necessary because a lack of ultraviolet sensitivity resulting

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**Fig. 1.** Spectral irradiance, as measured using a LI-1800UW (underwater) spectroradiometer (Licor), for photopic light conditions of salmonids in holding tanks at 10 cm depth, for the tungsten-white background for photopic adaptation and for the chromatic adaptation background used to test for the presence of an ultraviolet-cone mechanism. UV, ultraviolet.

**Fig. 2.** (A) Dorsal and (B) lateral views of the brain of a 7 g rainbow trout. The insertion tract of the recording electrode is indicated by - - - -. An, anterior; Co, corpus cerebellum; OT, optic tectum; T, telencephalon; V, ventral surface; I, olfactory nerve; II, optic nerve. (C) Camera lucida drawing of a cross section of the proximal region of the optic tectum at the junction with the optic nerve of a juvenile rainbow trout. The arrow indicates a puncture or tract from the recording electrode accentuated by Methylene Blue. (D) Multi-unit recording from the optic nerve of a juvenile rainbow trout. (E) Multi-unit recording from the optic tectum of the same fish. The bar indicates light-stimulus on. 

250 µV
from misplacement of electrodes could mimic the pattern of spectral and polarisation sensitivity of fish that have lost ultraviolet sensitivity through ontogenetic processes. This was an important consideration because colour-opponent neural units are present in the optic tectum. However, few ultraviolet- and polarisation-sensitive units appear to be present in this region of the brain in rainbow trout (Coughlin and Hawryshyn, 1994b). In contrast, ultraviolet and polarisation sensitivity are present in the optic nerve (Parkyn and Hawryshyn, 1993, 1999) and the torus semicircularis, within the central nervous system (Coughlin and Hawryshyn, 1994b, 1995). An example of the insertion tract of a recording electrode is shown in Fig. 2A,B. Fig. 2C shows the electrode tract in the optic nerve. Optic nerve responses (ONRs) to light stimulation typically differed from responses recorded from the tectum. In general, the degree of negative deflection observed in optic nerve recordings (Fig. 2D) was much less than that observed from tectal recordings (Fig. 2E). In addition, the latency from time of stimulation to time of response was less in optic nerve recordings than in tectal responses for a given wavelength at a given intensity. For example, the maximum ON-response was observed to be at 64 ms for an optic nerve recording versus 105 ms for a tectal recording for a 380 nm stimulus and a white-light background adaptation condition. The resultant ONRs were amplified by a Grass Instrument P5 pre-amplifier (3 Hz low-frequency and 300 Hz high-frequency filters). The signal was then exported to a computer via an A/D port for on-line analysis, display, and storage. Sampling of ONRs was randomised so that each response for a series of intensity increments at a particular wavelength was obtained with an inter-stimulus interval of between 20 and 30 s. Stimulus duration for each test wavelength of light was 750 ms.

Analysis of ganglion cell responses

Three ONRs were recorded and averaged for each increment of intensity of light. The stimulus was increased in increments of 0.2 ND over a range of up to 3–4 ND (depending on the preparation). The peak amplitudes of the ON- and OFF-responses at each of these intensities were plotted against increasing irradiance to generate a response versus intensity function (Parkyn and Hawryshyn, 1993, 1999). A third-order polynomial function was fitted to the data (e.g. Fig. 3A,B; Table 2). The criterion response level for threshold determination was 20 μV above the baseline. This criterion was selected because the sensitivity of the centre of a receptive field of an individual ganglion cell is thought to dominate its surround region near threshold (Daw, 1968; Spekreijse et al., 1972). Hence, although multi-unit recordings receive input from many ganglion cells, the integrated response reflects primarily the responses of the central region of the ganglion cell receptive field (Demarco and Powers, 1991). This reduces the effect of lateral inhibition from the surround region of the receptive field, which may actually result in a decrease in sensitivity as the stimulus increases to a suprathreshold level (Beaudet et al., 1993). In addition, this criterion level falls within the region where the response versus intensity curve approximates a linear function. This facilitated determination of the photon irradiance at the criterion level among different wavelengths or orientations of the polariser. Sensitivity was defined as $-\log_{10}$ of the photon irradiance at the criterion response voltage for each test wavelength (e.g. Fig. 3C) or the orientation angle of plane-polarised light. Sensitivity values were normalised to the median of each individual curve to generate relationships for relative sensitivity versus wavelength and relative sensitivity versus the orientation angle of polarised light. Relative sensitivity was used in these experiments because the absolute voltage of the response varied among individual fish as a result of electrode placement. Hence, it was not possible to obtain absolute sensitivity. Means ± one standard error of the mean (S.E.M.) of the replicates were then determined.

<table>
<thead>
<tr>
<th>Response</th>
<th>Wavelength (nm)</th>
<th>$r^2$</th>
<th>$F_{3,12}$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>ON</td>
<td>360</td>
<td>0.96</td>
<td>111.28</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>ON</td>
<td>440</td>
<td>0.97</td>
<td>123.30</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>ON</td>
<td>540</td>
<td>0.95</td>
<td>69.50</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>ON</td>
<td>620</td>
<td>0.98</td>
<td>179.10</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>OFF</td>
<td>360</td>
<td>0.95</td>
<td>73.12</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>OFF</td>
<td>440</td>
<td>0.92</td>
<td>47.90</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>OFF</td>
<td>540</td>
<td>0.92</td>
<td>44.90</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>OFF</td>
<td>620</td>
<td>0.96</td>
<td>100.20</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Spectral sensitivity of juvenile salmonids 1177

Contribution of cone photoreceptor classes to spectral sensitivity

The spectral sensitivity curves were compared both qualitatively and statistically (outlined below). Rainbow trout (Oncorhynchus mykiss) was chosen as the benchmark species for comparison because it has the most complete data set of microspectrophotometric-cone absorption spectra currently available for the salmonids (Hawryshyn and Hárosi, 1994). Qualitative assessments of the contribution of the different classes of photopic channels to spectral sensitivity were made by overlaying ultraviolet-, S-, M- and L-cone absorbance curves. These absorbance curves were obtained by converting microspectrophotometric absorbance (optical density) data for rainbow trout into absorbance values. First, the normalised microspectrophotometric data were multiplied by the specific absorbance data for each cone type obtained from Hawryshyn and Hárosi (1994). The product was then multiplied by the average length of the cone photoreceptor outer segments for rainbow trout (parr) (Novales Flamarique and Hawryshyn, 1997). These values were converted to absorbance values (Hárosi, 1975) and corrected for wavelength-specific losses in transmission by the ocular media using values obtained from juvenile (parr) rainbow trout (Hawryshyn et al., 1989).

