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**VISUAL BIOLOGY OF SALMONIDS WITH SPECIAL REFERENCE TO
POLARISED-LIGHT SENSITIVITY**

by

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B.Sc., University of Alberta, 1984

A Dissertation Submitted in Partial Fulfilment of the

Requirements for the Degree of

DOCTOR OF PHILOSOPHY

in the Department of Biology

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ABSTRACT

The visual biology and its relevance to orientation was examined in fishes in the subfamily Salmoninae (salmon, trout, and char) by characterising the spectral and polarisation sensitivity of their visual systems. In addition, the underlying neural mechanisms and biological relevance of polarisation sensitivity in the Salmoninae were examined with an emphasis on its role in orientation.

Integrated spike activity of axons from the optic nerve was used as a measure to determine the polarised-light sensitive mechanism underlying the ability of rainbow trout (*Oncorhynchus mykiss*) to orient in down-welling, linearly-polarised light. Relative sensitivity curves were then obtained for the five types of photoreceptor cells in this trout's retina: rods, ultraviolet sensitive cones (UV), short wavelength cones (blue-sensitive) (S), medium wavelength cones (green-sensitive) (M), and long wavelength cones (red-sensitive) (L).

Under scotopic conditions (dark adapted), no sensitivity to e-vector was apparent. Under photopic conditions (light adapted), trout parr exhibited on-responses with e-vector sensitivity in two orthogonal channels. No evidence of polarisation sensitivity was observed in the on-responses of larger fish (50-78 g smolts) under UV-isolating background conditions, whereas the off-responses were unchanged. M and L cones, in contrast, retained their unimodal response. The decrease in UV-polarisation sensitivity in larger fish was found not to be attributable to size-related changes in the ability of the ocular media of the eye to transmit polarised light.

Rainbow trout was used as a model species and spectral and polarisation sensitivity were compared with steelhead (an anadromous form of *O. mykiss*), cutthroat trout (*O. clarki*), kokanee (land-locked form of *O. nerka*), and brook char (*Salvelinus fontinalis*). Visual pigment templates from rainbow trout were corrected for ocular media absorbance and overlaid on the spectral sensitivity

curves for the purposes of comparison. Some differences in sensitivity were observed among species. In particular, on-responses were dominated by L-cones in most species. However, in kokanee, the M-cone was dominant. These differences may be related to the photic environments from which these fish originate. The first physiological evidence of near-UV sensitivity in the genus *Salvelinus* is also provided. It was therefore concluded that UV sensitivity is ubiquitous in the subfamily Salmoninae. Polarisation sensitivities in the above species were modelled using periodic regression analysis. Comparisons suggest that all of these species have a similar dual-channel polarisation detection system.

The effect of the tuberculostatic drug Ethambutol on the visual physiology of rainbow trout was also examined. This drug appears to cause a decrease in sensitivity analogous to chromatic adaptation. Fish were fed daily for one month and then spectral sensitivity and polarisation sensitivity of Ethambutol-treated fish were compared to control fish. Relative to controls the visual systems of treated fish were dominated by the M-cone mechanism. Spectral and polarised light sensitivity of UV and L-cones were reduced.

Finally, orientation responses of juvenile rainbow trout, steelhead, and brook char to a polarised-light stimulus were examined under controlled laboratory and semi-natural field conditions. Trained fish of all species oriented to the plane of polarised light, whereas untrained fish could not. Fish trained in the lab were tested at twilight and were found to be able to orient under natural skylight. Under semi-natural conditions, fish in floating net-pens in a lake were provided food rewards at a specific compass bearing. Their orientation responses were assessed under various natural and artificial conditions. When the sun was visible the fish typically had a unimodal distribution. However, they were typically not oriented when presented with only brightness and spectral cues at Zenith or on cloudy days. In contrast, both steelhead and sockeye were oriented correctly at civil twilight if the horizon was not obscured by heavy cloud or if blue sky was visible at Zenith.

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**This dissertation is dedicated to
to my parents, Charles and Patricia Parkyn**

Frontispiece

Patterns of polarised light reflected from the body surfaces of a parr rainbow trout (10g) (left column) and pre-smolt kokanee (12 g) (right column). Images were obtained by video-image subtraction of components of plane-polarised light using a Matrox PIP 512 image processing board. Images were then pseudo-coloured on the basis of intensity.

Rainbow Trout

Kokanee



Unpolarised Light

Rainbow Trout

Kokanee



Horizontal Polarised Light

Rainbow Trout

Kokanee



Vertical Polarised Light



0 10 20 30 40 50 60 70 80 90+

Percent Relative Reflected Polarised Light

CHAPTER I

GENERAL INTRODUCTION

The idea that other animals might perceive visual stimuli differently from humans was not formally recognised or perhaps even considered scientifically until the late 1800's. Certainly, the lay populace have long recognised that differences exist between the vision of various other animals and humans because of statements such as "he is as blind as a bat", "il est myope comme une taupe" (he is as near-sighted as a mole) or "she has the eyes of an eagle". Similarly, there is a general perception that many animals have better night vision than humans, e.g., cats and owls. One of the first scientific studies to compare vision of other animals to that of humans was conducted by Sir John Lubbock (1883). Lubbock took advantage of the photophobic response of ants. Ants will move their larvae away from illuminated areas. He used a carbon arc lamp as a light source a prism and various filters to provide a gradient of wavelengths of light. The light was projected onto a horizontal surface. He then placed an ant and a larva onto the projected rainbow. This experiment provided two insights into the spectral sensitivity of ants. First, the ants scurrying out of the light would stop carrying their larvae when they entered into the far red region of the spectrum. Lubbock concluded (correctly) that the ant's visual system has roughly similar sensitivity to that of the human eye to red light.

Second, the ants carrying larvae toward the violet end of the spectrum continued to carry their larvae into the dark region, corresponding to wavelengths in the near ultraviolet (UV)¹. He then concluded (also correctly) that ants can detect UV light. From this elegant and simple experiment came the first realisation that other species have different perceptual capabilities from humans. Schiemenz (1924) and Merker (1934) provided some of the first demonstrations that this phenomenon extended to fishes. Using colour choice and operant behaviour modification, their experiments demonstrated that the minnow (*Phoxinus laevis* and *Gasterosteus aculeatus* respectively) could discriminate near-UV from violet and other spectral hues. In spite of this, their studies were largely ignored, possibly because they were written in German. Starting in the 1970's, mounting experimental evidence suggested that all classes of vertebrates could detect UV light, including fish (Avery and Bowmaker 1982, Avery *et al.* 1983, Hárosi and Hashimoto 1983, Hawryshyn and Beauchamp 1985, Hawryshyn *et al.* 1989, Loew *et al.* 1993, Loew and McFarland 1994), amphibians (Dietz 1972, Govardovskii and Zueva 1974, Deutschlander and Phillips 1995), reptiles (Arnold and Neumeyer 1987, Fleishman and Loew 1993, Fleishman *et al.* 1997, Sillman *et al.* 1997), and birds (Huth and Burkhardt 1972, Kreithen and Eisner 1978, Goldsmith 1980, Chen *et al.* 1984, Jane and Bowmaker 1988, Maier 1994, Bennett *et al.* 1996). Recently, Jacobs *et al.* (1991) have provided

¹ In this dissertation, UV refers to the near UV region = 300 to 399 nm; sometimes it is termed UV A. UV A also causes a seasonal increase in skin melanin concentration in many diurnal vertebrates.

electrophysiological data that gerbils and other small mammals also can detect UV light.

The prevalence of UV light in aquatic ecosystems has not been examined in great detail until recently, perhaps because it was assumed that water attenuated UV light rapidly (McFarland 1986, Loew and McFarland 1990, McFarland 1991, Novales Flamarique *et al.* 1992). McFarland (1986) estimates that in blue oceanic waters a UV-cone mechanism in fish of similar sensitivity to the photopic mechanism in humans would function to depths of 100 m. Similarly, in more green coastal waters, such as those off British Columbia, this same mechanism would function to 30 m (McFarland 1986). Similar findings have been shown by Novales Flamarique *et al.* (1992) who calculated that UV sensitivity could be supported to at least 20 m in a mesotrophic freshwater system. For a review of light in the aquatic environment see Loew and McFarland (1990). Additionally, Novales Flamarique and Hawryshyn (1993) provide a detailed examination of UV transmission in neritic and estuarine waters of a British Columbia fjord.

Like UV light, the nature of polarised light in the aquatic environment has only recently been investigated, primarily as a result of early work by Talbot Waterman (Waterman 1954, 1955, Waterman and Westell 1956). Waterman (1981), Horvath and Varjú (1995), and Novales Flamarique and Hawryshyn (1997a) provide detailed information on the physical nature of aquatic polarisation fields under various conditions. Essentially, whether in the air or in the water, two forms of scattering contribute to polarisation of light: Rayleigh

scattering and/or Mie scattering. Rayleigh scattering operates when particles are small ($\leq \lambda/10$, where λ = wavelength).

$$[1] \quad I_s \propto \frac{1}{\lambda^4}$$

where I_s = intensity of scattering.

Thus, particles of this size strongly scatter short wavelengths, such as UV and the blue region of the spectrum (Bradbury and Verencamp 1998, Hailman 1977). In contrast, long wavelengths, such as the red region of the spectrum, scatter to a lesser extent. At sunset this is represented by the strong red light component in the West, with light in the East being scattered back toward the viewer and appearing blue (hence, the answer to the age old question of why the sky is blue). Spherical particles between $\lambda/10$ and $< 25\lambda$ in size are affected by Mie Scattering which is less wavelength dependent (Mie 1908, Hailman 1977, Bradbury and Verencamp 1998). In Mie scattering, wavelengths of light that are similar in size to a particle's diameter is scattered forward. Hence Mie scattering, unlike Rayleigh scattering, does not filter light to the same extent, although it does show a general trend to scatter more light with increasing wavelength, within the near UV and visible spectrum (Hailman 1977). An example of this phenomenon is the white appearance of clouds resulting from the scattering of light by water droplets in clouds. Geometry determines the direction of reflection and the degree of polarisation off of surfaces larger than 25λ , (Hailman 1977). The ultimate result of all of these forms of scattering is that the planes of propagation of photons scattered normal to the plane of incidence of the light

have their electric vectors (e-vector) that are polarised or vibrating in the same plane. If the plane of vibration of the e-vectors of the light source is partially polarised along one plane, it is referred to as elliptical-polarised light. If these e-vectors are almost totally polarised along one plane, the light source is said to be plane- or linearly- polarised.

Ants and their relatives also played a major role in the discovery of polarisation sensitivity in animals. An early study of orientation in ants suggested that they could orient in the correct direction when the sun was obscured and they could only view a small patch of blue sky (Santschi 1923). Santschi believed that the ants were using intensity gradients of light in the sky. This was in part true but it would be another 26 years until orientation using plane-polarised light (menotaxis) was formally described. These gradients were, in fact, the gradients of orientation and proportion of celestial polarised light. The first general acceptance of polarised light as a cue for orientation resulted from the pioneering work of von Frisch (1949) on orientation in honey bees (*Apis mellifera*). Since that time much research has been conducted on polarised-light sensitivity in various species [see Waterman (1981) and Rossel (1989) for reviews]. It appears that many arthropods show polarised-light sensitivity. In fact, some insects have gone to great lengths to make a portion of their ommatidia insensitive to polarised light (Wehner and Bernard 1993). This is apparently an aid to reduce the confounding of information from polarised-light distribution from information concerning the spectral hue of objects (Wehner and Bernard 1993). Another Phylum of invertebrates showing polarisation sensitivity

is Mollusca, in particular Class Cephalopoda (Rowell and Wells 1961, Jander *et al.* 1963, Tasaki and Karita 1966a,b, Saidel *et al.* 1983, Shashar and Cronin 1996). In short, the mechanism of polarisation sensitivity in invertebrates is always the same and arises as a consequence of the organisation of the photoreceptor cell's rhabdomeric-microvilli (Waterman 1966,1981, Wehner 1989). These microvilli are aligned perpendicular to light entering the ommatidea via the lens. This feature makes them sensitive to plane of linearly polarised light. In addition, these microvilli are positioned into intercalating stacks with alternating layers. Each alternating layer is contributed by one of two members of photoreceptors. Comparison of the photon catch between the two photoreceptors allows the invertebrates to discriminate the angular orientation of the plane of polarised light.

A limited form of polarisation sensitivity has also been described in humans (Hädinger 1844 *cited in* Gerharz 1982, Boehm 1940). It is generally believed that this results from scattering due to the macular pigment overlaying the human fovea (however, see Gerharz 1982). This form of polarisation sensitivity, however, has yet to be demonstrated in other animals. Können (1985) states that cordierite crystals were used for polarised-light navigation by Vikings. Such crystals or sunstones can serve as an analyser for polarised light and can be used to locate the band of maximally-polarised light in the sky.

Not long after the discovery of polarised-light orientation in bees, the first attempts were made to examine the sensitivity of non-human vertebrates to polarised light. Kramer (1950) studying the European starling (*Sturnus vulgaris*)

and Montgomery and Heinemann (1952) studying pigeons (*Columbia livia*), however, failed to show polarisation sensitivity in birds. To my knowledge, the first published observation of polarisation sensitivity in a vertebrate was in the freshwater angelfish (*Pteryophyllum scalare*), actually a cichlid (Waterman 1959). After Waterman's work, a second South American cichlid (*Pseudotropheus macropthalmus*) was shown to have polarisation sensitivity (Davitz and MacKaye 1978). Groot (1965) and Dill (1965, 1971) provided the first experimental evidence of polarisation sensitivity in a salmonid, the sockeye salmon (*Oncorhynchus nerka*), in the smolt stage of their life history. Specifically, they demonstrated that polarised light may be a cue used by salmonids during migration. Kawamura *et al.* (1981) demonstrated that rainbow trout (*O. mykiss*), tilapia (*Sarotherodon mosambicus*), and a carangid (*Trachurus*) could discriminate plane-polarised light, using innate heart rate responses. Similarly, Kleerekoper *et al.* (1973) demonstrated a locomotor response of goldfish (*Carassius auratus*) to linearly-polarised light. This work was followed by a study of the mechanisms contributing to polarisation sensitivity in goldfish (Hawryshyn and McFarland 1987). More recently, Hawryshyn *et al.* (1990) examined menotaxis in rainbow trout. Of particular significance, their studies provided the first tests of the characteristics of the mechanism(s) contributing to polarisation sensitivity, the minimal percent polarisation needed for orientation, and the ontogenetic differences in orientation responses. Parkyn and Hawryshyn (1993, Chapter II) and Coughlin and Hawryshyn (1994a,b) have

provided further evidence of ontogenetic trends and the photoreceptor mechanisms contributing to polarisation sensitivity in rainbow trout.

In contrast to invertebrates, the biophysical basis of polarisation sensitivity in vertebrates is not clearly understood (Hawryshyn 1992, Parkyn and Hawryshyn 1993). Numerous explanations have been invoked to account for polarisation sensitivity in vertebrates (Hawryshyn and McFarland 1987, Hawryshyn 1992, Rowe *et al.* 1994, Novales Flamarique 1997).

In reptiles it was first suggested that the refringent body in the cones of reptiles could act as a structure to scatter light and allow discrimination of the plane of polarised light (Underwood 1968, 1970). In birds, it has been suggested that the oil droplet of the double cone photoreceptor cell acts as a waveguide analyser (Young and Martin 1984). Young and Martin (1984) have presented some interesting explanations based on scattering of light in one of the members of a double-cone pair and dichroic absorption of the light by the second member of the pair. While this explanation may be plausible for many vertebrates, almost all teleost fishes lack such oil droplets (Walls 1942, Ali and Anctil 1976, Robinson 1994).

In fishes, some debate has ensued as to the biophysical basis of polarisation sensitivity. One of the first suggestions came from the observation that the adipose eyelid, a thickened cornea, is birefringent in sockeye salmon (Stewart 1963). However, this issue has not been investigated further in relation to polarisation sensitivity. Birefringent (iridescent) corneas are an almost universal feature of the corneas of the Gobiidae (gobies) and the

Scorpaeniformes (especially greenlings = Family Hexagramidae) (Lythgoe 1971, 1975a,c). The plate-like alignment of crystalline structures in the cornea cause the reflection of light and the splitting of this light into its two component planes of polarisation. One component of the light striking the cornea is reflected by guanine crystals. As a result, one orientation of resulting e-vector polarised light does not enter the eye. Its perpendicular component, however, passes through the cornea, illuminating the retina. Iridescent corneas can therefore act as a polarising reflector and therefore a polarising filter (Lythgoe 1975c). Hence, Lythgoe (1975a,c) believed that the corneas of these fish act like polarised sunglasses, reducing the incident light upon the retina. An additional interesting feature of corneal iridophores is that their ability to scatter light is under physiological control (Shand *et al.* 1990). This allows them to expand or contract depending on light intensity. At present, there is no evidence to support or preclude the idea that such corneal structures, located on the dorsal hemisphere of the eye, could be used as polarisation analysers. I speculate that under this scenario, a goby or greenling viewing up through Snell's window (see Horváth and Varjú 1995), or through the water column, could locate the band of maximally polarised light in the celestial hemisphere as a dark region in the sky. Such a dark region could be distinguished from less polarised regions of the sky with a retina that is pre-adapted evolutionarily for detection of increments and decrements of light. In addition, the lens is another component of the eye that has the potential to affect the polarisation state of transmitted light and hence

could serve as an analyser, at least in salmonids (C. Groot, personal communication). This line of research warrants further investigation.

Other explanations of polarisation sensitivity rely on the organisation of the retina and photoreceptors. The most clear example of this is in anchovies (Engraulidae: *Engraulis* spp.), in which it has been observed that the orientation of the outer segment disks of specialised cones are oriented parallel to light entering the retina and at right angles to each other (Fineran and Nichol 1978). The orientation of the outer segments is exactly perpendicular to the situation in most other vertebrates. Thus, these cones should be differentially-sensitive to different orientations of plane-polarised light (Fineran and Nichol 1978, Novales Flamarique 1997). The long-axis of the photoreceptor opsin molecule, 11-cis-retinal, would be aligned in the same plane as the vibration of the electric vector (e-vector) of some photons (Hawryshyn 1992). When this happens, the molecule absorbs one photon. This phenomenon, termed linear dichroism, was first discovered by Schmidt (1938) and later developed in more detail by Denton (1954) and Hárosi (1975). Under a polarised-light stimulus, the photopigment molecules in the photoreceptors with outer segments aligned in the direction of this plane will absorb more photons relative to cones with other alignments. If opponent connections of the cones exist at some level in the neural architecture of the fish, then it would be hypothesised that the fish should be able to detect the orientation of the maximum plane of polarised light. This hypothesis was investigated recently and evidence supporting polarisation sensitivity in *Engraulis anchoa* was presented (Novales Flamarique 1997). However, the biological

significance of polarisation sensitivity to *Engraulis* (like other fish) has yet to be examined.

Kunz and Callaghan (1989) speculated that UV photoreceptors aligned along the embryonic fissure may be involved in detection of polarised light by salmonids. However, they provided no test of polarisation sensitivity to support or refute their claim. Most recently, Cameron and Pugh (1991), Rowe *et al.* (1994), Novales Flamarique (1997), and Novales Flamarique *et al.* (1998) have presented variations on the Young and Martin (1984) theme using morphological elements of the fish cone, suggesting that double cones may serve as polarised-light analysers in fish. However, if double cones are involved, it is reasonable to speculate that they are not the only photoreceptors involved in detection of polarised light, at least in salmonids (Hawryshyn *et al.* 1990, Parkyn and Hawryshyn 1993, Coughlin and Hawryshyn 1994b, Novales *et al.* 1998). This is based on evidence that the ability to orient to polarised light is lost with the correlated ontogenetic loss of UV photoreceptors (Hawryshyn *et al.* 1990). Concomitantly, vertical polarisation sensitivity disappears with the loss of these same photoreceptors (Parkyn and Hawryshyn 1993, Chapter II). Snyder (1973), however, dismissed the role of double cones in polarisation sensitivity and suggested that rods were involved, but he did not provide a test of his hypothesis. Recently, however, rods have been shown to be insensitive to polarised light (Cameron and Pugh 1991, Parkyn and Hawryshyn 1993, Chapter II). In summary then, the mechanism(s) and biological relevance of polarisation sensitivity in fishes is equivocal and not at all well understood.

Salmon, trout, and char (Subfamily Salmoninae) are a hardy and readily available group of fishes for studies of comparative spectral and polarisation sensitivity. In the first instance, there is a considerable body of evidence documenting the ability of some salmon (e.g., sockeye salmon) (Dill 1965, Groot 1965) and trout (e.g., rainbow trout) (Kawamura *et al.* 1981, Hawryshyn *et al.* 1990) to orient using polarised light. In addition, this group of fishes represents genera, congeners, and conspecifics that often have very different life history traits [see Scott and Crossman (1973) and Groot and Margolis (1991) for reviews]. In particular, some salmonine fishes are known for extraordinary anadromous migrations, while others do not and thus spend their entire life in small streams or lakes. For example, although steelhead (*O. mykiss*) and sockeye salmon are anadromous migratory fish, their respective conspecifics, rainbow trout and kokanee (*O. nerka*), undergo only short-distance breeding migrations and do not enter coastal waters (Scott and Crossman 1973).

The central aim of my dissertation research was to examine the visual biology of fishes in the Subfamily Salmoninae, the salmon, trouts, and chars: 1) to characterise spectral and polarisation sensitivity in Pacific Northwest salmonines, including rainbow trout, steelhead, kokanee, cutthroat trout (*O. clarki clarki*), and brook char (*Salvelinus fontinalis*); and 2) to determine the mechanism(s) and biological relevance of polarisation sensitivity in the Salmoninae with respect to orientation.

Chapter II begins to address the occurrence and mechanism(s) of spectral and polarisation sensitivity in these fishes by examining the physiological

characterisation of UV sensitivity and polarisation sensitivity in a model species, the rainbow trout. This chapter also examines size-related polarisation transmittance of the ocular media of rainbow trout. A portion of the electrophysiology section of this chapter was published in a more preliminary form as Parkyn and Hawryshyn (1993). Chapter III provides a comparative examination of differences in spectral and polarisation sensitivity among the different species and forms of juvenile salmonines, reared under the same conditions. Relative differences in spectral sensitivity are addressed using templates derived from microspectrophotometric (MSP) measurements on rainbow trout (Hawryshyn and Hárosi 1994) that have been corrected for ocular media absorbance. Chapter IV examines the effects of the drug Ethambutol on spectral and polarisation sensitivity in rainbow trout. Understanding the effects of this drug on sensitivity may yield insight into the pathways that trout use to encode this information, as well as the cone mechanism(s) involved in polarisation sensitivity. The biological relevance of polarisation sensitivity in the Salmoninae is addressed in Chapter V using a behavioural study of the orientation of fish under both controlled laboratory conditions and semi-natural (field) conditions. This chapter serves as a link between laboratory studies on orientation (e.g., Dill 1971, Kawamura *et al.* 1981, Hawryshyn *et al.* 1990, Hawryshyn and Bolger 1990) and field-based studies (e.g., Groot 1965). In conclusion, Chapter VI provides a synthesis of preceding chapters and speculates on the most profitable areas for future research.

CHAPTER II

PHYSIOLOGICAL PROPERTIES OF POLARISED-LIGHT SENSITIVITY IN RAINBOW TROUT¹

INTRODUCTION

Polarisation sensitivity has been demonstrated in a diverse group of teleost fishes, using techniques ranging from innate orientation experiments to use of single-unit recording from the optic tectum (Table 1). The presence of polarised-light sensitivity in so many largely unrelated groups suggests that it may be a general feature of the visual system of teleosts. To date, there are two published accounts of fish species lacking polarisation sensitivity (*Lepomis cyanella* and *L. macrochirus*) (Novales Flamarique and Hawryshyn 1997b) and *Catostomus commersoni* (Novales 1997). However, one of these species, *L. cyanella*, has been previously described as having polarisation sensitivity using a Classical (Pavlovian) conditioning methodology (Cameron and Pugh 1991).

In spite of the number of studies since the 1960's, the mechanism by which vertebrates detect the plane of polarised light (e-vector) is not well understood (Forward and Waterman 1972, Waterman and Forward 1972, Hawryshyn 1992, Parkyn and Hawryshyn 1993). Hypotheses dealing with the biophysical basis of polarisation sensitivity in animals other than

¹ A portion of this paper was previously published as Parkyn and Hawryshyn (1993).

Table 1. Studies of fishes demonstrating polarised light sensitivity.

Family	Technique	Study
Carangidae	Innate heart-rate responses	Kawamura <i>et al.</i> (1981)
Centrarchidae	Heart-rate conditioning	Cameron and Pugh (1991)
	Optic ganglion cell responses	Novales Flamarique and Hawryshyn (1997b)
Cichlidae	Innate heart-rate responses	Kawamura <i>et al.</i> (1981)
	Operant conditioning	Davitz and MacKaye (1978)
Cyprinidae	Single-unit recording	Waterman and Hashimoto (1974)
	Single-unit recording	Waterman and Aoki (1974)
	Heart-rate conditioning	Hawryshyn and McFarland (1987)
	Innate orientation	Kleerekoper <i>et al.</i> (1973)
Engraulidae	Optic ganglion cell responses	Novales Flamarique (1997)
Hemiramphidae	Innate orientation	Forward and Waterman (1972)
	Innate orientation	Forward <i>et al.</i> (1972)
Salmonidae	Innate responses	Groot (1965)
	Heart-rate conditioning	Dill (1971)
	Innate responses	Kawamura <i>et al.</i> (1981)
	Operant conditioning	Hawryshyn <i>et al.</i> (1990)
	Operant conditioning	Hawryshyn and Bolger (1990)
	Optic ganglion cell responses	Parkyn and Hawryshyn (1993)
	Single-unit recording	Coughlin and Hawryshyn (1995)
	Optic ganglion cell responses	Novales Flamarique and Hawryshyn (1996)
	Optic ganglion cell responses	Present study

invertebrates and salamanders have limited experimental support (Hawryshyn 1992). These hypotheses include birefringence, refraction, reflection (Stewart 1962, Underwood 1968, Gerharz 1982, Novales Flamarique *et al.* 1998), wave guiding (Young and Martin 1984, Cameron and Pugh 1991, Rowe *et al.* 1994), and dichroism (Fineran and Nichol 1978, Novales Flamarique 1997). The cellular basis of polarisation sensitivity in invertebrates is inherent to their unique structural organisation (Wehner 1983), as discussed in Chapter 1. The mechanism mediating polarisation sensitivity in salamanders, the perpendicular orientation of pineal photoreceptors to incident light (Taylor and Adler 1973), was tested for its applicability in rainbow trout by Hawryshyn *et al.* (1990). By placing a mask over the pineal organ of rainbow trout, Hawryshyn *et al.* (1990) found that it did not mediate their polarisation sensitivity.

Regardless of the mechanism, one feature common to current models of polarisation sensitivity in fishes is the presence of orthogonally-opposing e-vector sensitive photoreceptors (Hawryshyn and McFarland 1987, Hawryshyn *et al.* 1990, Cameron and Pugh 1991, Hawryshyn 1992, Novales Flamarique and Hawryshyn 1997b). At the level of the neuron, such a model resembles the two-channel system first developed by Waterman (1966) and Waterman and Horch (1966) for invertebrates. Indeed, multi-unit recording from ganglion cells of the optic nerve and single-units from both the *torus semicircularis* and the optic nerve support this view of a dual channel system in fish (Parkyn and Hawryshyn 1993, Coughlin and Hawryshyn 1994b, 1995).

Not surprisingly, the roles of polarised light in the behaviour of fish are also not well understood. However, it is known that rainbow trout and other salmonids can make directed movements under a downwelling, polarised-light field (Groot 1965, Kawamura *et al.* 1981, Hawryshyn and Bolger 1990, Hawryshyn *et al.* 1990), but only if the photic environment includes UV (Hawryshyn *et al.* 1990). Elimination of longer wavelengths of light did not significantly decrease this ability (Hawryshyn *et al.* 1990). Conversely, removal of the UV portion of the spectrum seriously impaired the ability of the fish to orient. This indicated that perception of the plane of polarisation involves input of one or more types of UV-sensitive photoreceptors. On the basis of these findings, the present study was initiated to examine the neural basis for polarisation sensitivity in rainbow trout and to determine which photoreceptor mechanisms participate in polarisation sensitivity.

The first objective of the present study is to provide a characterisation of the polarisation-sensitive mechanisms in rainbow trout, a species known to display polarisation sensitivity (Groot 1965, Dill 1965, 1971, Kawamura *et al.* 1981) and e-vector orientation (Hawryshyn *et al.* 1990, Hawryshyn and Bolger 1990). Polarisation sensitivity of the on- and the off-response channels were examined by measuring multi-unit ganglion cell responses (GCR's) from axons in the optic nerve. Chromatic adaptation was used to identify the contribution of individual types of cone photoreceptors to polarisation sensitivity (Yager 1969, Beauchamp *et al.* 1979, Hawryshyn and McFarland 1987, Hawryshyn *et al.*

1989). Multi-unit on-responses of rainbow trout were first presented by Parkyn and Hawryshyn (1993) and resemble conditioned-behavioural responses from goldfish (*Carassius auratus*) (Hawryshyn and McFarland 1987). Several studies on spectral sensitivity have briefly discussed the role of off-responses as a shadow detector for brightness contrast functions (Wheeler 1982, Beaudet *et al.* 1993, McDonald and Hawryshyn 1995). However, polarisation sensitivity of off-responses have only been examined once previously, using single-unit recording (Coughlin and Hawryshyn 1995).

As a second objective, larger-size smolts were examined to determine if size and developmental status affects polarisation sensitivity, as suggested by Hawryshyn *et al.* (1990). During the process of growth, juvenile salmonids undergo a suite of developmental changes termed smoltification (Hoar 1989). Related to smoltification is a change in the retinal photoreceptor mosaic, which causes a decrease or loss of the accessory-corner cones or UV-cones (Lyll 1957, Bowmaker and Kunz 1987, Kunz 1987, Browman and Hawryshyn 1992, Hawryshyn and Hárosi 1994, Kunz and Callaghan 1989, Kunz *et al.* 1994). Therefore, this study also addressed whether physiological changes in polarisation sensitivity underlie observed decreases in behavioural responsiveness of *O. mykiss* to the orientation of polarised light by Hawryshyn *et al.* (1990).

As a third and final objective, polarised-light transmission of the ocular media was examined as a function of body weight. The effects of fish size on

spectral transmission of ocular media are known for trout (McCandless *et al.* 1969, Hawryshyn *et al.* 1989, Thorpe and Douglas 1993). However, to date, the effects of fish size on transmission of polarised light in the eye are unknown. Studies of the transmission of polarised light as a function of wavelength have also not been undertaken in fish of any size or age. Jagger (1996) stated that the lens of the trout eye transmits polarised light well. Although this may be true, empirical evidence to date is entirely lacking. It has been hypothesised by some researchers that changes in wavelength-specific transmission of light may account for size/age-related differences in spectral sensitivity, especially in the UV region (Thorpe and Douglas 1993). It was therefore important to understand whether growth-related changes might also affect polarisation transmission, and thus also affect wavelength-specific polarisation sensitivity. This paper is the first study to address these issues.

METHODS

Polarised-light Sensitivity

Study Animals

Non-anadromous (Badger Lake, British Columbia), wild-stock rainbow trout, 8-10 g body weight, were obtained from the British Columbia Ministry of Environment's Fraser Valley Hatchery, Abbotsford, British Columbia, Canada. Fish were fed Biodiet Grower[®] (Bioproducts Inc., Warrenton, Oregon) every

second day in the fish-holding facility at the University of Victoria. Fish were held under a 12-h dark:12-h light photoperiod and a mean water temperature of $15 \pm 1^\circ\text{C}$ for a minimum of 8 weeks prior to the initiation of the study. The photic conditions during rearing were broad spectrum (fluorescent lights), containing wavelengths from 360-700 nm. Two size-classes of fish, 9-14 g (small), and 70-100 g (large), were examined to assess the effect of size and hence, developmental status on e-vector sensitivity. Typically, rainbow trout at sizes greater than 30g undergo a suite of morphological and physiological changes. Hoar (1976) termed these changes "pseudomoltification". At this point UV photoreceptors appear to largely lost from the retina (Hawryshyn *et al.* 1989, Browman and Hawryshyn 1992, Beaudet *et al.* 1993).