The fit of the absorbance curves to the resultant spectral sensitivity data was made by vertically adjusting the rainbow trout cone absorbance curves until the best visible fit was obtained (Parkyn and Hawryshyn, 1999). This admittedly simplistic method was employed after attempts had been made
to use an additive model which provided an unrealistic and non-descriptive fit of the data (perhaps because of possible inhibitory interactions among cone mechanisms). Thus, rather than being a quantitative test of the cone contribution (e.g. Sperling and Harwerth, 1971; Coughlin and Hawryshyn, 1994a; Vorobyev and Osario, 1998), these templates served simply as a qualitative guide for comparison of the described rainbow trout cone pigments with the observed photopic spectral sensitivity. In this respect, the comparison indicates which cone mechanisms may be contributing to sensitivity and whether the observed peaks in sensitivity correspond to the known peaks of absorptance ($\lambda_{\text{max}}$) for rainbow trout cone pigments.

**Modelling the relationship between sensitivity and angular orientation of a linear polariser**

Periodic regression analysis (Mardia, 1976; Batschelet, 1981; Fisher, 1993; Zar, 1996) was used to explore the relationship between relative sensitivity ($S$) and the angular orientation of the polariser e-vector ($\theta$). Inspection of the data, resulting curves, regression coefficients of determination ($r^2$) and sums of squares (Batschelet, 1981; Zar, 1996) suggested that ON-responses were composed of a first and a second harmonic:

$$S = M + A_1 \cos(\omega \theta - \phi_1) + A_2 \cos(2\omega \theta - \phi_2),$$

(1) where $M$ is the mesor (the mean of the curve), $A_1$ is the amplitude of the first harmonic, $A_2$ is the amplitude of the second harmonic, $\omega = 2\pi / \tau$, where $\tau$ is the period of the function, $\phi_1$ is the acrophase (the angle associated with maximal value of $S$) of the first function, and $\phi_2$ is the acrophase of the second function.

An additional parameter, $\delta$, the width of the peak at the mesor, was calculated to facilitate further comparisons of the $S$ versus polariser orientation curves (Batschelet, 1981):

$$\delta = 2\psi,$$

(2) where $\psi = 2\pi / \tau (\theta - \phi)$.

A similar procedure was used to fit curves to the OFF-response data. Inspection of the means of the data and a test of the model fit indicated, however, that the function was unimodal. For curves with unimodal characteristics, Batschelet (1981) recommends the use of the function:

$$S = M + A \cos(\omega \theta),$$

(3) where $M$ is the mesor, $A$ is amplitude and $\omega = 2\pi / \tau$, where $\tau$ is the period of the function.

**Comparative statistical analyses**

As a preliminary requirement for statistical analyses of spectral and polarisation sensitivity data, a Kolmogorov–Smirnov goodness-of-fit test (Conover, 1980; Norusis and SPSS Inc., 1993; Zar, 1996) was used to determine that the data used in this study did not deviate significantly from a normal distribution (two-tailed test, $\alpha = 0.1$). The significance of all other statistical tests in this study was defined as $P \leq 0.05$.

Sample sizes precluded statistical examination of sensitivity in all species and forms of fish (anadromous/non-anadromous) simultaneously; thus, two groups of comparisons were made. In the first group of comparisons, the non-anadromous rainbow trout was compared with its anadromous form, the steelhead. In the second group, comparisons were made among $O. \text{mykiss}$, $O. \text{nerka}$ and $S. \text{fontinalis}$. Differences in sensitivity ($S$) versus wavelength ($\lambda$) and in $S$ versus the angular orientation of polarised light (e-vector, $\phi$) were tested using a parametric analysis of variance with repeated measures (ANOVAR) (SPSS V6.1) following the recommendations of Huynh and Mandeville (1979), Norusis and SPSS Inc. (1993) and Zar (1996). The sample size of $O. \text{c. clarki}$ was small and, hence, not appropriate for inclusion in statistical analyses.

**Results**

**Spectral sensitivity under white-light background conditions: rainbow trout versus steelhead**

The amplitude of ganglion-cell responses from the optic nerve was sufficient to allow determination of the spectral sensitivity of both rainbow trout and steelhead from 340 to 660 nm. No significant differences were detected between rainbow trout and steelhead in either the ON- or OFF-responses (ANOVAR; rainbow trout, $F_{0.05,19} = 1.45, P = 0.25$; steelhead, $F_{0.05,19} = 3.66, P = 0.082$). In addition, there was no significant statistical interaction between anodromous (steelhead) and non-anadromous (rainbow trout) forms and the sensitivity of either the ON- or OFF-response as a function of wavelength (ANOVAR; steelhead, $F_{0.05,112} = 0.89, P = 0.569$; rainbow trout, $F_{0.05,112} = 1.03, P = 0.61$). In summary, the spectral sensitivities of these two forms of $O. \text{mykiss}$ were not statistically distinguishable. As a result, the samples of these conspecifics were pooled to aid further comparisons among species. For $O. \text{mykiss}$ (rainbow trout and steelhead pooled), there was an overall difference in spectral sensitivity between the ON- and the OFF-responses under white-light background conditions (ANOVAR; $F_{0.05,19} = 9.26, P = 0.03$) (Figs 4A, 5A). Similarly, the relative sensitivity of both the ON- and OFF-responses differed significantly as a function of wavelength (ANOVAR; ON-responses, $F_{0.05,12} = 11.44, P < 0.01$; OFF-responses, $F_{0.05,12} = 27.20, P < 0.001$).