Photopic experiments were conducted between 1000 h and 2000 h to obviate possible variability resulting from diel retinomotor movements (Ali 1975, LaVail 1976, Douglas and Wagner 1982). Similarly, a scotopic-vision experiment was conducted between 2200 h and 0200 h to correspond with the time period during which the fish were normally dark-adapted.

Preparation

Fish were immersed in a $100 \text{ mg}\cdot\text{l}^{-1}$ solution of tricaine methanesulfonate (MS-222) to Stage 4 anaesthesia (Jolly *et al.* 1972), and total length and weight were recorded. Fish were then administered intramuscular injections of an immobilising agent (Pavulon®, $0.0005 \text{ mg}\cdot\text{g}^{-1}$ body weight) at several sites,

restrained, and irrigated with a $20 \text{ mg}\cdot\text{l}^{-1}$ solution of tricaine at $300 \text{ ml}\cdot\text{min}^{-1}$ at 15°C . After 10 min, the skin overlying the cranium and the right frontal bone was removed to expose the right optic tectum and provide access to the optic nerve. A local-anaesthetic salve (Pontocaine®) was applied to the remaining exposed bone. Following completion of an experiment, the fish was sacrificed by a brain and spinal pith. All procedures and care of the animals in this study were in accordance with the guidelines of the Canadian Council for Animal Care.

Apparatus

Prepared fish were placed in a Faraday cage (Fig. 1) and irrigated with 15°C water at $240 \text{ ml}\cdot\text{min}^{-1}$. Relative threshold of individual photoreceptor mechanism were manipulated using chromatic adaptation from two background channels. Background lighting conditions were produced using 250 W Tungsten-Halogen (EJH Spectro) lamps and longwave- and shortwave-pass interference filters (Corion®). Liquid-filled light-guides permitted two background channels of different spectral composition to be simultaneously superimposed onto the eye. Coloured backgrounds were used to isolate each of the four cone mechanisms [UV, short wavelength (S), middle wavelength (M), and long wavelength (L)] (Fig. 2) following Beauchamp *et al.* (1979) and Hawryshyn *et al.* (1989). Inconel-coated neutral-density filters (Corion®) were used to control background-channel intensity. Fish were adapted to the background conditions for 60 min prior to sampling of e-vector sensitivity. Liquid-filled, UV-transmissive

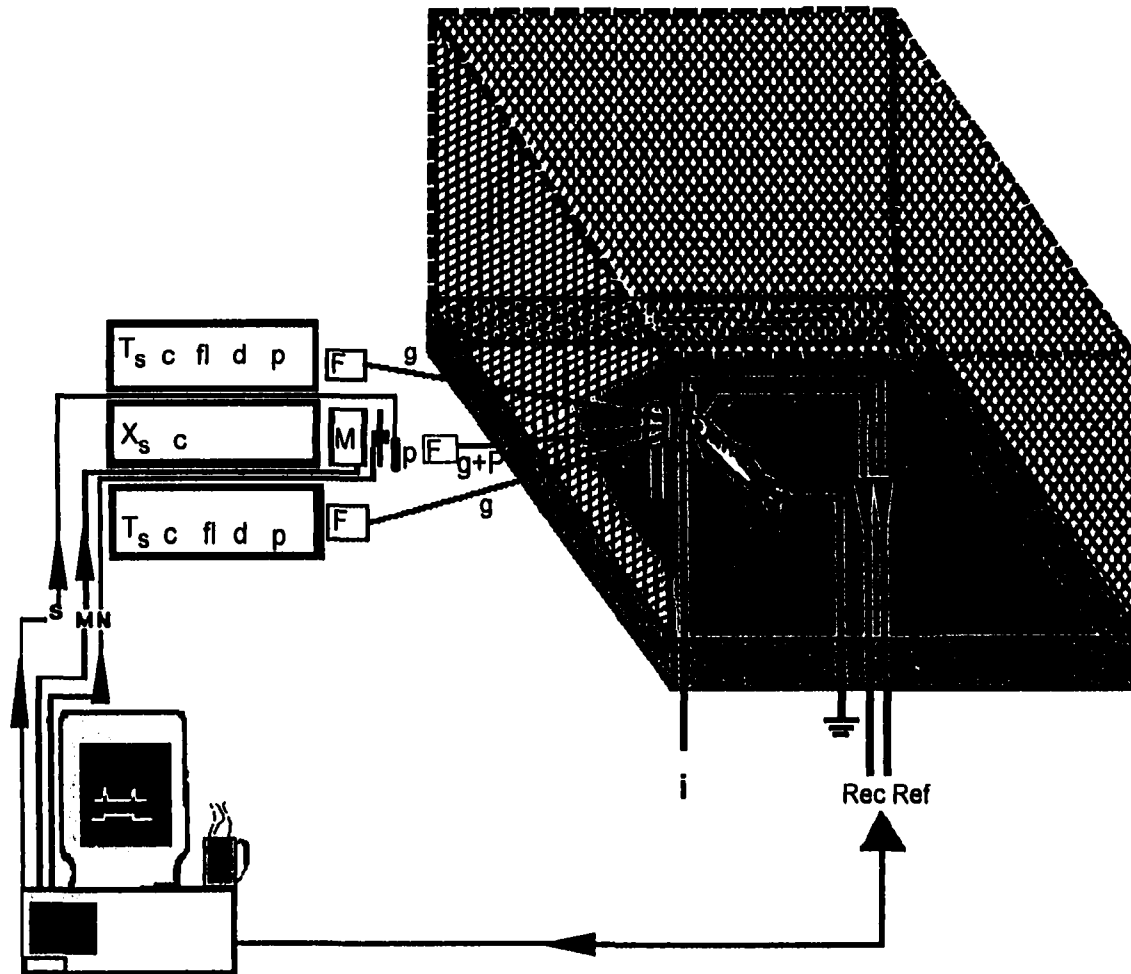


Fig. 1. Schematic of the apparatus for recording multi-unit ganglion cell responses (GCRs) from the optic nerve: c = condenser lens, d = diaphragm, F = filter tray, fl = field lens, g = light guide, i = irrigation system, M = monochromator, N = neutral density wedge, p = projection lens, P = polarising filter (on end of light guide), Rec = recording electrode, Ref = reference electrode, s = electronic shutter, T_s = tungsten lamp, and X_s = xenon lamp.

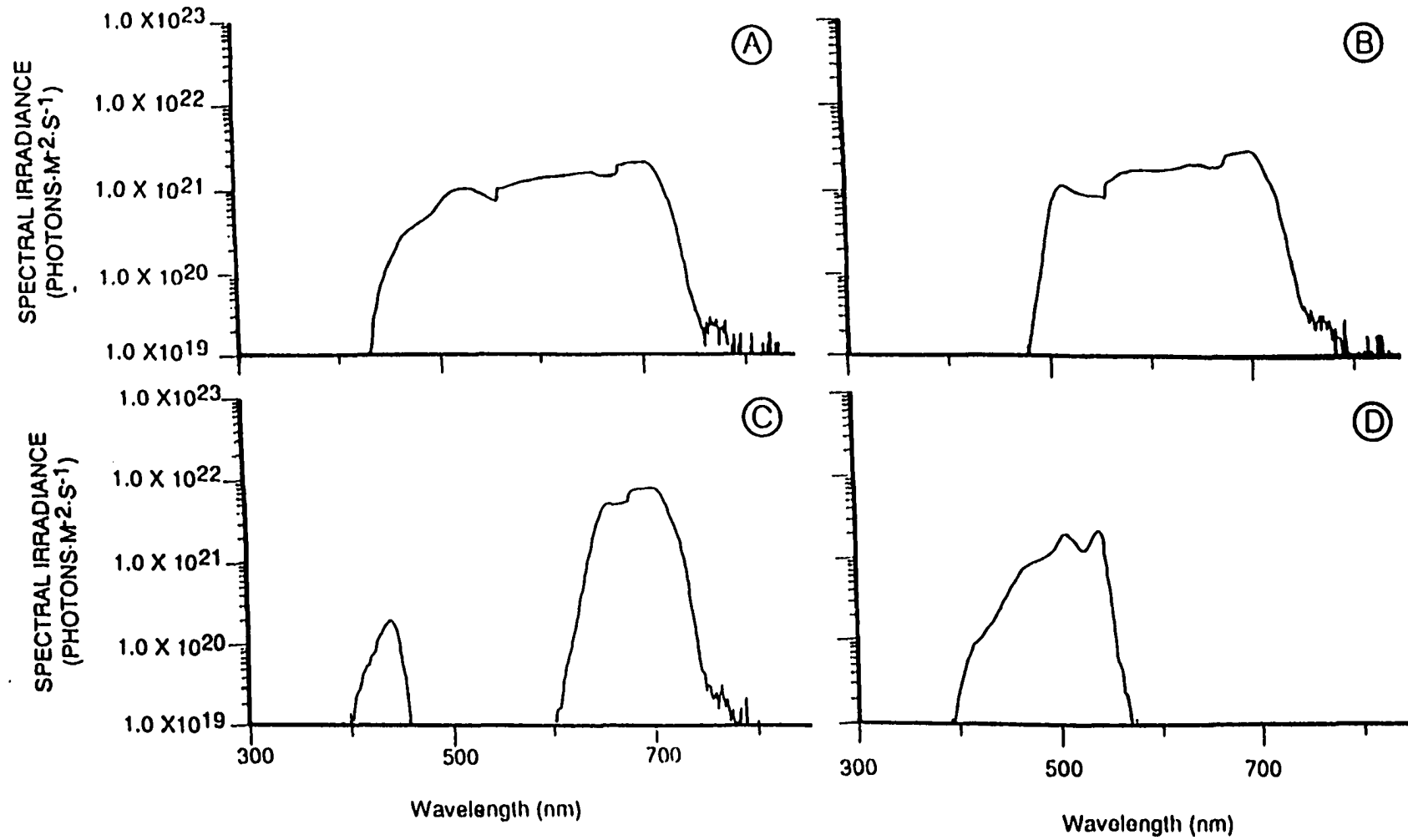


Fig. 2. Photic conditions of background channels used to isolate cone mechanisms, as measured by a model Li1800 UW spectroradiometer (LiCor): A) UV-cone isolation; B) S-cone isolation; C) M-cone isolation; and D) L-cone isolation.

optical guides were used to project light from a remote three-channel optical system. The light-pipe from the Xenon-stimulus channel was used to project light through a UV-grade linear-polarising filter (Polaroid HNP'B) to cover the entire eye (Fig. 1). To ensure that the polarised-light field was illuminating the ventral surface of the retina in a manner similar to the downwelling light in behavioural experiments, the lenses of the light-guides were positioned 2.5 cm from the left eye of the fish and at a 20° angle above the horizontal midline of the pupil to project the stimulus and background channels onto the ventral surface of the retina. A 750 msec light stimulus, controlled by an electronic shutter (Uniblitz®), was projected from a 300 W Xenon lamp (Oriol®). Sampling of GCR's was at 20 sec intervals and was controlled by the experimenter through a microcomputer interface. The wavelength of the stimulus beam was controlled by an interference monochrometer (SA Instruments) and the intensity of the stimulus (irradiance) was controlled by a neutral density wedge (Optikon®). The plane of polarisation was manipulated from 0° to 180° , in 30° increments, where 0° and 180° represent vertically-polarised light fields, and 90° represents a horizontal orientation. To eliminate the potential of intensity differences at different positions of the polariser (Coemans *et al.* 1990, Martin 1991), the result of interaction of the filter and inherent polarisation of the optical system, stimulus intensity was calibrated at each angular position of the polariser for each set of stimulus conditions (Hawryshyn and McFarland 1987) using a Photodyne

radiometer. In addition, a diffuser was placed between the exit of the light pipe and the polariser to remove any inherent polarisation in the optical system generated by the liquid-light pipes.

Extracellular recording of light-evoked potentials from the optic nerve has been used by Wheeler (1979a,b) and DeMarco and Powers (1991) to assess spectral sensitivity in goldfish. However, recordings from suction electrodes, such as those used by DeMarco and Powers (1991), have the potential to be highly variable as a result of experimentally-induced anoxia within the electrode, making quantitative analysis and interpretation difficult (Stys *et al.* 1991).

Therefore, electrodes were made from a sharpened 0.15-mm diameter Ag_(s) Teflon-coated wire (0.5 mm tip exposure). The recording electrode was inserted into the lumen of the optic nerve near the juncture of the nerve and the optic tectum. A reference electrode was then placed in the right naris. The ground wire was affixed to the fish at the caudal peduncle (Fig. 1). The signal was amplified through a Grass Instrument P5 pre-amplifier (3-Hz low frequency filter and 300-Hz high frequency filter) and exported to the computer via an A/D port for on-line analysis, display, and storage using ASYST® (Keithly-Asyst Software).

Analyses

Three GCR's were recorded and averaged for each intensity to reduce spurious noise (Fig. 3A). The amplitude of the GCR's was plotted against increasing irradiance to generate an intensity/response curve (Fig. 3B). A third-

order polynomial was generated to fit the data. Threshold intensity was determined at the criterion response level of 30 μV using the equation for the third-order polynomial. Sensitivity was then determined as the reciprocal of irradiance at the criterion response level for a given angle of e-vector. The \log_{10} of the sensitivity values were normalised to generate relative sensitivity versus e-vector orientation curves. Data were fitted using weighted least-squares regression (Axum 5.0, Zar 1996). Data were analysed with a one-way ANOVA with repeated-measures to examine within-subjects effects (ANOVAR) (Norušis and SPSS Inc. 1993, Zar 1996). This procedure tested the null hypothesis (H_0) that sensitivity did not differ as a function of different angular orientations of a polariser. Significance of all statistical tests in this study were assigned a criterion level of $\alpha = 0.05$.

Polarisation Transmission of Ocular Media

A randomly selected eye was dissected following euthanasia of rainbow trout of varying sizes, ranging from 5-372 g. The intact eye was rinsed in Ringer's solution (Russell 1990) to remove excess mucus and blood. The sclera at the back of the eye was removed, along with the pigmented epithelium, and the retina. The posterior (open) portion of the eye was placed in a carefully-fitted holder against a quartz coverslip (Fig. 3). If the eye was smaller than the

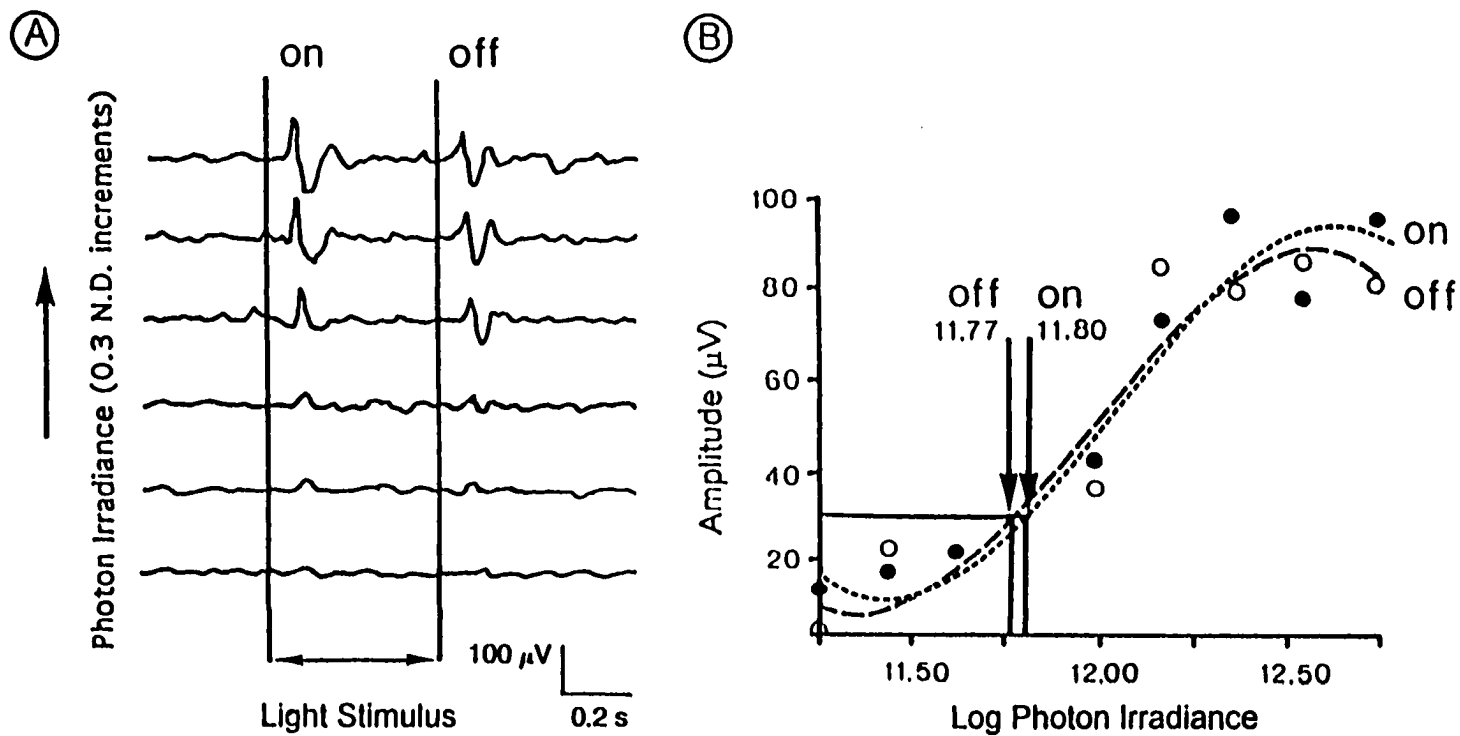


Fig. 3. A) Increases in amplitude of on- (solid lines) and off-responses (dashed lines) recorded from the optic nerve ganglion cells with increasing photon irradiance; and B) A plot of amplitude of response against intensity of stimulus. Intensity of light at threshold ($30 \mu\text{V}$) is indicated by the intersection of the slope and the *solid black lines*, as indicated by the *arrows* (on-response = 11.80, off-response = 11.77).

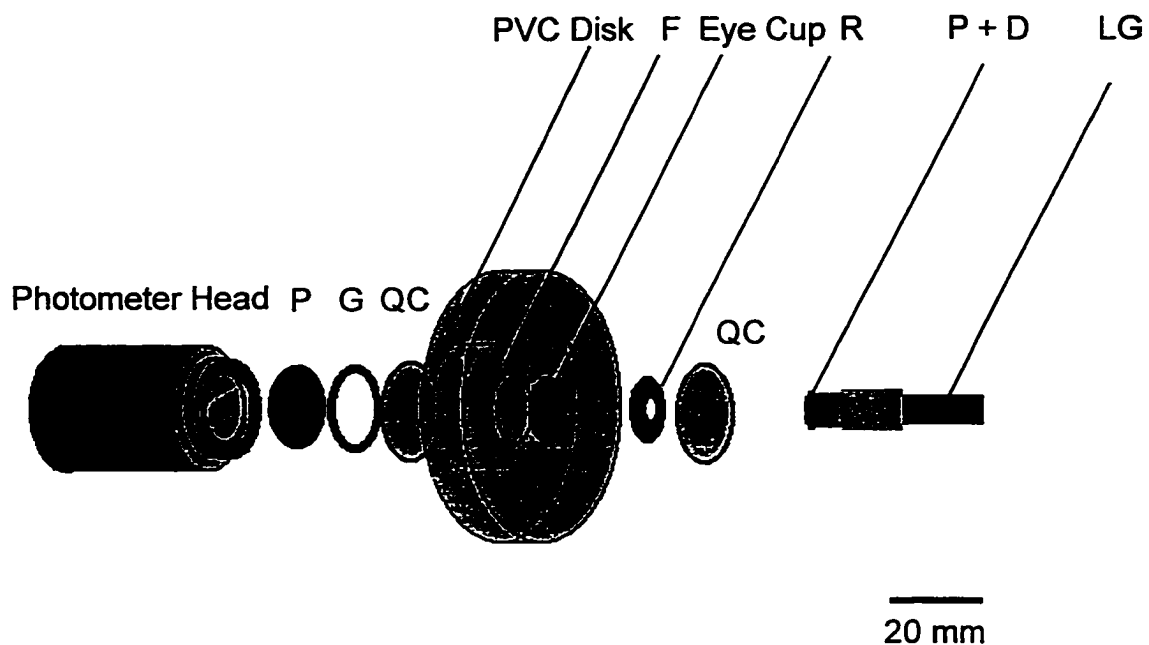


Fig. 4. The apparatus for measuring polarisation transmission properties of the ocular media of the trout eye. D = Albanene diffuser, Eye cup = sample holder, F = threaded flange, G = rubber gasket, LG = liquid light guide, P = HNP'B (Polaroid) polarising filter, PVC Disk = body of apparatus, QC = quartz cover slip, Photometer Head = detector head of photodyne radiometer, R = black rubber eye retaining ring/light baffle.

diameter of the sample holder, the space between the eye and the holder was filled with chilled Vaseline. This was extruded from a syringe as a ring around the eye. A black, non-transmissive retainer ring was placed on the iris to hold the eye in the holder and to serve as a barrier to stray light. This barrier ensured that light striking the photodiode could enter the eye only through the aperture of the pupil. The anterior portion of the sample holder was then filled with water. A quartz coverslip was attached with a ring of Vaseline over the sample cup. Two polarisers (a polariser and an analyser) were used to assess the optical properties of the preparation. One polarising filter (HNP'B, Polaroid) was placed over the window of a photometer to serve as an analyser (Photodyne, Optikon®). The eyeholder was then placed on end of the detector over the photodiode and the polariser. A diffuser (Albanene®) was placed on the stimulus-channel light guide to diffuse any inherent polarisation. A polariser (HNP'B, Polaroid) was placed over this diffuser. The polariser was rotated through 360° at each of six wavelengths (360, 380, 440, 540, 620, and 660 nm). These wavelengths correspond to the maximum sensitivity of each of the four photopic mechanisms currently known for rainbow trout (Hawryshyn *et al.* 1989, Hawryshyn and Hárosi 1994, Beaudet *et al.* 1993, present study). The maximal and minimal light transmitted through the eye was recorded and the % polarised-light transmittance ($\%P_{tran}$) was calculated following Hawryshyn and Bolger (1990):

$$[2] \quad \%P_{tran} = \frac{\Pi - \perp}{\Pi + \perp} * 100$$

where Π = intensity of light at λ_n with the polariser and the analyser parallel, and \perp = Intensity of light at λ_n with polariser and the analyser perpendicular.

The $\%P_{tran}$ was also determined for an HNP'B polarising filter in the absence of an eye. The values obtained from these measurements were used to correct for wavelength specific differences in the $\%P_{tran}$ of the polarising filter by dividing $\%P_{tran}$ of the eye at a particular wavelength by the $\%P_{tran}$ of the polarising filter at the same wavelength and multiplying by 100.

Prior to statistical examination of $\%P_{tran}$, the data were arcsine-transformed to correct for non-normality (Zar 1996). An ANOVAR, (Norušis and SPSS Inc. 1993) was then used to test the H_0 that $\%P_{tran}$ did not differ between fish of two size categories: <30 g and >30 g. This break point in the data was used because it has been observed previously that fish <30 g can orient to plane-polarised light, whereas larger fish (>30 g) cannot (Hawryshyn *et al.* 1990). Thus, this test examines whether a loss of orientation behaviour might be attributable to a change in $\%P_{tran}$ of the eyes in small versus large fish. In addition, differences in $\%P_{tran}$ at different wavelengths were tested. Finally, the statistical interaction of the wavelength and size of fish on $\%P_{tran}$ was tested.

RESULTS

E-vector Sensitivity of Rainbow Trout Parr under Photopic Conditions

White-light Background Adaptation

On-responses

An incremental UV (380 nm) stimulus superimposed on a white background yielded a bimodal response for the on-response with two maxima at 90° (horizontal) and 0/180° (vertical), and two minima at 30° and 150° (Fig. 5). The resultant W-shaped function had a maximum difference in sensitivity of 0.41 of a log unit. Sensitivity differed significantly with angle of orientation of the polariser (One-way ANOVA: $F_{0.05, 72} = 6.73$, $P < 0.001$, $n=12$).

Off-responses

In contrast to the on-responses, off-responses yielded a sensitivity with a single maximum at 90° and two minima at 0° and 180° (Fig. 5). The bell-shaped response of the off-response manifested a difference in sensitivity of 0.33 of a log unit. The sensitivity of the on-responses differed significantly with angle of orientation of the polariser (One-way ANOVA: $F_{0.05, 72} = 10.95$, $P < 0.001$, $n=12$).

Chromatic Adaptation

On-responses

In contrast to observations made under a white background, the on-responses of

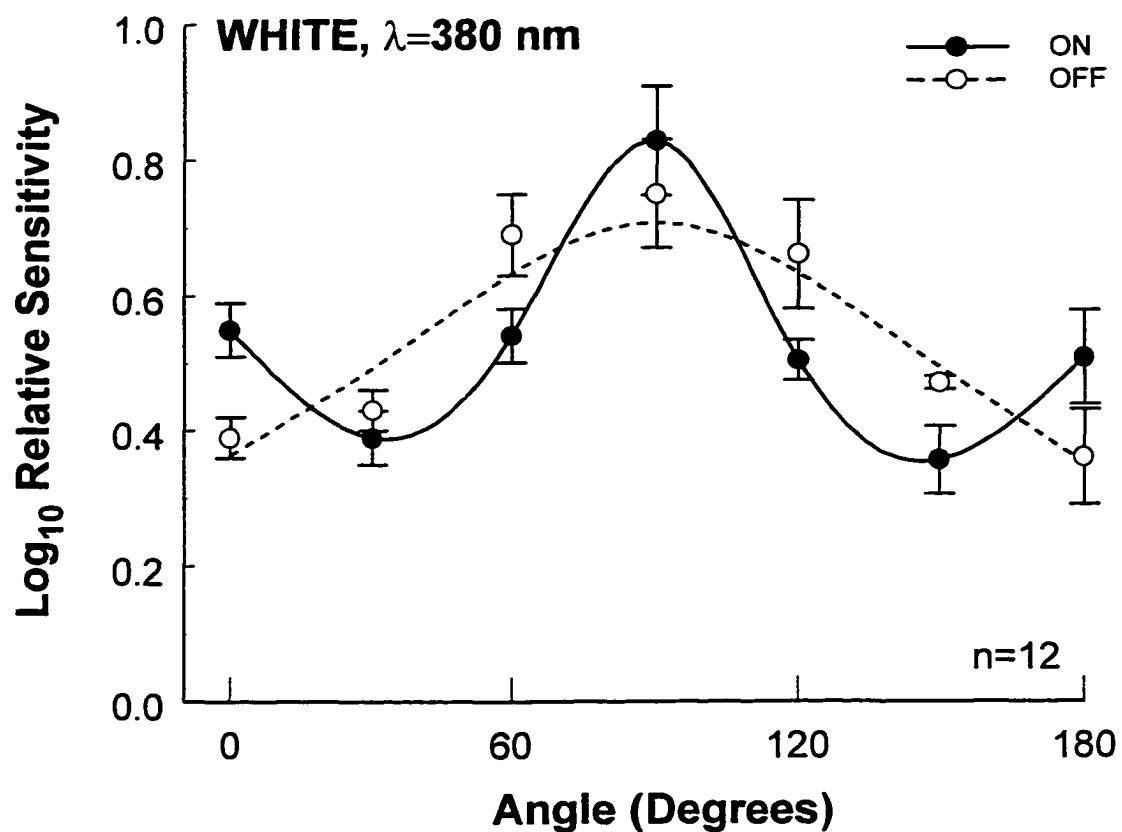


Fig. 5. Relative sensitivity of on- (solid line) and off-responses (dashed line) in rainbow trout parr (<30 g) to changes in orientation of linearly-polarised light under photopic conditions without chromatic adaptation, as measured using compound ganglion cell responses. Error bars represent ± 1 S.E.

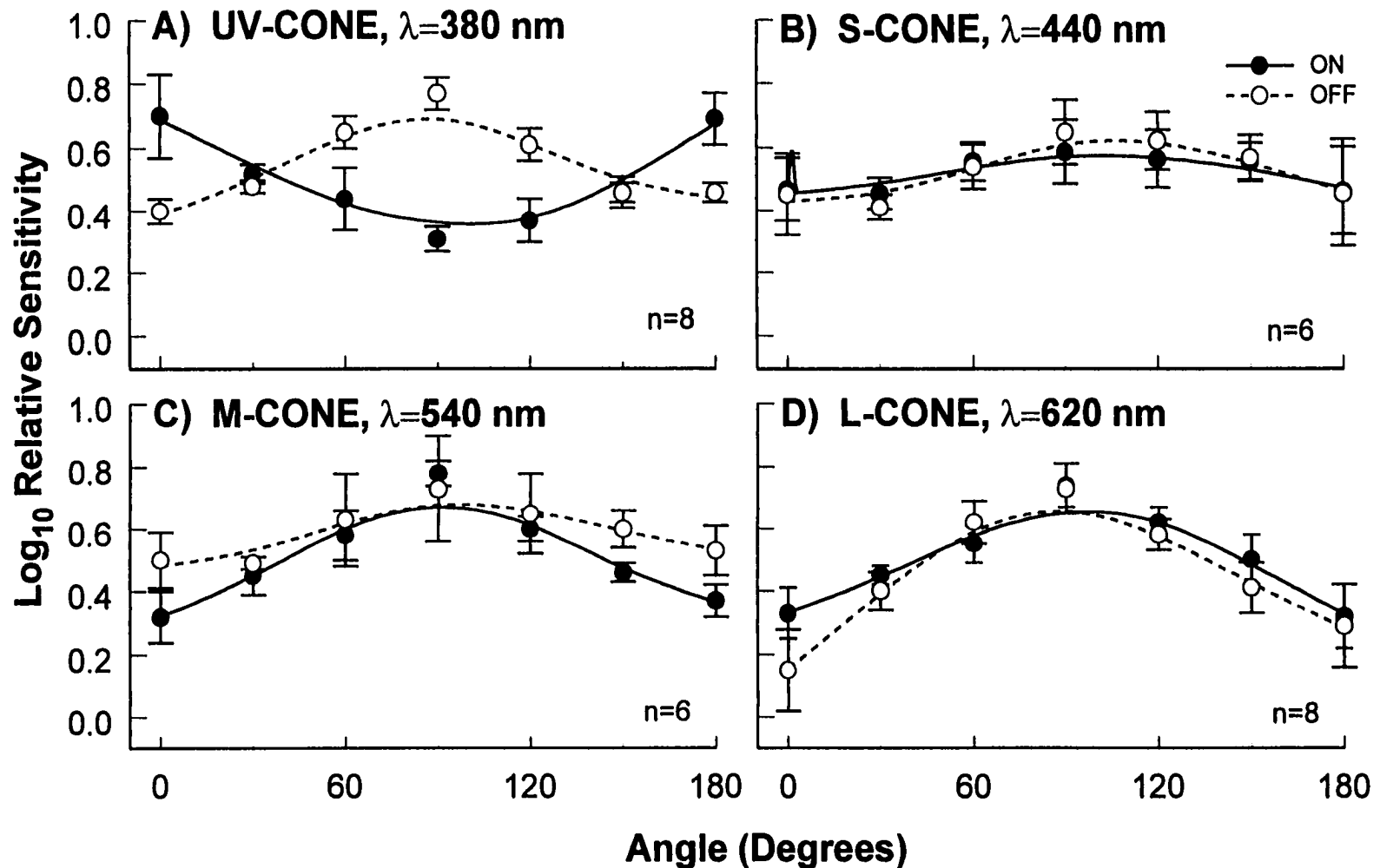


Fig. 6. Relative sensitivity of on- (solid lines) and off-responses (dashed lines) in rainbow trout parr (<30 g) to changes in orientation of linearly-polarised light under photopic conditions with chromatic adaptation, as measured using compound ganglion cell responses: A) UV-cone isolation, stimulus = 380 nm; B) S-cone isolation, stimulus = 440 nm; C) M-cone isolation, stimulus = 540 nm; and D) L-cone isolation, stimulus = 620 nm. Error bars represent ± 1 S.E.

UV-, M-, and L- cones to a series of incremental stimuli near the λ_{max} of their corresponding pigments demonstrated a unimodal maximum sensitivity (Fig. 6). However, ϕ (the e-vector orientation of the stimulus eliciting the maximum response from a given cone mechanism, which equals the acrophase of the function) differed between the isolated UV-cone mechanism ($0^\circ/180^\circ$ = vertically sensitive) (Fig. 6A) and the M- and L- cones (90° = horizontally sensitive) (Fig. 6C and 6D, respectively). Sensitivity differed significantly as a function of the angular orientation of the polariser (One-way ANOVA: $F_{0.05, 54} = 3.56$, $P = 0.005$; $F_{0.05, 30} = 7.16$, $P < 0.001$; and $F_{0.05, 42} = 3.48$, $P = 0.007$, respectively). Isolated M- and L- cones showed the same ϕ when presented with a UV stimulus (Fig. 7A and 7B, respectively). Unlike the other cone mechanisms, the S-cones showed no relationship between sensitivity and e-vector orientation (One-way ANOVA: $F_{0.05, 36} = 1.44$, $P = 0.232$) (Fig. 6B).