For ON-responses, the maximal difference in spectral sensitivity observed among test wavelengths was approximately 1.2 log unit, although two broad regions of maximal sensitivity were apparent (Fig. 4A). On the basis of the overlay of absorptance templates, one of these regions appeared to be dominated by contributions from both the L- (red) and M- (green) cone mechanism sensitivity, while the other was dominated by the S- (blue) and ultraviolet-channel sensitivity. In this second region, the relative sensitivity did not vary from 380 nm in the near-ultraviolet to 480 nm in the blue region of the spectrum. In contrast to ON-responses, OFF-responses of $O. \text{mykiss}$ were dominated by the M-cone mechanism (Fig. 5A). Under white-light adaptation conditions, ultraviolet sensitivity was potentially present, as indicated by the ultraviolet-absorptance template in the
ultraviolet region of the test wavelengths for both ON- and OFF-responses (Figs 4A, 5A). This is addressed further under the specific conditions of the ultraviolet-isolation background.

**Interspecific comparisons under white-light background conditions**

Under the same white-light adaptation conditions, the spectral sensitivities of the ON-responses for cutthroat *O. c. clarki*, kokanee *O. nerka* and brook char *Salvelinus fontinalis* were generally similar to those of rainbow trout and steelhead: all species had visual capability across the visible spectrum and into the near-ultraviolet (Fig. 4A–D). Overall, differences in sensitivity among wavelengths were not greater than 1.3 log unit for any of the species, with the greatest variability of responses for all species at 600–660 nm. The ON-responses varied significantly with wavelength (*F*\(_{0.05,13}=18.29, P<0.001*). Specifically, sensitivity in the region between 500 and 640 nm (green to red) of the spectrum was consistently highest. ON-responses among *O. mykiss*, *O. nerka* and *S. fontinalis* were significantly different (*F*\(_{0.05,2}=3.59, P=0.04*). However, there was a statistical interaction that precluded further statistical examination of the main effects. Inspection of the graphs for the ON-responses revealed that the ON-responses differ primarily in the region 520–640 nm (Fig. 4A–D). In particular, the ON-responses of *O. mykiss*, *O. c. clarki* and *S. fontinalis* appeared to have spectral input from the L- and M-mechanisms, with contributions from the S- and ultraviolet-mechanisms (Fig. 4A,B,D). Unlike the other species, the spectral sensitivity of the ON-responses for *O. nerka* appeared to be dominated almost entirely by an M-cone mechanism (Fig. 4C), while the L-cone mechanism made no contribution to the total spectral sensitivity curve. The S-cone and ultraviolet-cone mechanisms also appeared to contribute to sensitivity on the basis of the overlay of these templates. In addition, the position of *λ*\(_{max}\) for the S-cone template was not coincident with the observed peak in sensitivity for *O. c. clarki* and *S. fontinalis*, although it was coincident with the *λ*\(_{max}\) for *O. mykiss* and *O. nerka*. This suggests that the spectral sensitivity of the S-cone mechanisms of the former two species may be shifted relative to that of *O. mykiss*. A similar phenomenon may be occurring with either the M-cone or L-cone mechanism in *S. fontinalis* since neither absorptance template matches the *λ*\(_{max}\) observed from the spectral sensitivity data.

In comparison with *O. mykiss*, another difference was apparent in the red region of the spectrum for *O. c. clarki* (Fig. 4B). With alignment of the L-cone absorptance template to the descending long-wave limb of the data, a sensitivity difference of 0.5 log unit was observed. Notably, this dip in sensitivity was near where peak sensitivity would be predicted by the L-cone template. Instead, a peak at 620 nm in the red region of the spectrum was observed. In spite of the lack of fit of the L-cone template in *O. c. clarki*, the fit of an M-cone template was relatively good, even in the region that should correspond to the region of maximum sensitivity of the L-cone (Fig. 4B).

As with ON-responses, the sensitivity of OFF-responses also varied significantly as a function of wavelength (*F*\(_{0.05,13}=18.22, P<0.001*). Unlike the ON-responses, the OFF-responses showed no statistically detectable, species-specific differences (*F*\(_{0.05,2}=2.54, P=0.102*). In general, all species displayed a broad-band sensitivity for the OFF-response with maxima in the region from 500 to 600 nm of the spectrum (Fig. 5A–D). This coincided with the *λ*\(_{max}\) of the M-cone absorptance template. However, this template did not fit...
Fig. 4. Spectral sensitivity of ON-responses recorded from the optic nerve of (A) Oncorhynchus mykiss, (B) O. c. clarki, (C) O. nerka and (D) Salvelinus fontinalis. Fish were adapted to a white-light tungsten background. Values represent means ± 1 S.E.M. of standardised and normalised data. Lines represent rainbow trout absorptance templates. An asterisk indicates a 'pseudo peak'. UV, ultraviolet.

Fig. 5. Spectral sensitivity of OFF-responses recorded from the optic nerve of (A) Oncorhynchus mykiss, (B) O. c. clarki, (C) O. nerka and (D) Salvelinus fontinalis. Fish were adapted to a white-light tungsten background. Values represent means ± 1 S.E.M. of standardised and normalised data. Lines represent absorptance templates of rainbow trout cone photopigments. UV, ultraviolet.
well, particularly for wavelengths below 450 nm, for all species excluding *O. nerka* (Fig. 5A,B,D). The OFF-responses also had a second, smaller maximum in the near-ultraviolet region. This second peak was centred at 360–380 nm (Fig. 5A–D). In addition to this ultraviolet sensitivity, *O. c. clarki* and *S. fontinalis* appeared to have some OFF-response sensitivity at 620–660 nm.

It is important to note that, while ultraviolet sensitivity was observed in the responses of the fish under this adaptation condition, the mechanism(s) mediating ultraviolet-sensitivity (i.e. an ultraviolet-cone versus β-band absorbance by another cone mechanism) could not be determined without further chromatic adaptation to attempt to isolate the ultraviolet mechanism.

**Spectral sensitivity under ultraviolet-isolating background conditions**

Following chromatic adaptation of the S-, M- and L-cone mechanisms with a yellow–orange ultraviolet-isolating background (Fig. 1), a peak in ultraviolet sensitivity consistent with the rainbow trout ultraviolet absorbance template was observed in steelhead *O. mykiss* (Fig. 6A). Sensitivity in the ultraviolet region of the spectrum increased relative to wavelengths greater than 400 nm, indicating the presence of an independent ultraviolet mechanism (Hawryshyn and Beauchamp, 1985). In general, the shape of the absorbance templates for all cone classes was consistent with the spectral sensitivity curve of steelhead, except in the region of the spectral sensitivity curve where the absorbance templates of the L- and M-cones overlapped (Fig. 6A). In this region, the L-cone template overestimated sensitivity and the M-cone template was a better approximation of the shape of the sensitivity response.

Chromatic adaptation affected the shape of the spectral sensitivity curves of *O. c. clarki*, *O. nerka* and *S. fontinalis* more than that of *O. mykiss* (Fig. 6A–D). As with *O. mykiss*, ultraviolet sensitivity was also present in the ON-responses of these species (Fig. 6B–D). In contrast to *O. mykiss*, however, the spectral sensitivity of the ON-responses in *O. c. clarki* showed not only a peak in the ultraviolet but a second maximum in the region from 400 to 460 nm of the spectrum. In addition, the sensitivity of *O. c. clarki* in the ultraviolet and the violet regions of the spectrum corresponded well with λ_max of the ultraviolet- and S-cones of the absorbance template, respectively (Fig. 6B). An additional peak in sensitivity was also present at 600–640 nm (marked by an asterisk). The shape of the spectral sensitivity curve in this region did not correspond to the shape of the absorbance template for the L-cone and could be aligned only with the descending limb of this template. The ON-responses in the ultraviolet region of the spectrum in *O. nerka* and *S. fontinalis* were larger than those in steelhead (*O. mykiss*) (Fig. 6C,D). However, the correspondence of the ultraviolet- and S-cone absorbance templates...
templates to the spectral sensitivity was relatively poor in *O. nerka* compared with *S. fontinalis*. In addition, the \( \lambda_{\text{max}} \) of the ultraviolet absorptance template at 365 nm (Fig. 6C,D) did not match the peak sensitivity of the ON-responses (390 nm for *O. nerka* and 380 nm for *S. fontinalis*) for either of these species. The overlay of the M-cone and L-cone templates suggested that these mechanisms contribute to spectral sensitivity for both *O. nerka* and *S. fontinalis* under this adaptation regime. In *O. nerka*, these cone mechanisms appeared to be 1 log unit less sensitive than the ultraviolet-cone mechanism, whereas in *S. fontinalis*, the L- and M-cone mechanisms were more sensitive. Under adaptation conditions to increase the relative sensitivity of the ultraviolet-cone mechanism, the sensitivity of *S. fontinalis* in the red region of the spectrum, like that of *O. c. clarki*, was lower than that predicted by the absorptance template for the L-cone. However, the observed peak at around 640 nm was smaller than that observed for *O. c. clarki*.

In contrast to the ON-responses, the OFF-responses decreased in sensitivity in the ultraviolet and violet region of the spectrum following adaptation to the yellow–orange (ultraviolet-isolating) background and compared with observations with a white-light background (Fig. 7A–D). The absorptance template for the M-cone underestimated the observed spectral sensitivity curves at wavelengths below 460 nm for all species except *O. nerka* (Fig. 7A–D). In addition, the L-cone absorptance template suggested that the L-cone mechanism may also have contributed to OFF-sensitivity for both *O. c. clarki* and *O. nerka*.

In summary, under white-light adaptation conditions, the spectral sensitivity of ON- and OFF-responses differed. Similarly, the spectral sensitivity of ON-responses differed significantly among species. Specifically, ON-responses were dominated by both L- and M-cone mechanisms in all species except *O. nerka*, in which the M-cone mechanism alone contributed most to sensitivity. In contrast, OFF-responses were not significantly different among species, with sensitivity depending primarily on the M-cone mechanism. Under the yellow–orange (ultraviolet-isolating) background, all species appeared to show ON-responses indicative of ultraviolet sensitivity with varying degrees of contribution from the L-, M- and S-cone mechanisms. The OFF-responses showed no evidence of a contribution from the ultraviolet cone mechanism, but instead were dominated by the M-cone mechanism with possibly some contribution from the L-cone mechanism.

**Polarisation sensitivity: ON-responses**

Sensitivity of the ON-responses of *O. mykiss*, *O. c. clarki*, *D. C. Parkyn AND C. W. Hawryshyn*
O. nerka and S. fontinalis to the orientation of the linear polariser was a characteristic W-shaped function (Parkyn and Hawryshyn, 1993, 1999) (Fig. 8A–D). Overall, ON- and OFF-responses were found to be significantly different (ANOVAR; \( F_{0.05,17}=8.78, P<0.05 \)). The sensitivity of ON-responses varied significantly with the angular orientation of the polariser (within-subjects effect) (ANOVAR, \( F_{0.05,6}=5.31, P<0.001 \)). The sensitivity of the ON-response to the ultraviolet-polarised-light stimulus varied significantly with the angle of the stimulus for O. mykiss, O. nerka and S. fontinalis (Fig. 8A–D; Table 3). In addition, no differences were detected among species for the relative sensitivity of ON-responses and the angular orientation of the polarised ultraviolet stimulus (ANOVAR, \( F_{0.05,2}=1.63, P=0.222 \)). A test of the interaction effects of species by angle of polariser was also non-significant (ANOVAR, \( F_{0.05,12}=1.25, P=0.257 \)).

Periodic regression analysis of the means of the ON-responses to the angle of the polarised-light stimulus of all species examined had a W-shaped response with \( \phi=0^\circ, 90^\circ \) and \( 180^\circ \), consistent with the model outlined in equation 1. However, the angular correlation coefficient \( (r^2) \) ranged from 0.53 for O. c. clarki to 0.98 for O. mykiss (Fig. 8A–D). All periodic regressions of the ON-responses were nonetheless significant (Fig. 8A–D). The peak width (\( \delta \)) at the mesor was also observed to vary from a minimum of 53.6° in O. nerka to a maximum of 68.6° in O. c. clarki.