Off-responses

Off-responses under all chromatic adaptation conditions were unimodal (Fig. 6 A-D, Fig. 7 A-B) and similar to the on-responses observed for the M- and L-cone mechanisms (Fig. 6 C-D). As with on-responses, sensitivity of the off responses of the UV and L-cones differed significantly with the angular orientation of the polariser (One-way ANOVA: $F_{0.05, 42} = 13.63$, $P < 0.001$; and $F_{0.05, 42} = 8.02$, $P < 0.001$, respectively) (Fig. 6A, 6D). In contrast, the sensitivity of the M-cones did not differ as a function of polariser orientation (One-way

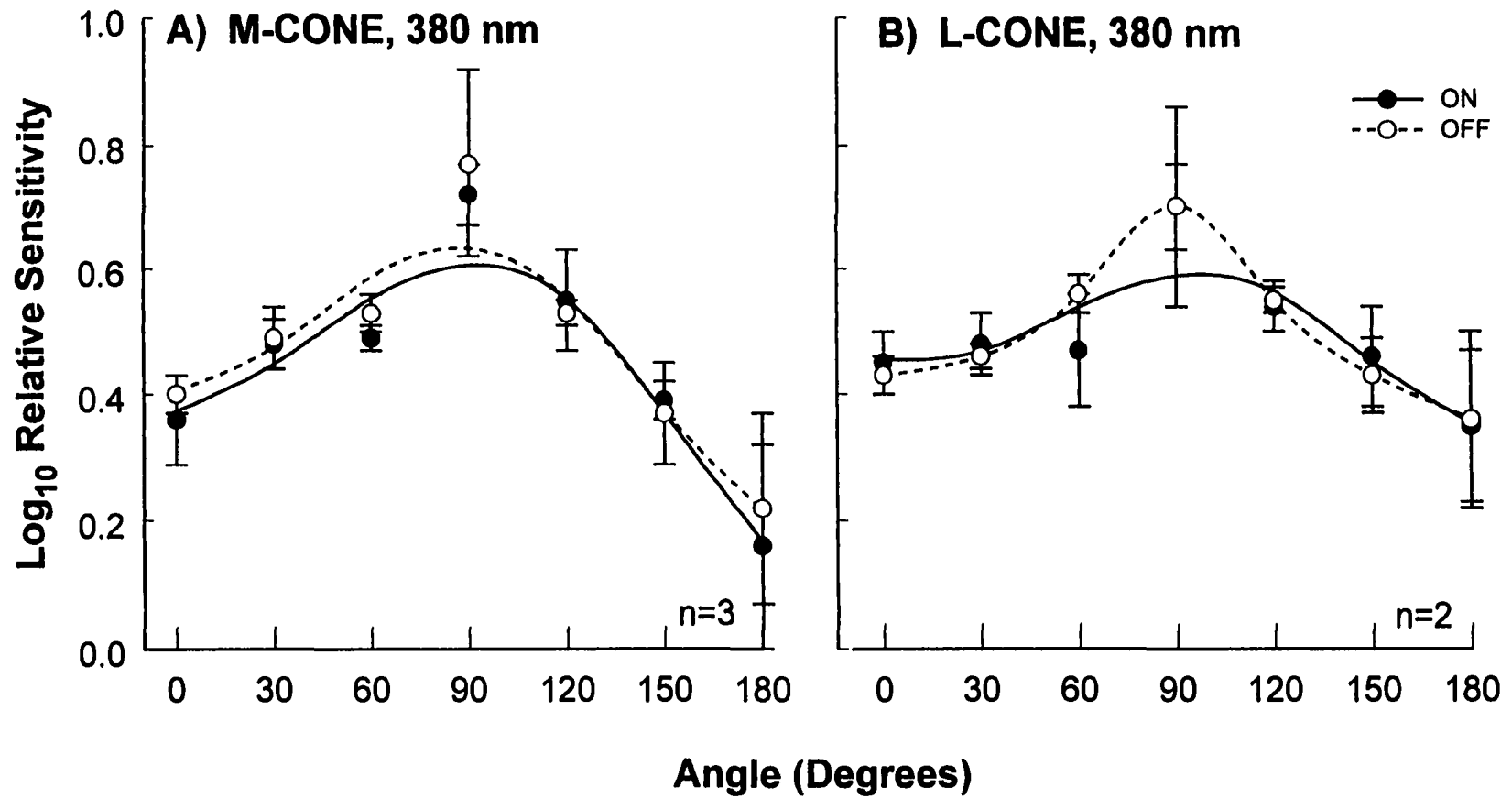


Fig. 7. Relative sensitivity of on- and off-responses in rainbow trout parr (<30 g) to changes in orientation of linearly-polarised UV light (380 nm) under: A) M-cone isolation conditions; and B) L- cone isolation conditions, as measured using compound ganglion cell responses.

ANOVAR: $F_{0.05, 30} = 1.84$, $P = 0.124$) (Fig. 6C). Similarly, the S-cones also showed no relationship between sensitivity and orientation of the polariser (One-way ANOVAR: $F_{0.05, 26} = 0.35$, $P = 0.904$) (Fig. 6B).

Scotopic Sensitivity

No differences were observed in the relative sensitivity of the on-response in rods with changes in the plane of polarisation (Fig. 8). This indicated that rods lack polarisation sensitivity. Variability in the response versus intensity functions of the off-response prevented an accurate determination of off-response sensitivity.

Size-related Changes in e-vector Sensitivity

On-responses

No polarisation sensitivity was observed among the different orientations of the polariser for the on-responses of both the UV- and S-cones in fish >30 g (Fig. 9A and 9B, respectively). This indicated that there was a loss of polarisation sensitivity under UV-cone isolation conditions and a continued lack of polarisation sensitivity in the S-cones in larger fish. In contrast, the M- and L-cone mechanisms exposed to a 540 nm and a 620 nm stimulus, respectively, had a ϕ (Fig. 9C and 9D, respectively) consistent with that observed in smaller fish (Fig. 6C and 6D, respectively). This indicated that there was no loss of polarisation sensitivity of the on-response of these cone mechanisms with an increase in size of fish.

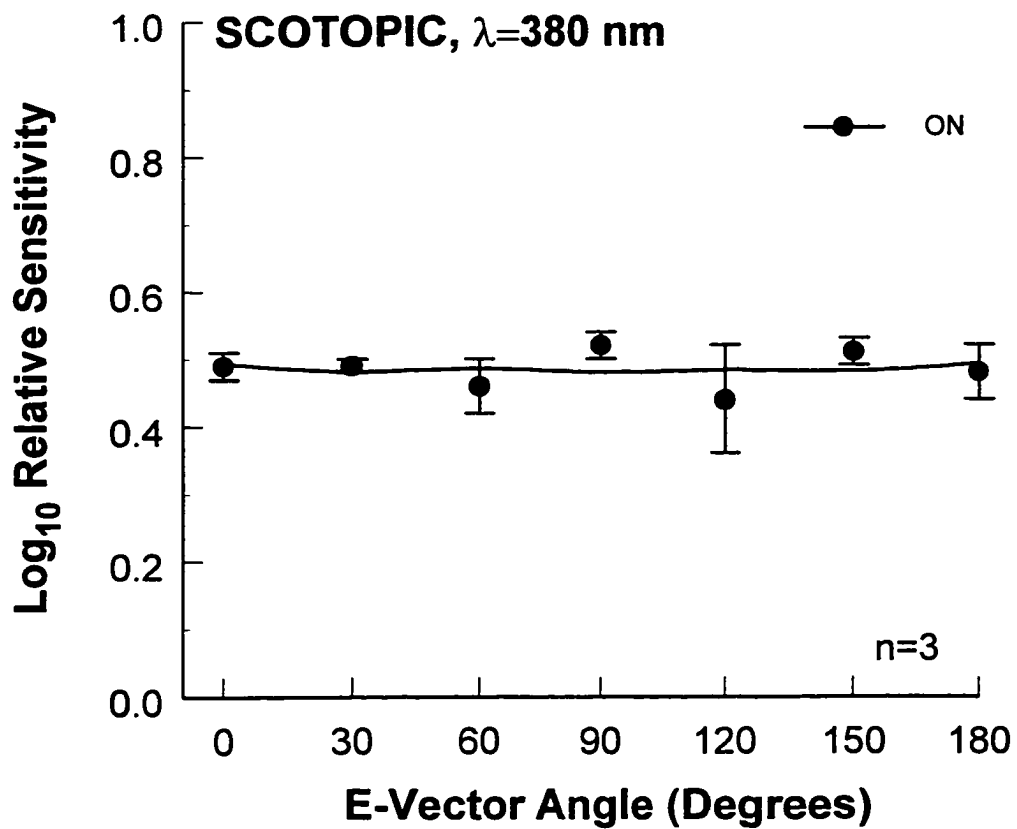


Fig. 8. Lack of polarisation sensitivity of rainbow trout parr (<30 g) with changes in orientation of linearly-polarised light under scotopic conditions, as measured using multi-unit ganglion cell responses.

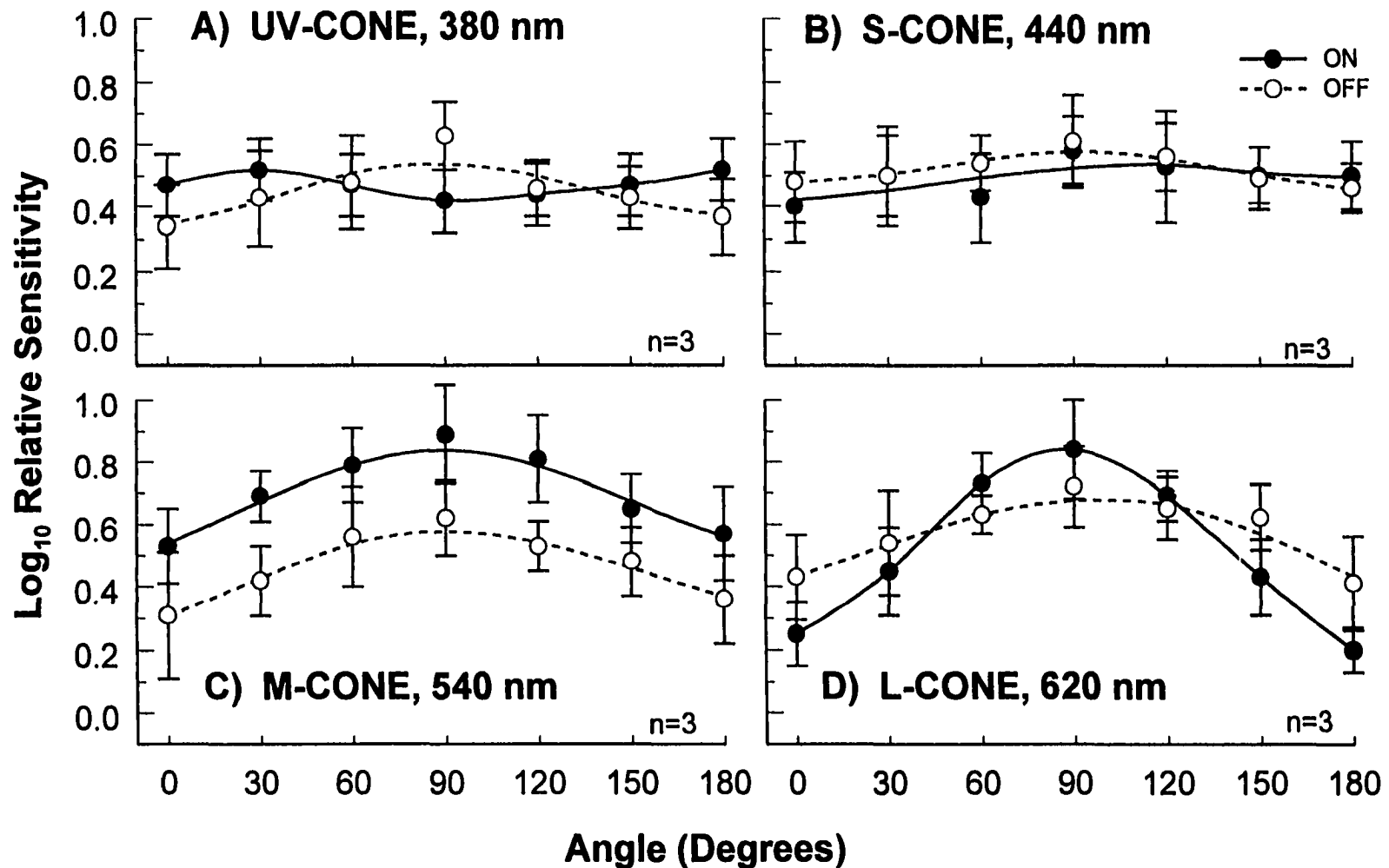


Fig. 9. Relative sensitivity of on- and off-responses in large (>30 g) rainbow trout to changes in orientation of linearly-polarised light under photopic conditions with chromatic adaptation, as measured using multi-unit ganglion cell responses: A) UV-cone isolation, stimulus = 380 nm; B) S-cone isolation, stimulus = 440 nm; C) M-cone isolation, stimulus = 540 nm; and D) L-cone isolation, stimulus = 620 nm. Error bars represent ± 1 S.E.

Off-responses

As with the on-responses of the M- and L-cones, the sensitivity of the off-responses of the UV-, M-, and L-cones with changes in orientation of the polariser in larger fish were unimodal (Fig. 9 A,C,D). In contrast, the off-response of the S-cone was relatively flat (Fig. 9B).