Polarisation sensitivity: ON-responses

In contrast to the ON-responses, the OFF-responses yielded a bell-shaped function (Fig. 9A–D). As with ON-responses, the sensitivity of OFF-responses varied significantly with polariser orientation (Table 3). Observed OFF-responses in all species were unimodal with \( \phi=90^\circ \) and \( \delta \) ranging from a minimum of 64.3° in O. nerka to a maximum of 87.9° in O. c. clarki (Fig. 9A–D). As a result, the relationship between sensitivity and the angle of the polariser (\( \theta \)) in O. mykiss, O. c. clarki and S. fontinalis was described by equation 3, a simple cosine function (Fig. 9A,B,D). The data for O. nerka were more sharply peaked than could be described by a cosine function. An alternative model suggested to attain a more descriptive fit for sharply peaked data (Batschelet, 1981) was:

\[
S = M + Acos[\omega\theta - \phi + vsin(\omega\theta - \phi)],
\]

where \( M \) is the mesor, \( A \) is amplitude, \( \omega=2\pi/\tau \), where \( \tau \) is the period of the function, \( \phi \) is the acrophase (the angle associated with maximal sensitivity) and \( v \) is the coefficient of skewing (Batschelet, 1981). This equation provided a descriptive and significant fit for O. nerka (Fig. 9C).

In summary, ON and OFF polarisation curves differed significantly. However, small differences in amplitude and peak width at the mesor were apparent among species. In general, the ON-responses of the salmonids were sensitive to both horizontally and vertically polarised light, while the OFF-responses were sensitive to horizontal plane-polarised light only.
Discussion

In addition to extending our current knowledge on the phyletic distribution of the ultraviolet photoreceptor within the salmonid Genus *Oncorhynchus*, this research provides the first evidence of an independent ultraviolet-sensitive mechanism in the Genus *Salvelinus*. Little work has been conducted on the visual physiology of *Salvelinus* spp., although the basic retinal morphology and development of Arctic char (*S. alpinus*) is known (Vigh-Teichmann et al., 1991). It has been shown that the photoreceptor mosaic of Arctic char is similar to that of other salmonids (Lyall, 1957; Ahlbert, 1976; Kunz, 1987; Vigh-Teichmann et al., 1991; Browman and Hawryshyn, 1992). These similarities include the presence of small single corner cones (sometimes termed accessory corner cones); these have been associated with ultraviolet sensitivity in *O. mykiss* (Browman and Hawryshyn, 1992; Beaudet et al., 1993; Hawryshyn and Hárosi, 1994). Thus, the presence of ultraviolet sensitivity in *S. fontinalis* was predictable.

Electrophysiological evidence for an ultraviolet-cone mechanism from the present study supports the view that ultraviolet sensitivity is a shared ancestral character (a plesiomorphism) present in all clades of the subfamily Salmoninae. In addition, the ultraviolet-cone mechanism appeared to contribute to the ON-response but not to the OFF-response for all fishes in the current study. Even under chromatic adaptation to increase the relative sensitivity of the ultraviolet-cone mechanism, its contribution to the OFF-response could not be demonstrated conclusively. The nature of ultraviolet sensitivity in salmonids with respect to ON and OFF channels may reflect differences in the ultraviolet content of the veiling irradiance and reflection from targets in the water column. In addition, or alternatively, this may be a consequence of a specialised function of ultraviolet sensitivity, for example polarisation sensitivity.

The observation of an independent ultraviolet-cone mechanism in this study has also been addressed recently by a search for evidence of ultraviolet-cone pigments in other fish taxa (Hisatomi et al., 1996). Their study suggested the presence

<table>
<thead>
<tr>
<th>Species</th>
<th>Ganglion cell responses</th>
<th>N</th>
<th>F</th>
<th>P</th>
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</thead>
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<tr>
<td><em>Oncorhynchus mykiss</em></td>
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<td>10</td>
<td>5.78</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>OFF</td>
<td>10</td>
<td>11.32</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><em>O. nerka</em></td>
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<td>5.10</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>OFF</td>
<td>5</td>
<td>5.12</td>
<td>0.003</td>
</tr>
<tr>
<td><em>Salvelinus fontinalis</em></td>
<td>ON</td>
<td>7</td>
<td>7.15</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>OFF</td>
<td>7</td>
<td>3.54</td>
<td>&lt;0.030</td>
</tr>
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Table 3. Summary statistics of ANOVA with repeated measures tests for differences in sensitivity as a function of angular position of a linear polariser.

Fig. 9. Polariisation sensitivity of OFF-responses recorded from the optic nerve to a plane-polarised ultraviolet stimulus (360 nm + HNP’B ultraviolet linear polariser) of (A) *Oncorhynchus mykiss*, (B) *O. c. clarki*, (C) *O. nerka* and (D) *Salvelinus fontinalis*. Values represent means ± 1 S.E.M. The solid line represents the periodic regression model. The dashed line represents the mesor.
of amino acid sequences in salmonids with properties similar to those of the ultraviolet photopigment in goldfish (Carassius auratus). On the basis of molecular data, Hisatomi et al. (1996) stated that similar sequences have been identified in (i) the Ostariophysi, Family Cyprinidae (goldfish and zebra danio Danio rerio); (ii) a protacanthopterygian, Family Salmonidae (chum salmon, O. keta), mistakenly ascribed to the Clupeiformes (herrings) by Hisatomi et al. (1996); and (iii) an acanthopterygian, Family Oryzidae (the medaka, Oryzias sp.). Hisatomi et al. (1996) mistakenly called this fish a killifish (Family Cyprinodontidae) of the top-minnow family (which is in fact Family Atherinidae). Correction of these taxonomic inaccuracies leads to a significant conclusion. The gene for the ultraviolet pigment is present in several distantly related groups of teleost fishes and may be an ancestral feature of the entire Euteleostii. Because this group contains over 20 000 species and is the most speciose group of vertebrates (Nelson, 1994), the number of species with ultraviolet sensitivity is potentially large.

Regardless of the phyletic distribution of ultraviolet sensitivity among fishes, the selective pressures that gave rise to an independent ultraviolet photoreceptor mechanism is a matter of speculation. The presence of ultraviolet light in veiling illumination is known to increase zooplankton capture rate and thus presumably detection (Loew et al., 1993; Browman et al., 1994). In addition, ultraviolet sensitivity appears to contribute to colour vision through opponent interactions (Hárosi and Hashimoto, 1983; Coughlin and Hawryshyn, 1994b). These findings are corroborated by behavioural evidence (Neumeyer, 1985) and are consistent with the idea that ultraviolet sensitivity extends the spectral bandwidth of vision. The reason why a fish should evolve a wide bandwidth of sensitivity is a matter of speculation, but some evidence suggests it is a mechanism to allow contrast under a variety of photic conditions or in complex light environments (Munz and McFarland, 1977) and may reflect the ‘generalist’ visual system of salmonids. Finally, ultraviolet sensitivity has a role in polarisation sensitivity in salmonids that may be exclusive of its role in colour vision (Hawryshyn et al., 1989, 1990; Hawryshyn and Bolger, 1990; Parkyn and Hawryshyn, 1993, 1999; Coughlin and Hawryshyn, 1995).

While all salmonids examined herein demonstrated photosensitivity generally consistent with the rainbow trout absorbance templates, differences were observed in the relative sensitivity of individual cone mechanisms among species and forms of fish. The fish retina processes output from the cones; hence, the interactions among mechanisms do not represent a simple summation of all the cone outputs for both the ON- and OFF-responses. In salmonids, these interactions result in regions of the spectrum in which one cone mechanism appears to mask or inhibit the spectral sensitivity of another cone mechanism, as in the case of the ON-responses under white-light conditions or the reduced sensitivity in the ultraviolet region of the OFF-responses under chromatic adaptation. This is also certainly the case for polarisation sensitivity, since the measured and calculated dichroic ratios at the level of the cone photoreceptor are well below the level of modulation observed from behavioural and physiological experiments (Hawryshyn and McFarland, 1987; Novales Flamarique and Hawryshyn, 1997; Novales Flamarique et al., 1998).

Observations of differences among species may also represent species-specific characteristics that have evolved under different selective pressures. This is demonstrated by the observation that, while the spectral sensitivities of the OFF-responses were dominated by a single mechanism and were not statistically different, the ON-responses of the various species were different under both white light and ultraviolet-isolating conditions. As juveniles, O. mykiss, O. c. clarki and S. fontinalis live in riffles, primarily in streams and rivers, as well as in the shallow littoral zone in lakes (Scott and Crossman, 1973; Behnke, 1992). In contrast, juvenile O. nerka are found in lakes in the limnetic zone (Scott and Crossman, 1973). The detection of biologically relevant stimuli in the photic environment of kokanee (O. nerka) should place different demands on their visual system. In spite of this, it appears that the classes of photoreceptor in salmonids do not differ, on the basis of the fit of the a-absorbance peak of the absorbance templates. Instead, it is the relative contribution of individual classes of cone mechanism to sensitivity that appears to differ.

This issue of relatively small differences in the visual sensitivity among salmonids was also addressed by Bridges and Yoshikami (1970) in an extensive study of salmonid rod photopigments. However, this result should not be surprising. First, salmonid species are closely related (Behnke, 1992; Stearley and Smith, 1993). Coastal rainbow trout, steelhead O. mykiss and coastal cutthroat trout O. c. clarki, in particular, have been separate species since the late Pliocene (Behnke, 1992). In addition, a large degree of natural introgression has occurred between O. mykiss and O. c. clarki (Campion and Utter, 1985). Such events undoubtedly should reduce the genetic and phenotypic differences between these species. A second factor contributing to the non-specificity of the visual system in salmonids is their wide geographic range and the diversity of environments they occupy. Salmonids in the present study should be considered to have a ‘generalist’ visual system. Following the scheme of Levine and McNichol (1979) for the ecological and behavioural classification of fish, many salmonids live in three of the four possible visual environments (Groups I, II and III) during their lifespan. In particular, the visual environment of anadromous salmonids, while not as complex as coral reef environments, nonetheless exposes the retina of a salmonid to a range of different spectral backgrounds (Novales Flamarique et al., 1992; Novales Flamarique and Hawryshyn, 1993). The data from the present study indicate little specialisation among the species. In contrast, lake char (S. namaycush) represent a salmonid with a typically deep-water lacustrine distribution (Scott and Crossman, 1973). A comparison with its close relative S. fontinalis (present study) would provide useful insight into the degree to which the visual system of lake char has adapted to its less variable environment.

Interestingly, the maximum sensitivity (λ_{max}) of the photopic mechanisms varied considerably among salmonid
species from different studies (Table 4). This may in part be due to differences in methodology. Douglas and Hawryshyn (1990) discuss this issue at length with respect to behavioural methods of assessing spectral sensitivity. However, much variability has been observed in these fish even when using similar techniques. In the present study, in which fish were reared under the same conditions and tested using the same technique, the differences in the $\lambda_{\text{max}}$ were not large. Hence, it is important to stress documentation of the culture conditions during rearing of the fish. This may shed light on factors influencing sensitivity and help in differentiating the effects of differences in rearing environment from differences in data collection methodology or species. For example, environmental factors, such as temperature and photoperiod, affect the relative proportions of two retinal photopigments, rhodopsin ($A_1$) and porphyropsin ($A_2$) (Dartnall et al., 1961; Beatty, 1966; Tsin and Beatty, 1977; Allen and Munz, 1983). The relative ratio of these pigments, in turn, may affect spectral sensitivity (Dartnall et al., 1961; Munz and McFarland, 1977; Whitmore and Bowmaker, 1989). However, some experimental evidence suggests that the differences in absorbance accompanying such pigment changes may affect spectral sensitivity only slightly, at least near threshold (Muntz and Northmore, 1973; Muntz, 1975). Other factors can increase absorbance (optical density), e.g. the length of the cone outer segment or an increase in the density of photopigment. These increases will result in a broadened bandwidth of photon capture by an individual photoreceptor (Allen et al., 1973). This would further decrease differences in wavelengths of photon capture by two photopigments differing in $\lambda_{\text{max}}$ by 10 or 15 nm. Control of rearing conditions is, therefore, necessary to reduce the possible effects of phenotypic variation on differences in visual sensitivity. Sampling bias may also be a potential factor contributing to differences among replicates and fish species. When present, this bias is due to placement of the recording electrode where it may collect only a fraction of ganglion cell axonal output. However, the diameter of the recording electrode in the present study was very large relative to the diameter of the optic nerve (approximately 0.5 mm:1 mm). In addition, sampling replication in the white-light regime tests would have controlled for this potential problem.