Polarised-light Transmission of Ocular Media

Polarised-light transmission of trout eyes did not differ significantly between fish <30 g and >30 g (ANOVAR: $F_{0.05,20} = 1.17$, $P = 0.294$) (Fig. 10A). However, $\%P_{tran}$ did vary significantly as a function of wavelength (ANOVAR: $F_{0.05, 105} = 24.15$, $P = 0.001$). The interaction of wavelength and size, however, was not significant (ANOVAR: $F_{0.05, 100} = 0.74$, $P = 0.592$). No statistical differences in polarisation transmission were detected between large and small fish, therefore $\%P_{tran}$ data were pooled and plotted (mean \pm 1SE) as a function of wavelength (Fig. 9B). Polarised-light transmission varied from a minimum of 74% (uncorrected data) to 78% (corrected data) at 360 nm, to a maximum of 86% and 87% (uncorrected and corrected data, respectively) at both 620 and 660 nm. Spectral transmittance of ocular media for fish <30 g and >30 g (Hawryshyn *et al.* 1990) were overlaid for comparison (Fig. 10B). It was apparent that the observed decrements of $\%P_{tran}$ in the UV region for both corrected and uncorrected data paralleled the decrements observed in their

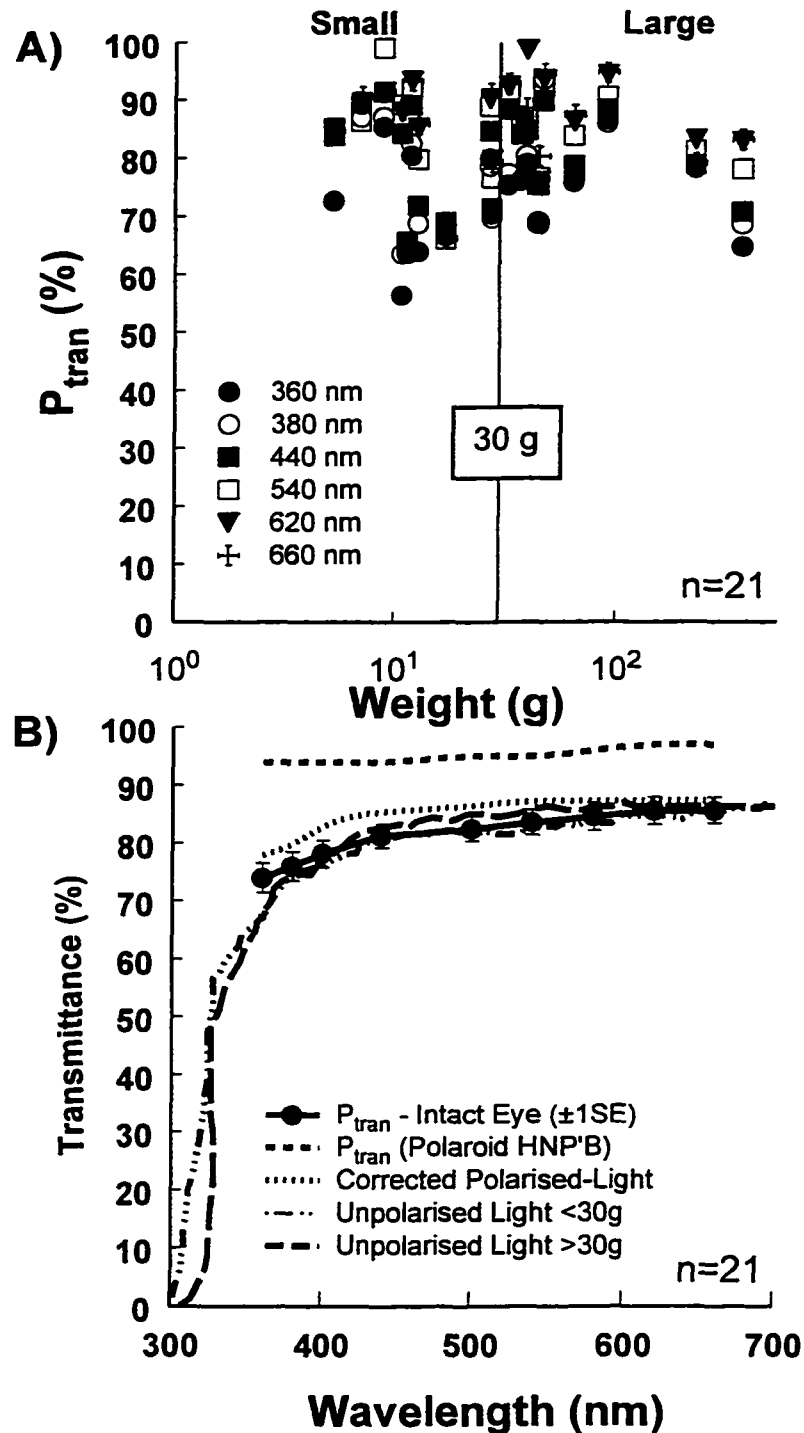


Fig. 10. A) Percent polarised-light transmission of small (<30 g) and large (>30 g) rainbow trout as a function of weight and wavelength; and B) Mean percent polarised-light transmission as a function of wavelength for: ocular media of the trout eye; an HNP'B polarising filter; and ocular media corrected for transmission of the polarising filter. ¹Spectral transmittance of ocular media of small and large rainbow trout (Hawryshyn *et al.* 1989) are overlaid for comparison.

spectral transmittance data (Fig. 10B).

DISCUSSION

This study documents two classes of polarisation-sensitive cones defined by differences in their ϕ (vertical and horizontal) and links this sensitivity to the UV-cones (vertical ϕ) and the M- and L-cones (horizontal ϕ). In addition, two other types of photoreceptors were found to be polarisation-insensitive (S-cones and rods). Whereas the responses of the UV-cones are the result of α -band absorption of UV-light, the responses of the M- and L- cones to UV-light are best attributed to β -band absorption through the Schiff-base linkage of the photopigment molecule (Hawryshyn 1992, Fein and Szuts 1982). The β -band can contribute a significant amount of sensitivity, from 0.3-0.6 of the α -band (ϕ). Thus, two channels for the detection of polarised UV-light appear to present in *O. mykiss*. Evidence of opponency between these two channels has been described by Coughlin and Hawryshyn (1995). In their study, the most predominant form of polarisation opponency was between UV-cone on-responses and L-cone off-responses (biphasic unit).

Some evidence for opponency of polarisation-sensitive mechanisms also exists in the present study. Under white-light conditions with a UV stimulus, the central portion of the on-response curve with an acrophase of 90° (Fig. 5) is undoubtedly contributed by one or both of the L-cone or M-cone mechanisms.

One or both of these mechanisms is also responsible for the off-response. However, note that the half-band width of the central portion of the on-response is more narrow than the corresponding region of the off-response. It is also narrower than the on-response of either the M- or L-cones under chromatic adaptation (Fig. 6C, 6D). This suggests that under white-light conditions the off-response may be inhibiting the on-response at these angular orientations of polarised light. The function of such an interaction is a matter of speculation, and it is possible that it may serve to sharpen the contrast of the on-response to horizontally-polarised light. Thus, the response to horizontally-polarised light would be dampened unless the e-vector is very close to 90°.

The opponent interaction of the two e-vector mechanisms has been suggested to function in a manner that mimics colour vision in insects (Bernard and Wehner 1977, Wehner 1983). Such an interaction has been suggested by the work of Coughlin and Hawryshyn (1995) at the level of the torus semicircularis. However, it is likely that if perception of e-vector resembles hue discrimination, it does so only superficially, as it may only involve discrimination of polarised from non-polarised light, and possibly (though not necessarily) the degree or orientation of polarisation. In such a scheme, an extraspectral hue could be assigned to detectable polarised light; thus the pseudosaturation of this hue would then correspond to the degree of polarisation. Under such a scenario, menotaxis (orientation to polarised light; also see Chapter V) would involve orienting relative to the strongest band of this non-spectral colour.

Orthogonal (vertical and horizontal) filters for line-orientation discrimination have long been known for humans (Foster and Ward 1991), and would be useful in fish if their visual system operates in an analogous manner. A system operating in this manner could take advantage of the on- and off-channels in the processing of e-vector. Furthermore, the opponent interactions of the polarisation-sensitive mechanisms that have been described from single-unit recording (Coughlin and Hawryshyn 1995) would aid in discrimination of various orientations of polarised light. However, more detailed discrimination of e-vector orientation is possible. If vertical- and horizontal-polarised light can be distinguished from one another, then the possibility of polarisation-contrast discrimination exists. Such a system has been described in *Octopus vulgaris* by Shashar and Cronin (1996). The potential for this type of processing is that the animal can discriminate between different patterns of polarised light. This would be particularly useful in complex polarised-light fields or for object recognition. Further investigation into retinal processing/coding and behaviour of fish must continue before these issues, as they apply to fish, can be fully addressed.

The observation that the trout retina is polarisation sensitive in the UV-region of the spectrum also allows some speculation on how fish might differentiate features of hue and polarisation. If the major role of the UV-cone is the perception of e-vector and UV sensitivity is a dedicated channel, then spectral information could be determined from the comparison of non-UV cones, whereas the polarisation cues would be encoded by comparison of the UV-cone

and either the L-cone or the M-cone. This view is based largely on the body of evidence that indicates that UV light should serve poorly in the role of image formation because: 1) corneal and lens dispersion increases logarithmically with shorter wavelengths in fish (Lythgoe 1979, Sivak and Mandelman 1982), resulting in strong chromatic aberration of the lens in the short wavelengths (Sivak and Mandelman 1982, Jagger 1992); 2) UV is scattered in the water column more strongly than longer wavelengths (Jerlov 1976, Loew and McFarland 1990); 3) UV-cones have poor brightness contrast (Hawryshyn 1991a); and 4) UV-cones have large critical diameters and large receptive-field diameters that result in decreased spatial resolution and lower acuity in the UV (Hawryshyn 1991b). As a contrast to this view, two recent studies have demonstrated that the presence of UV light augments prey detection (Loew *et al.* 1991, Browman *et al.* 1994). However, neither of these studies examined if the increased prey capture in the presence of UV resulted from photon capture by the M- and the L-cones, and not involve the UV-cones. For example, in Browman *et al.* (1994) foraging was assessed in the presence or absence of UV light in a white-light envelope. A necessary control would have been to estimate the photon capture by the β -band of the non-UV mechanisms and then repeat the experiment in the absence of UV with the light intensity increased to provide the same total photon catch. If this increased brightness caused an increment in prey capture at the same level as when UV was present, then it would not have

been possible to conclude that the UV photoreceptors were contributing to the fish's increased abilities for prey detection.

This naturally leads into the issue of how fish unconfound polarisation sensitivity from spectral sensitivity. This is a particularly interesting problem, if much of the same neural architecture is shared between the systems. Recent evidence from single-unit recording suggests that the axons of the optic nerve ganglia project UV information not only to the optic tectum but also the *torus semicircularis* (Coughlin and Hawryshyn 1994b, 1995). This suggests that processing of UV-polarised light and spectral information may be occurring in physically separate portions of the brain and that differential processing or extraction of sensory information may be occurring.

The absence of e-vector sensitivity in the on-response of larger rainbow trout (smolts), under UV-cone isolation conditions, provides physiological corroboration that larger fish fail to orient relative to the dominant plane of polarised light (Hawryshyn *et al.* 1990). The present study also suggests that the sensitivity decrements are not due to decreases in polarisation transmittance of the ocular media in fish less than 372 g (maximum size examined in this study). This does not preclude the possibility that decreases in polarisation transmittance may occur as the fish grows and ages further. The same factors that affect dispersion of a retinal image, such as lenticular scattering, will also affect polarised-light transmittance. Scattering will affect partially-polarised light incident upon the eye by reducing the proportion of linearly-polarised light (i.e.,

the light is diffused). Consequently, decreases in polarisation transmittance would be predicted with an increase in dispersion with age, such as may result with a deposition of lenticular pigments (Thorpe and Douglas 1993).

Development of a Model of Polarisation Sensitivity in Fishes

Through chromatic adaptation experiments, it was determined that polarisation sensitivity in rainbow trout does not arise from a simple function of the orthogonal juxtaposition of double cones, as suggested by Cameron and Pugh (1991) and Rowe *et al.* (1994). If e-vector sensitivity in trout follows Cameron and Pugh's model, then chromatic adaptation of orthogonal L/M double cones to isolate UV-cones would result in a reduction or absence of e-vector sensitivity rather than the observed vertical sensitivity observed in the present study. Furthermore, chromatic adaptation used to isolate L-or M- cones reduces sensitivity to vertical e-vector and results in a curve showing a ϕ to horizontal e-vector and not a "W"-function as in Fig. 5 or Cameron and Pugh (1991).

Current models for the induction of polarisation sensitivity in invertebrates do not explain polarisation sensitivity in fishes since outer segment disks of vertebrate cones typically lack the vertical orientation of invertebrate rhabdomeric-microvilli (Eakin 1968). Similarly, Cameron and Pugh's (1991) model does not fit the results of the present study. Thus, it is prudent to outline the features that a model of e-vector sensitivity should contain to satisfy

observations about polarisation sensitivity in rainbow trout (the present study, Hawryshyn *et al.* 1990, Parkyn and Hawryshyn 1993, Coughlin and Hawryshyn 1995), as well as those previously observed for goldfish (Hawryshyn and McFarland 1987). The model must: 1) include a mechanism that is independent of cone orientation in the mosaic. This is necessary because a 180° modulation in sensitivity was observed in spite of the orthogonal L/M double-cone mosaic of the trout retina. It is here that vertical and horizontal filters could operate as in Foster and Ward (1991); 2) include a mechanism for induction of polarisation sensitivity in the small, single UV-cones; 3) have e-vector sensitivity begin operating near 60% polarisation, the lower limit for observed orientation in rainbow trout (Hawryshyn and Bolger 1990); and 4) must be applicable to UV wavelengths of light as well as longer wavelengths, since polarisation sensitivity can be recorded from chromatically adapted M- and L-cones, regardless of whether they are stimulated by UV or the wavelength at the λ_{max} of their α -peak absorption.

SUMMARY

Integrated spike activity of axons from the optic nerve was measured in an investigation of the e-vector sensitive mechanism underlying the ability of rainbow trout to orient in downwelling, linearly-polarised light. In anaesthetised and immobilised fish, one eye was exposed to incremental light flashes that were

superimposed over closely controlled background conditions. Under scotopic and various photopic conditions, intensity/response curves were generated from the on- and off-responses of the optic nerve. Relative sensitivity curves were then obtained as a function of e-vector direction for the five types of receptor cells in this trout's retina: rods, ultraviolet cones (UV), short wavelength cones (S), medium wavelength cones (M), and long wavelength cones (L).

Under scotopic conditions, no sensitivity to e-vector was apparent: thus, rods do not mediate polarisation sensitivity. Under photopic conditions, parr weighing 8-10 g exhibited on-responses with e-vector sensitivity in two orthogonal channels. A UV stimulus (380 nm) on a white background evoked a three-peaked response (0° , 90° , and 180°) to the e-vector orientation presented in 30° increments between 0° and 180° . In contrast, off-responses resulted in a unimodal bell-shaped curve with $\phi = 90^\circ$. When the background was illuminated with appropriate short- and long- wavelength cut-off filters, on- and off-responses of the M- and L-cones showed maximum responses only to the horizontal (90°) plane, whether they were stimulated at their α -absorption band or their β -absorption band in the near-UV. On-responses of isolated UV-cones had maximum responses to the vertical (0° and 180°) e-vector, thus corresponding to a second channel. The blue sensitive S-cones did not show evidence of polarisation sensitivity. As well, no evidence of polarisation sensitivity was observed in the on-responses of larger individuals (50-78 g smolts) under UV-isolating background conditions, whereas the off-responses appeared to be

relatively unchanged. The loss in UV-polarisation sensitivity in larger fish was not attributable to size-related changes in the ability of the ocular media of the eye to transmit polarised light.

CHAPTER III

PHYSIOLOGICAL COMPARISONS OF SPECTRAL AND POLARISATION SENSITIVITY AMONG JUVENILE SALMONINAE

INTRODUCTION

A recurring theme in vision science is that the visual system in fish has adapted to maximise sensitivity (i.e. photon capture) in a light-limiting environment (i.e., the Sensitivity Hypothesis) (Bayliss *et al.* 1936, Clarke 1936, Munz 1958, Lythgoe 1966, 1968, Dartnall 1975, Crescitelli *et al.* 1985, 1991). According to this hypothesis the peak sensitivity of photoreceptor pigments should coincide with the most photon-rich wavelength in the photic environment. This view was inherently biased, however, because the photopigment extraction methods used in these early studies allowed the characterisation of only rod photopigments, and not photopigments from cones (Fernald 1988, Bowmaker 1990). As a result, the majority of early comparative vision studies in fish have examined the properties of rod-based (typically scotopic) vision (Wald 1936-37, 1938-39, 1941, Wald *et al.* 1957, Beatty 1966, Bridges and Yoshikami 1970, Crescitelli *et al.* 1985). Undoubtedly, the Sensitivity Hypothesis has merit for mesopelagic (mid-water), crepuscular, and nocturnal fish, as a result of the limited amounts of light penetrating the water under these conditions. This is especially the case where light has a more restricted bandwidth, such as in the

open ocean at depths of 50 m or more (Bayliss *et al.* 1936, Dartnall 1975, Munz and McFarland 1977, Partridge *et al.* 1988, 1992, Lythgoe and Partridge 1989, Loew and McFarland 1990, McFarland 1991). In addition, such a strategy would be important for fish that forage by detecting the silhouette of their prey (Tamura and Wisby 1963, McFarland and Munz 1975, McFarland 1991). A tabulation of Ali and Anctil's (1976) extensive summary of teleost fish retinal morphology reveals, however, that only 31 of 146 families they included possess pure rod retinae. The remaining 113 out of the 146 families they examined had both rods and cones (duplex retinae). In addition, representatives of the most speciose teleost families, such as the Cyprinidae, Gobiidae, and Percidae, were found to have duplex retinae. Thus, numerically, the majority of teleost species would be predicted to have duplex retinae.

One advantage that multiple pigment vision can provide is contrast of objects against the different spectral backgrounds provided by sidewelling, upwelling, and downwelling light (Lythgoe 1966, 1968, Dartnall 1975, Munz and McFarland 1977, Loew and Lythgoe 1978, Loew and McFarland 1990, Cronin *et al.* 1994). This is of particular relevance in the neritic zone (coastal and shallow waters) and the epipelagic zone where the differences among upwelling, downwelling, and sidewelling light are the greatest (see Munz and McFarland 1977, Novales Flamarique and Hawryshyn 1993). While comparative examinations of the visual sensitivity of fishes have been conducted using microspectrophotometry (MSP) (Bridges and Yoshikami 1970, Lythgoe and

Partridge 1989), only a few studies have made comparisons among fish that are closely related (Lythgoe *et al.* 1994). Comparative physiological studies on intact, functioning visual systems are even more rare (e.g., McDonald and Hawryshyn 1995, Smit and Anker 1997; also see Fleischman *et al.* 1997 with reference to the Iguanid genus *Anolis*). Comparative aspects of polarisation sensitivity regardless of methodology are also largely unknown as this is still an emerging topic of research [summarised in Chapter I and Parkyn and Hawryshyn (1993)].

However, several detailed, comparative studies of particular features of the fish visual system have been made (Bridges and Yoshikami 1970, Thorpe and Douglas 1993, Douglas and Thorpe 1993, Lythgoe *et al.* 1994, Bowmaker *et al.* 1994). Two studies, one by McCandless *et al.* (1969) on the lens and ocular-media transmission of several trout species, and another by Bridges and Yoshikami (1970) on rod-photopigments composition in the Salmonidae. Most recently, Beaudet *et al.* (1997) quantified the various morphological types of cone photoreceptors of some adult *Oncorhynchus* spp. in the anadromous phase of their migration. However, few physiological data are available on the comparative photopic (daylight) vision of the various trout, salmon and char. Additionally, statistical comparisons of visual sensitivity are generally lacking.

All juvenile salmonids examined thus far appear 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, Kusmic *et al.* 1993,

Hawryshyn and Hárosi 1994, Novales Flamarique and Hawryshyn 1996). In at least four species, rainbow trout, brown trout (*Salmo trutta*), Atlantic salmon (*S. salar*), and sockeye salmon, one of these mechanisms is sensitive in the ultraviolet (UV) (Bowmaker and Kunz 1987, Kunz 1987, Douglas 1989, Kunz and Callaghan 1989, Browman and Hawryshyn 1992, Beaudet *et al.* 1993, Hawryshyn and Hárosi 1994, Novales Flamarique and Hawryshyn 1996). In addition to the cones, an additional single class of cells (rods) contributes to scotopic or night vision of salmonids (Allen and Munz 1983).

Electrophysiological studies also have determined that responses of the salmonid retina to light can be classified into two additional forms: on-responses and off-responses (Fig. 3A) (Allen and Munz 1983, Beaudet *et al.* 1993, Parkyn and Hawryshyn 1993, Coughlin and Hawryshyn 1994a,b). These two classes of responses operate as separate channels at the level of the ganglion cells (Wheeler 1982, Ewart 1997). The on-response detects increments of light (e.g., the flash of reflected light from the side of another fish), whereas the off-response detects decrements of light (e.g., a passing shadow of an overhead predator). Under photopic or daylight conditions, cones contribute to the on- and off-responses (Wheeler 1982, Hawryshyn *et al.* 1989, Beaudet *et al.* 1993, Bilotta *et al.* 1995, Coughlin and Hawryshyn 1994a, Novales Flamarique and Hawryshyn 1996). Rods also respond with on- and off- responses under scotopic (dark) conditions (Allen and Munz 1983, Parkyn and Hawryshyn 1993, Chapter II). In amphibians, properties of ganglion cells mediating on-responses

can be further subdivided into three more sub-categories based on excitatory receptive field size. In contrast, off-responses appear to be mediated by a single identifiable class of ganglion cells.

Characterisations of photopic spectral and polarisation sensitivity exist for some species of salmonids, using various methodologies (Douglas 1983, Bowmaker and Kunz 1987, Hawryshyn *et al.* 1989, Browman and Hawryshyn 1992, Beaudet *et al.* 1993, Parkyn and Hawryshyn 1993, Coughlin and Hawryshyn 1994a,b, Novales Flamarique and Hawryshyn 1996). One method, multi-unit recordings, provides an effective and rapid method to quantify spectral and polarisation sensitivity (Allen and Munz 1983, DeMarco and Powers 1991, Beaudet *et al.* 1993, Parkyn and Hawryshyn 1993, McDonald and Hawryshyn 1995, Novales Flamarique and Hawryshyn 1997b). To date, however, few comparative studies of fish vision have used this technique.

Methodologically, there are several reasons comparisons among different species of fish should control for differences in rearing conditions. The first is to eliminate differences that could result from phenotypic plasticity. Environmental factors, such as temperature and photoperiod, affect the relative proportions of two retinal photopigments, rhodopsin and porphyropsin (A_1 and A_2 , respectively) (Beatty 1966, Tsin and Beatty 1977, Allen and Munz 1983). The relative ratio of these pigments, in turn, appears to have the potential to affect spectral sensitivity (Dartnall *et al.* 1961, Munz and McFarland 1977, Whitmore and Bowmaker 1989). However, experimental evidence suggests that the differences in

absorption accompanying such pigment changes only slightly affects spectral sensitivity (Muntz and Northmore 1973). Similarly, an increase in optical density through an increase in the density of photopigment or the length of the outer segment will result in the broadening of the wavelengths of photon capture by an individual photoreceptor (Knowles and Dartnall 1975, Allen *et al.* 1982). This further decreases the differences in wavelengths of photon capture by two photopigments differing by 10 or 15 nm.

Another reason for controlling for environmental effects was suggested by a recent study of three-spined stickleback (*Gasterosteus aculeatus*) (McDonald and Hawryshyn 1995). This study demonstrated that maximal spectral sensitivity of the off-response, as determined electrophysiologically, corresponded well with the transmission characteristics of the natal water body of the fish (McDonald and Hawryshyn 1995). This suggested some form of spectral tuning. This finding was consistent with the contrast sensitivity hypothesis (Lythgoe 1966, 1968, Dartnall 1975, Munz and McFarland 1977, Loew and Lythgoe 1978). McDonald and Hawryshyn (1995) suggested that this may represent a plastic or phenotypic response that allowed fish to detect maximally the shadows of visually relevant cues. Alternatively, this may represent evidence of micro-scale adaptation to the photic environment (McDonald and Hawryshyn 1995, Fleishman *et al.* 1997). Control of rearing conditions is therefore necessary to eliminate the confounding factor of phenotypic variation in differences in visual sensitivity.

The primary objective of the present study was to compare spectral and polarisation sensitivity of juvenile trout, char, and salmon in the subfamily Salmoninae. For comparative purposes, rainbow trout and steelhead (both *Oncorhynchus mykiss*), coastal cutthroat trout (*O. clarki clarki*), kokanee (*O. nerka*), and brook char (*Salvelinus fontinalis*) were examined. Visual sensitivity of steelhead, the anadromous form of *O. mykiss*. Thus it is interesting to compare spectral and polarisation sensitivity in this fish to the non-anadromous form of *O. mykiss*, the rainbow trout. Similarly, the visual sensitivity of kokanee, the non-anadromous form of *O. nerka*, was characterised for the first time. Kokanee visual sensitivity was then compared quantitatively with its congeners, as well as qualitatively with the visual sensitivity of the anadromous form of *O. nerka*, the sockeye salmon (reported by Novales Flamarique and Hawryshyn 1996). Clearly, the habitat of ocean-migrating adult salmonids (Hart 1973) is vastly different from the small coastal lakes and streams of their non-anadromous counterparts, and it was hypothesised that this would be reflected in visual sensitivities. Coastal cutthroat trout is a closely related species to rainbow trout (Behnke 1992) and the two species hybridise extensively throughout their sympatric native-range (Campton and Utter 1985). Cutthroat are a weakly migratory species that makes short-term use of the oceans and estuaries for only a portion of the year (Scott and Crossman 1973). It was hypothesised that because *O. c. clarki* occupies much of the same stream habitat as *O. mykiss* (rainbow trout), both species should have similar spectral

sensitivities. Finally, brook char, a member of the genus *Salvelinus* (a group distantly related to *Oncorhynchus*), was examined for the first time. Examination of brook char should allow us to assess if UV and polarisation sensitivity are ubiquitous features among the salmonines.

MATERIALS AND METHODS

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 2). Fish were maintained on a 12-h dark: 12-h light photoperiod at a mean temperature of $14 \pm 1^\circ \text{C}$ for 10 wks prior to experimentation. Lighting in the rearing facility was provided by broad-spectrum fluorescent lights containing wavelengths from 360-700 nm (integrated energy of $400 \text{ W cm}^{-2} \text{ s}^{-1}$).

Details of surgical procedures are outlined in Parkyn and Hawryshyn (1993) and Chapter II. Following completion of an individual experiment, the fish was sacrificed by severance of the spine at the brainstem. All procedures and care of the animals in this study were in accordance with the guidelines of the Canadian Council for Animal Care.

Experimental Protocol

Background lighting conditions consisted of a voltage-regulated D.C. source, 250 W Tungsten-Halogen lamp (EJH Spectro®), and longwave- and

Table 2. Species, geographic origins, and migratory patterns of salmonids used in spectral and polarisation sensitivity experiments.

Species ¹	Common Name ¹	Origin	Anadromous
<i>Oncorhynchus clarki clarki</i>	coastal cutthroat	Sooke Lake, B.C.	Yes
<i>O. mykiss</i> ²	coastal rainbow	Badger Lake, B.C.	No
<i>O. mykiss</i> ²	steelhead	Cowichan River, B.C.	Yes
<i>O. nerka</i>	kokanee	Kooteney Lake, B.C.	No
<i>Salvelinus fontinalis</i> ³	brook char	Aylmer Lake, B.C.	No

¹ Robins *et al.* (1991)

² Behnke (1992) classifies coastal rainbow and steelhead as *O. mykiss irideus*. However, this subspecies designation has not been recognised formally.

³ Introduced from Lake Nipigon, Ontario, stock in the early 1900's.

shortwave-pass interference filters (Corion®). Inconel-coated neutral-density filters (Corion®) were used to control background-channel intensity. UV/visible light guides (Oriol®) were used to project light from the background channels onto the surface of the eye. To ensure even illumination of the eye, a light spot with a diameter twice the size of the pupil was focused onto the cornea of the fish. The stimulus channel was superimposed onto the pupil using a third light pipe. The light stimulus was controlled by a computer-controlled shutter (Uniblitz®) and was projected from a 300 W Xenon lamp (Oriol®). The wavelength of the stimulus beam was controlled by an holographic-grating monochromator (SA Instruments, New Jersey) and the intensity of the stimulus (irradiance) was controlled by a neutral density wedge (Optikon®).

Fish were placed in a cradle apparatus housed within a Faraday cage. The buccal cavity was irrigated with 15 °C water at 240 ml/min. Epoxy-coated NiCrO₄ electrodes (0.6 mm diameter, 0.5 mm exposed tip) were inserted into the right naris and the optic nerve (Fig. 1) following Parkyn and Hawryshyn (1993) (Chapter II). The caudal peduncle of the fish was attached to ground via a metal alligator clip. Stimulus duration of the test wavelength of light was 750 ms. The resultant ganglion cell responses (GCR's) were amplified through a Grass Instrument P5 pre-amplifier (3 Hz low frequency filter and 300 Hz high frequency filter) and exported to a computer via an A/D port for on-line analysis, display, and storage. Sampling of GCR's was at 30 sec intervals.

Testing Conditions

White-light Background Conditions

Fish were adapted to one of two background conditions for 60 min prior to sampling. With the first background condition, fish were exposed to a quartz-halogen-tungsten background (250 W). Two light-channels, each with a 650 nm shortpass filter with a 2.0 neutral density (ND) Inconel filter, were used to adapt the eye of the study fish to white-light conditions. This filter was selected because, unlike other shortpass filters, such as 700 and 750 nm shortpass filters, this filter provides transmission of violet and some near-UV light (Corion Corporation 1990). This is relevant to the study because of the potential role of the S- (blue/violet) photoreceptors in detection of veiling illumination (Douglas and Hawryshyn 1990). Thus, the fish visual system was photopic and all classes of cones underwent some adaptation.

Ultraviolet-Isolating Background

The second background condition, UV, was used to test for the presence of an independent UV-sensitive mechanism. Under this condition, fish were adapted to a background provided by a 450 nm longpass and a 650 nm longpass filter (Corion®). Further details of this background condition is provided in Parkyn and Hawryshyn (1993) and Chapter II. When UV-cones are present, these isolating conditions allow the characterisation of UV sensitivity (Hawryshyn

and Beauchamp 1985, Beaudet *et al.* 1993, Parkyn and Hawryshyn 1993, Coughlin and Hawryshyn 1994a).

Recording from the Optic Nerve

Anatomical and physiological information was used to ensure recording was from multi-unit responses of the optic nerve (Fig. 11). To determine the correct anatomical position of the electrode in the brain, the head of a trout, with intact brain and inserted recording electrode, was removed. The head was then 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 stain for a minimum of 1 h. The brain was then excised from the cranium. The brain was coarsely sectioned at ~0.5 mm intervals. The sections were then examined to determine if the electrode had been inserted into the optic nerve. A stereotaxic apparatus was used to provide repeatability of electrode position in subsequent experimental preparations. Physiological comparisons of optic nerve and tectal responses provided an additional verification of electrode placement in the optic nerve. A lack of UV sensitivity resulting from misplacement of electrodes could mimic the pattern of spectral and polarisation sensitivity of fish that have lost UV sensitivity resulting from ontogenetic processes. This was an important consideration because

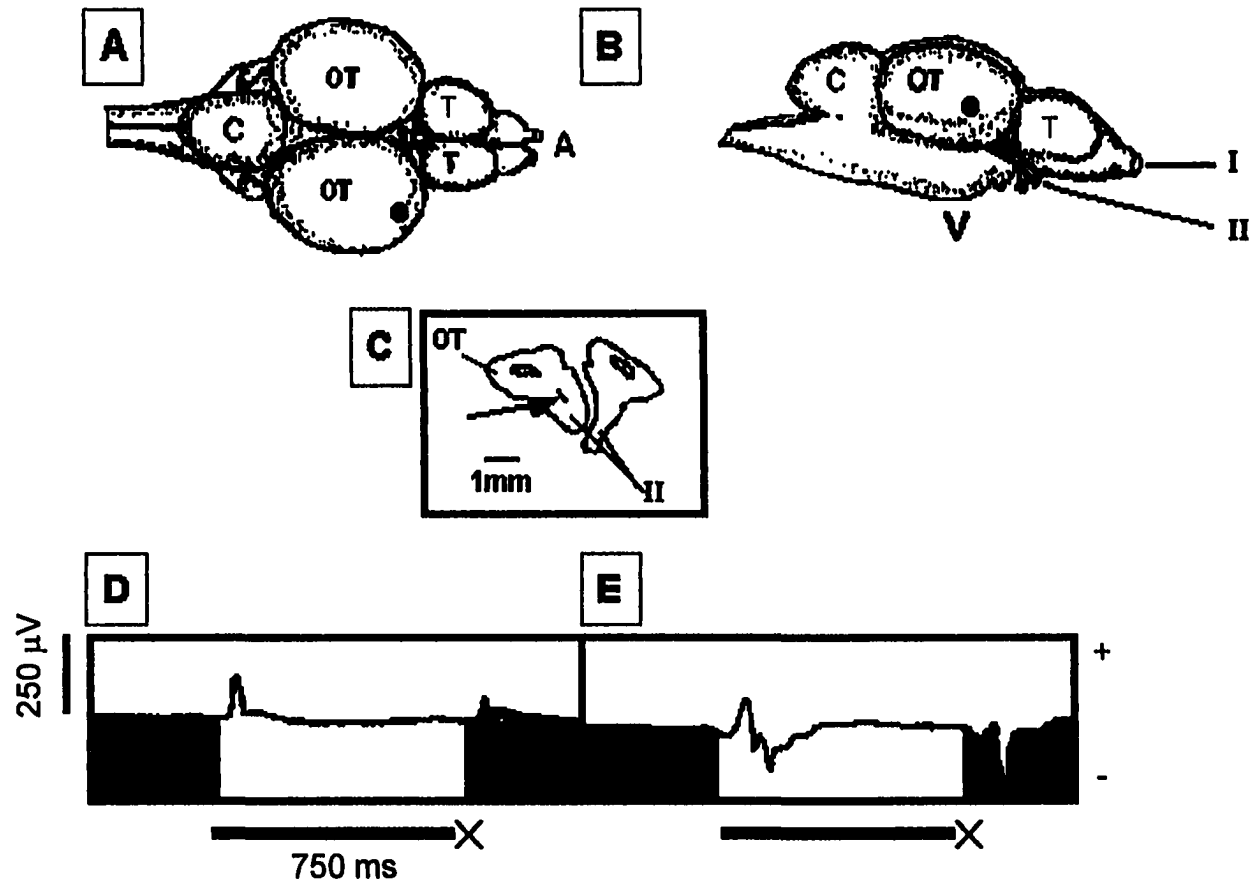


Fig. 11. A) Dorsal and B) lateral view of the brain of a 7 g rainbow trout. Insertion point of the recording electrode is indicated by ●—; C) *camera lucida* drawing of a cross-sectional view of the proximal region of the optic tectum and the optic nerve of a juvenile rainbow trout. Arrow indicates insertion tract of electrode in the optic nerve; D) Multi-unit recording from the optic nerve of a rainbow trout; and E) Multi-unit recording from the optic tectum of the same fish. Dark bar indicates light-stimulus on, X indicates cessation of stimulus. A = anterior, C = corpus cerebellum, OT = optic tectum, T = telencephalon, V = ventral surface, I = olfactory nerve, and II = optic nerve.

although colour-opponent neural units are known to be present in the optic tectum, few UV- and polarisation-sensitive units have been demonstrated in this region for rainbow trout (Coughlin and Hawryshyn 1994b). However, UV and polarisation sensitivity is present in the optic nerve (Parkyn and Hawryshyn 1993) and the *torus semicircularis* (Coughlin and Hawryshyn 1994b, 1995).