Another feature of particular interest is the presence of high red-light sensitivity of the ON-response and high green-light sensitivity of the OFF-response under white-light background conditions. The L-cone mechanism is known to dominate the ON-response of many fish species under tungsten white-light conditions (Daw, 1968; Easter, 1975; Beauchamp and Rowe, 1977; Coughlin and Hawryshyn, 1994b). However, it is notable that, in the present study, the wavelength of this peak sensitivity, in the orange-red end of the spectrum, did not match previous observations for $O. \text{mykiss}$ by either Hawryshyn et al. (1989) or Coughlin and Hawryshyn (1994b). Several factors could account for this apparent discrepancy including: (i) microspectrophotometric/absorptance-template availability; and (ii) the rearing conditions of the fish. When these previous two studies were conducted, the microspectrophotometric absorbance curves for $O. \text{mykiss}$ were only beginning to become available (Kusmic et al., 1993; Hawryshyn and Hárosi, 1994). Instead, an eighth-order

<table>
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<th>GCR</th>
<th>MSP</th>
<th>Behaviour</th>
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<td>$O. \text{mykiss}$</td>
<td>370–380&lt;sup&gt;1&lt;/sup&gt;</td>
<td>390&lt;sup&gt;2&lt;/sup&gt;</td>
<td>400&lt;sup&gt;3&lt;/sup&gt;</td>
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<td>510 (520)&lt;sup&gt;*&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>580</td>
<td>NE</td>
<td>598</td>
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<td>580–600</td>
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<td>$S. \text{trutta}$</td>
<td>355&lt;sup&gt;8&lt;/sup&gt;</td>
<td>441</td>
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<tr>
<td>$S. \text{fontinalis}$</td>
<td>360–380&lt;sup&gt;1&lt;/sup&gt;</td>
<td>420</td>
<td>540</td>
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</table>

<sup>1</sup>Present study; <sup>2</sup>Beaudet et al. (1993); <sup>3</sup>Kusmic et al. (1993); <sup>4</sup>Hawryshyn and Hárosi (1994); <sup>5</sup>Douglas (1983); <sup>6</sup>Hawryshyn et al. (1989); <sup>7</sup>Novales Flamarique and Hawryshyn (1996); <sup>8</sup>Bowmaker and Kunz (1987); NE, not examined; <sup>*</sup>values differed between juveniles and adults (in parentheses).
polynomial nomogram (Bernard, 1987; Browman and Hawryshyn, 1992; Beaudet et al., 1993) was aligned to what was interpreted to be the $\lambda_{\text{max}}$ of the data.

Actual microspectrophotometric absorption curves are now available, and correcting them for ocular media transmission and converting them to absorbance has given us a guideline for placement of templates that was not previously available. ‘Pseudo peaks’ in the red region of the spectrum have been described previously for humans and macaques (far from the $\lambda_{\text{max}}$ of known primate retinal pigments; Splerling and Harwerth, 1971) and for goldfish (Beauchamp and Rowe, 1977; Neumeyer, 1984). With alignment of the absorbance template, a similar conclusion is reached about the nature of the channel contributing to the observed spectral sensitivity. Specifically, a factor, such as lateral inhibition, is decreasing the L-cone mechanism sensitivity over part of the range of this mechanism. In $O. clarki$, this was apparent because the template for the M-cone mechanism appeared to provide a better description of the sensitivity in part of the range where one would expect the L-cone mechanism to dominate sensitivity.

Re-examination of spectral sensitivity curves from Coughlin and Hawryshyn (1994b; their Figs 2, 4A–C), where the fish were cultured under similar conditions to those used in the present study, suggested that the sensitivity in the region from 500 to 660 nm could be subject to the same phenomenon. Under this scenario, the dichromatic unit (their Fig. 4A) would become monochromatic, the trichromatic unit (their Fig. 4B) would become dichromatic, and their tetrachromatic unit (their Fig. 4C) would become trichromatic. Compelling evidence for the peak sensitivity of the L-cone mechanism being near 585 nm under these conditions was also apparent in Coughlin and Hawryshyn (1995, their Fig. 10A). In their figure, a complete curve without any inhibition of the peak sensitivity in the red region of the spectrum is observed. Such a curve was similar to the ON-response spectral sensitivity under white-light conditions for $O. mykiss$ in the present study. Again, an alternative that may aid in explaining the disparity between the present experiment and that of Hawryshyn et al. (1989), in particular, is that the longer-wavelength red-sensitivity of the L-cone mechanism in Hawryshyn et al. (1989) may have resulted from their fish having a higher proportion of A2-based photopigment. Such shifts are known to occur seasonally in some species of fishes (Beatty, 1966, 1984; Loew and Dartnall, 1976; Whitmore and Bowmaker, 1989). However, adult fish in the holding facility used in the present study did maintain their seasonal reproductive cycles, in spite of a controlled photoperiod (D. C. Parkyn, personal observation). This suggests an endogenous rhythm to this cycle or seasonal fluctuation of another potential Zeitgeber (see Tsin and Beatty, 1977).

The white-light background conditions in this study approximate the background conditions of a fish viewing downwelling illumination in the neritic zone or epilimnion (Novales Flamarique et al., 1992; Hawryshyn, 1992). The dominance of the L-cone mechanism (red) ON-responses and the M-cone mechanism (green) OFF-responses suggests that, under these conditions, these wavelengths have particular utility for the detection of objects in the water column. There are several explanations for this phenomenon. First, the dominant contribution of the L-cone mechanism to sensitivity may constitute a form of physiological compensation. This may result from the relatively rapid attenuation of red light in the environments of these fish (Novales Flamarique et al., 1992; Novales Flamarique and Hawryshyn, 1993). Second, the transparency of water to red and green light is much less variable than that at wavelengths in the blue or violet portion of the spectrum (Lythgoe, 1975). For example, dissolved organic matter (e.g. humic and fulvic acids) absorbs strongly in the blue and violet regions and yet is transparent in the red (Lythgoe, 1975). Third, such a physiological compensation may be important because these wavelengths are offset from the prevailing downwelling light, which is typically green or blue in the lakes and coastal waters of North America where these fish are endemic. This may, therefore, be an example of an offset in sensitivity to enhance contrast (Lythgoe, 1966, 1968, 1975, 1979; Easter, 1975). This would enhance the detection of photons in the 585–660 nm region of the spectrum reflected by a nearby target relative to the light background surrounding that target, since these same wavelengths would also be rapidly attenuated from the water column (Lythgoe, 1966, 1968, 1979). In addition, this high red-sensitivity is probably exploited by the almost exclusively red nuptial coloration of many salmonine species. Typically, these species spawn in shallow clear-water streams and along lake shores where the effects of wavelength-specific attenuation are reduced. Thus, a conspecific reflecting light in the long-wavelength portion of the visible spectrum would be conspicuous to a fish with high spectral sensitivity in this region.

The OFF-responses of all species examined with both the white-light background and ultraviolet-isolating conditions were dominated by the M-cone mechanism. Beaudet et al. (1993) observed similar results under ultraviolet-cone isolating conditions, as did McDonald and Hawryshyn (1995) under S-cone-mechanism isolating conditions. Wheeler (1979) speculated that this shadow detector would have utility in the detection of predators and prey. What is of particular interest is the matching of the ON- and OFF-responses in kokanee ($O. nerka$) under a white-light background. This would suggest that, in the relatively more monochromatic limnetic environment of this species, colour contrast of objects may not be as important as it is in streams or the neritic zone.

Polarisation sensitivity

The similarity of spectral sensitivity among the salmonids examined was also mirrored in polarisation sensitivity. Relative sensitivity to the angular orientation of linearly polarised ultraviolet light has been examined for rainbow trout and sockeye salmon (Parkyn and Hawryshyn, 1993, 1999; Coughlin and Hawryshyn, 1994a; Novales Flamarique and Hawryshyn, 1996). The findings of the present study are consistent with previous work and represent an extension of it. Differences in shape of the ON and OFF polarisation-sensitivity curves
sensitivity to polarised light is also dominated by the M- and L-cone mechanisms, which are preferentially sensitive to horizontally polarised light, at least in the axons of optic nerve ganglion cells (Coughlin and Hawryshyn, 1995; Parkyn, 1998). Coughlin and Hawryshyn (1995) found little polarisation sensitivity in the ON-response of the M-cone (one of three single-unit recordings) or L-cone (one of nine single-unit recordings) mechanism, as recorded from the torus semicircularis. This is perplexing in the light of their observation that horizontal polarisation sensitivity is present in the ON-response of the optic nerve using multi-unit recording (as was also found by Parkyn and Hawryshyn, 1993) as well as in the OFF-response (Coughlin and Hawryshyn, 1994b; Parkyn, 1998). This suggests one of several possibilities.

First, differences existed between the adaptation conditions of the present study and that of Coughlin and Hawryshyn (1995). In particular, the description of polarisation sensitivity in the ON-responses of M- and L-cone mechanisms in Parkyn and Hawryshyn (1993) and Parkyn (1998) was obtained under chromatic adaptation conditions designed to isolate for these respective mechanisms physiologically. The present examination of spectral sensitivity (Figs 4, 6) suggested that, under white-light or yellow–orange background conditions, the M-cone as well as the L-cone do at least contribute to ON-response spectral sensitivity in all species of salmonids examined. In addition, in some regions of the spectrum, the M-cone appears to display inhibition of what would otherwise be a dominant L-cone mechanism if the spectral sensitivity conformed to the shape of the L-cone absorptance curve (e.g. 560–620 nm in Fig. 4B).

Second, Coughlin and Hawryshyn (1994b) examined only nine single units with L-cone mechanism input in the torus semicircularis, so the sampling bias of examining a few single units versus the entire ganglion cell population of the optic nerve may have contributed to their observations.

A third possibility is that ON- and OFF-responses of the M-cone mechanism and the ON-responses of the L-cone mechanism may be processed in an area of the brain that they did not sample. Because horizontal polarisation sensitivity is present in these ON-responses in the optic nerve and not the torus semicircularis (Parkyn and Hawryshyn, 1993; Coughlin and Hawryshyn, 1994b), these signals may project to some other structure in the brain. One speculative role for this information would be to unconfound spectral sensitivity from polarisation sensitivity. This would be important since, at present, there is limited evidence to suggest that different populations of photoreceptors are subserving spectral and polarisation sensitivity in fish (Novales Flamarique and Hawryshyn, 1997) or hymenopterans (Wehner, 1976). Alternatively, this polarisation sensitivity in the M-cone and in the ON-response of the L-cone may serve no purpose, but merely be a byproduct of the mechanism that gives rise to polarisation sensitivity in the L-cone.

The roles of polarisation sensitivity in fish are a matter of speculation and debate (Groot, 1965; Dill, 1971; Hawryshyn and Bolger, 1990; Hawryshyn et al., 1990; Rowe et al., 1994; Bains, 1996). However, polarisation sensitivity, and in...
particular ultraviolet-polarisation sensitivity, does appear to have a role in spatial orientation in salmonids (Groot, 1965; Dill, 1971; Hawryshyn and Bolger, 1990; Hawryshyn et al., 1990; Parkyn, 1998). However, relative sensitivity to plane- polarised light appears to be similar among species of salmonids, regardless of whether the species is migratory or non-migratory. This suggests that, for these species, polarised-light sensitivity may have biological roles other than solely providing a mechanism for orientation behaviour.

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References