An example of the entrance position and insertion tract of a recording electrode is shown in Fig. 11A and 11B. Figure 11C shows the electrode tract in the optic nerve as enhanced by methylene blue stain. Optic nerve responses to light stimulation typically differed from responses recorded from the tectum. In general, the degree of negative deflection observed in optic nerve recordings (Fig. 11D) was much less than that observed from tectal recordings (Fig. 11E). A previous study of goldfish also found that tectal responses were far more variable than the more stereotypical responses from optic nerve axons (O'Benar 1976). Finally, 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 (e.g., with a 380 nm stimulus, the maximum on-response was observed to be at 64 msec for an optic nerve recording versus 105 msec for a tectal recording). Therefore, if the characteristics of the response were not like those in Fig. 11D during the preparation of an experiment, then the recording electrode was repositioned.

Analysis of Ganglion Cell Responses (GCR's)

Three GCR's were recorded and averaged for each increment of intensity of light as a method to reduce spurious noise. The amplitudes of the peaks 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, Chapter II). A third-order polynomial function was fit to the data following Parkyn and Hawryshyn (1993) (Chapter II). The criterion response level for determination was $20 \mu\text{V}$, near the detectable threshold of the experimental preparation. 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, Spekrijse *et al.* 1972). Hence, although multi-unit recordings receive input from many ganglion cells, the integrated response primarily reflects the responses of the centre region of the ganglion cell receptive field. 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 suprathreshold (Beaudet *et al.* 1993).

Sensitivity was defined as the $-\log_{10}$ of the photon irradiance at the criterion response voltage for each test wavelength or angle of e-vector. Sensitivity values were normalised to the median of each individual curve to generate relative sensitivity versus wavelength curves and relative sensitivity versus e-vector orientation curves. Relative sensitivity was used in these experiments because the absolute voltage of the response varied between experimental

replicates as a result of the extracellular recording technique and hence it was not possible to obtain absolute sensitivity. The means ± 1 standard error of the mean [SE] were then determined.

Contribution of Cone Photoreceptors

The contribution of the different cone photoreceptors in the spectral sensitivity was assessed by overlaying templates based on microspectrophotometric (MSP) data for rainbow trout (Hawryshyn and Hárosi 1994), after correcting for ocular media transmission. Rainbow trout was chosen as the benchmark species for comparison because it has the most complete data set of MSP-cone absorption spectra currently available for the salmonids.

Relative MSP-absorption curves for each of the four spectral classes of cones in rainbow trout (Hawryshyn and Hárosi 1994) were corrected for wavelength-specific light losses caused by ocular-media absorption in the eye of juvenile rainbow trout. These losses were calculated by fitting a model based on the Michaelis-Menton function (Lehninger 1982, Dowling 1987) to data from Hawryshyn *et al.* (1989):

$$[3] \quad T = \frac{T_{max} \lambda^a}{\lambda + \sigma^a}$$

where T = the dependent variable (%), T_{max} = the maximum value of the dependent variable (%), λ = the independent variable (nm), a = the slope of the

function, and σ = the value of λ (nm) at 50% of T_{max} (termed the Michaelis-Menton constant or the wavelength at half-maximum transmittance).

Equation [3] was modified to adjust the origin of the function because the x-intercept (the wavelength at which the ocular media ceases to transmit light) was not zero:

$$[4] \quad T = \frac{T_{max}(\lambda - c)^a}{(\lambda - c)^a + (\sigma - c)^a}$$

where c = wavelength (nm) of the x-intercept.

The terms of Equation [3] were then reduced to:

$$[5] \quad T = \frac{T_{max}}{\left(1 + \frac{(\sigma - c)^a}{(\lambda - c)^a}\right)}$$

The parameters of Equation [5], (T_{max} , σ , c , a), 95% confidence intervals, and significance of the fit of Equation [5] were then estimated using an iterative non-linear-regression technique (Levenburg-Marquart algorithm, Axum 5.0) together with the data from the independent (λ) and dependent (T) variables.

Substitution of the parameter estimates into Equation [5] permitted calculation of any value of T , the correction factor, for any wavelength within the range of optical transmission of the trout ocular media (300-800 nm). Values of T were used as the correction factor by dividing T by 100 and multiplying the quotient by the absorption values of individual cone-photoreceptor types in Hawryshyn and

Hárosi (1994). These corrected MSP-absorption values were converted to a relative scale of 0 to 1 and overlaid on spectral-sensitivity curves obtained in the present study. Rather than being a quantitative test of the cone contribution, such as in Sperling and Harwerth (1971) or Coughlin and Hawryshyn (1994a), these templates served as a qualitative guide for examination of the cone photoreceptors contributing to spectral sensitivity. Additionally, eighth order polynomials were generated using MSP values from Hawryshyn and Hárosi (1994) following Bernard (1987). The fit of these models was examined based on the correlation coefficient (r^2) and the sums of squares (SS). *F*-statistics and *P*-values also were also calculated (Zar 1996).

Modelling the Relationship between Sensitivity and Angular Orientation of a Polariser

Periodic regression analysis (Mardia 1976, Batschelet 1981, Fisher 1993, Zar 1996) was used to model the relationship between \log_{10} -relative sensitivity (*S*) and the angular orientation of the polariser (*Angle*). Inspection of the data, resulting curves, regression coefficients of determination (R^2), and sums of squares (SS) (Batschelet 1981, Zar 1996) suggested that on-responses were composed of a first and a second harmonic:

$$[6] \quad S = M + A_1 \cos(\omega \text{ Angle} - \phi_1) + A_2 \cos(2\omega \text{ Angle} - \phi_2)$$

where M = mesor (the mean of the curve), A_1 = amplitude of first harmonic,

A_2 = amplitude of second harmonic, $\omega = 2\pi/\tau$ where τ = period of the function,

ϕ_1 = acrophase (the angle associated with maximal value of y) of 1st function, and

ϕ_2 = acrophase of 2nd function.

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

$$[7] \quad \delta = 2 \psi$$

where $\psi = 2\pi/\tau$ (Angle - ϕ).

A similar procedure was used to curve-fit the off-response data.

Inspection of the means of the data and a test of model fit indicated, however,

that the function was unimodal. For curves with unimodal characteristics,

Batschelet (1981) suggested the use of the function:

$$[8] \quad S = M + A \cos(\omega \text{ Angle})$$

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

Comparative Analyses

As a preliminary requirement to all statistical analyses of spectral and polarisation sensitivity data, a Kolmogrov-Smirnov Goodness of Fit Test

(Conover 1980, Zar 1996, Norušis and SPSS Inc. 1993) was used to determine that the data used in this study did not deviate significantly from a normal distribution (2-tail test, $\alpha \geq 0.1$). Significance of all other statistical tests in this chapter was defined as $P \leq 0.05$.

Sample sizes precluded statistical examination of sensitivity in all species and forms of fish (anadromous/nonanadromous) simultaneously; thus two groups of comparisons were made. In the first group of comparisons, non-anadromous rainbow trout was compared to its anadromous form, steelhead. In the second group, comparisons were made among *O. mykiss*, *O. nerka*, and *S. fontinalis*. Differences in sensitivity (S) versus wavelength (λ), and S versus angular orientation of polarised light (e-vector, ϕ), were tested using a parametric repeated measures analysis of variance (ANOVAR) (SPSS V6.1) following the recommendations of Huynh and Mandeville (1979), Norušis and SPSS Inc. (1993), and Zar (1996). Numbers of *O. c. clarki* were too few to allow for statistical analysis but cutthroat were included for the purposes of graphical comparison.

RESULTS

Cone Photoreceptor Templates with Correction for Ocular Media Transmittance

Eighth-order polynomials have been previously used as templates to examine spectral sensitivity (Browman and Hawryshyn 1992, Beaudet *et al.*

1993, Coughlin and Hawryshyn 1994a,b). Initially, therefore, eighth order polynomials were generated using the MSP data from Hawryshyn and Hárosi (1994), without any correction for ocular transmission. The resulting polynomials were predictive and significant for both the L- and M-cone photopigments ($r^2 = 0.95$, $F_{0.05,35} = 6.01$, $P < 0.01$ and $r^2 = 0.97$, $F_{0.05,37} = 387.90$, $P < 0.005$, respectively). However, the fit was less descriptive for the S- and UV- cones. In particular, the match of the model using an eighth-order polynomial was poor for the S-cone photopigment ($r^2 = 0.28$, $F_{0.05,31} = 0.43$, $P > 0.05$). The shoulders of the function overestimated the absorbance of this photopigment. Omission of the constant term from the polynomial equation aligned the polynomial equation with the shoulders of the MSP data but the function underestimated the region of the peak of absorbance by ~ 0.1 of a log unit of absorbance ($r^2 = 0.94$, $F_{0.05,31} = 5.56$, $P < 0.01$). The eighth-order polynomial fit of the UV-cone pigment was also not predictive ($r^2 = 0.58$, $F_{0.05,26} = 0.86$, $P > 0.05$). This appeared to be due to the narrow bandwidth of the UV-cone pigment.

Based on the discrepancies in the fit of the eighth-order polynomials with the MSP data for the UV- and S-cone mechanisms, in particular, MSP-templates were made directly from Hawryshyn and Hárosi's (1994) MSP data and were then used to examine spectral sensitivity. These data were then corrected for ocular media transmittance to allow comparison directly with spectral sensitivity data. Equation [5], in conjunction with ocular media transmittance data from Hawryshyn *et al.* (1989), was used as the correction factor. Equation [5] was

predictive and significant using an F-test for non-linear regressions (Zar 1996) ($r^2 = 0.95$, $F_{0.05, 103} = 78548$, $P < 0.001$) (Fig. 12A). Parameter estimates for the equation were $T_{max} = 88.5\%$, $\alpha = 1.5$, and $\sigma = 326.20$ nm. In addition, the predicted x -intercept (c) was 300 nm.

Spectral Sensitivity under White-light Background Conditions

Rainbow Trout versus Steelhead

Both rainbow trout and steelhead displayed physiological responses to light from the near-UV at 340 nm to at least 660 nm. No significant differences were detected between rainbow trout and steelhead in either the on- or off-responses (ANOVAR; $F_{0.05, 19} = 1.45$, $P = 0.25$ and $F_{0.05, 19} = 3.66$, $P = 0.082$, respectively). In addition, there was no significant statistical interaction between the form of fish (rainbow trout or steelhead) and sensitivity of either the on- or off-response as a function of wavelength (ANOVAR; $F_{0.05, 112} = 0.89$, $P = 0.569$ and $F_{0.05, 112} = 1.03$, $P = 0.61$, respectively). In summary, the spectral sensitivity of these two forms of *O. mykiss* were not statistically distinguishable. As a result, the samples of these conspecifics were pooled to aid further comparisons among species. For *O. 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; $F_{0.05, 59, 1} = 9.26$, $P = 0.03$) (Fig. 13A, 14A). Similarly, relative sensitivity of both the on- and off-responses

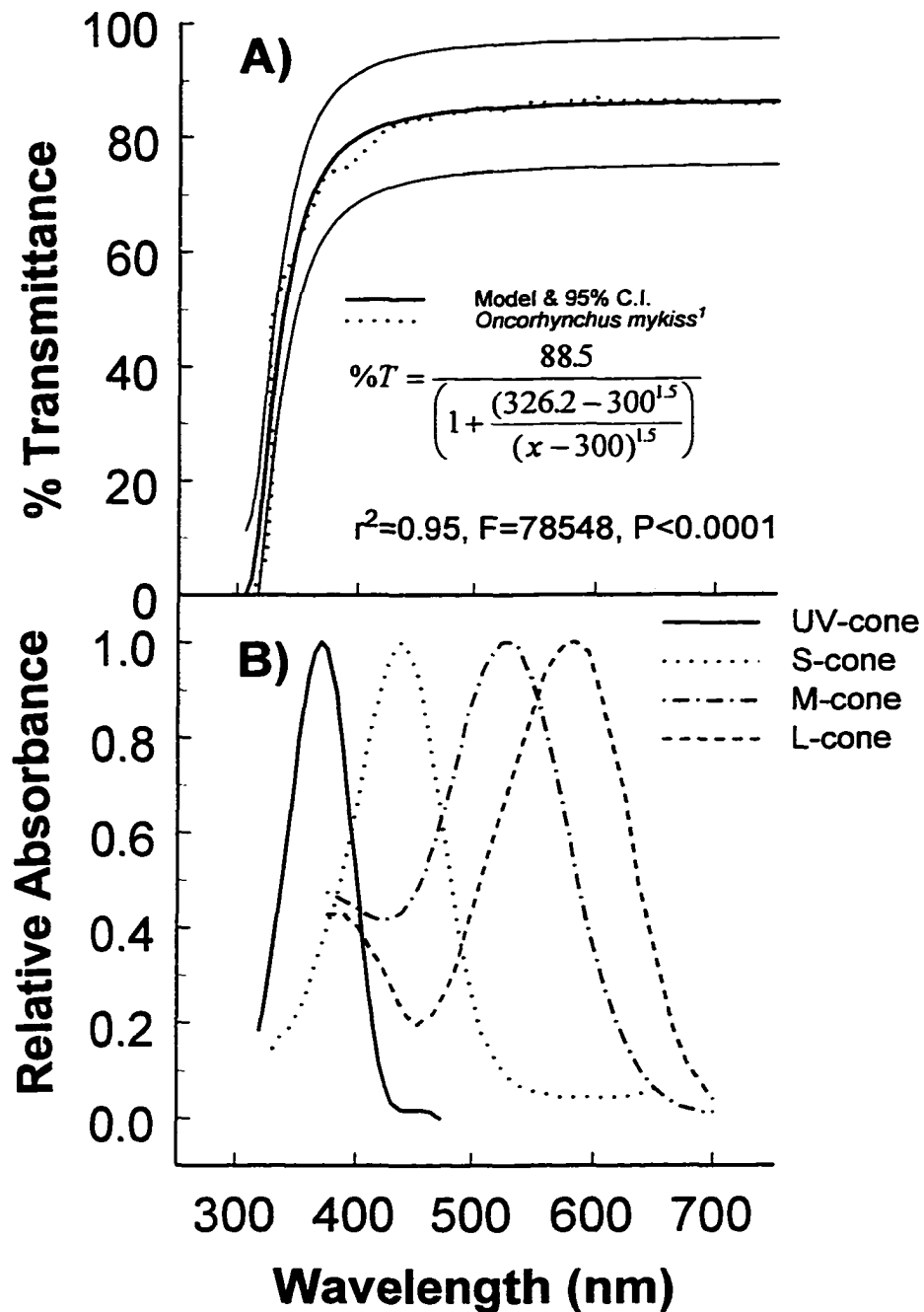


Fig. 12. A) Percent transmittance of ocular media in juvenile rainbow trout as a function of wavelength. A modified Michaelis-Menton function was fit to this data. ¹Data obtained from Hawryshyn *et al.* (1989); and B) Relative absorbance of rainbow trout cone photoreceptor pigments corrected for ocular-media absorbance. Data obtained from Hawryshyn and Hárosi (1994).

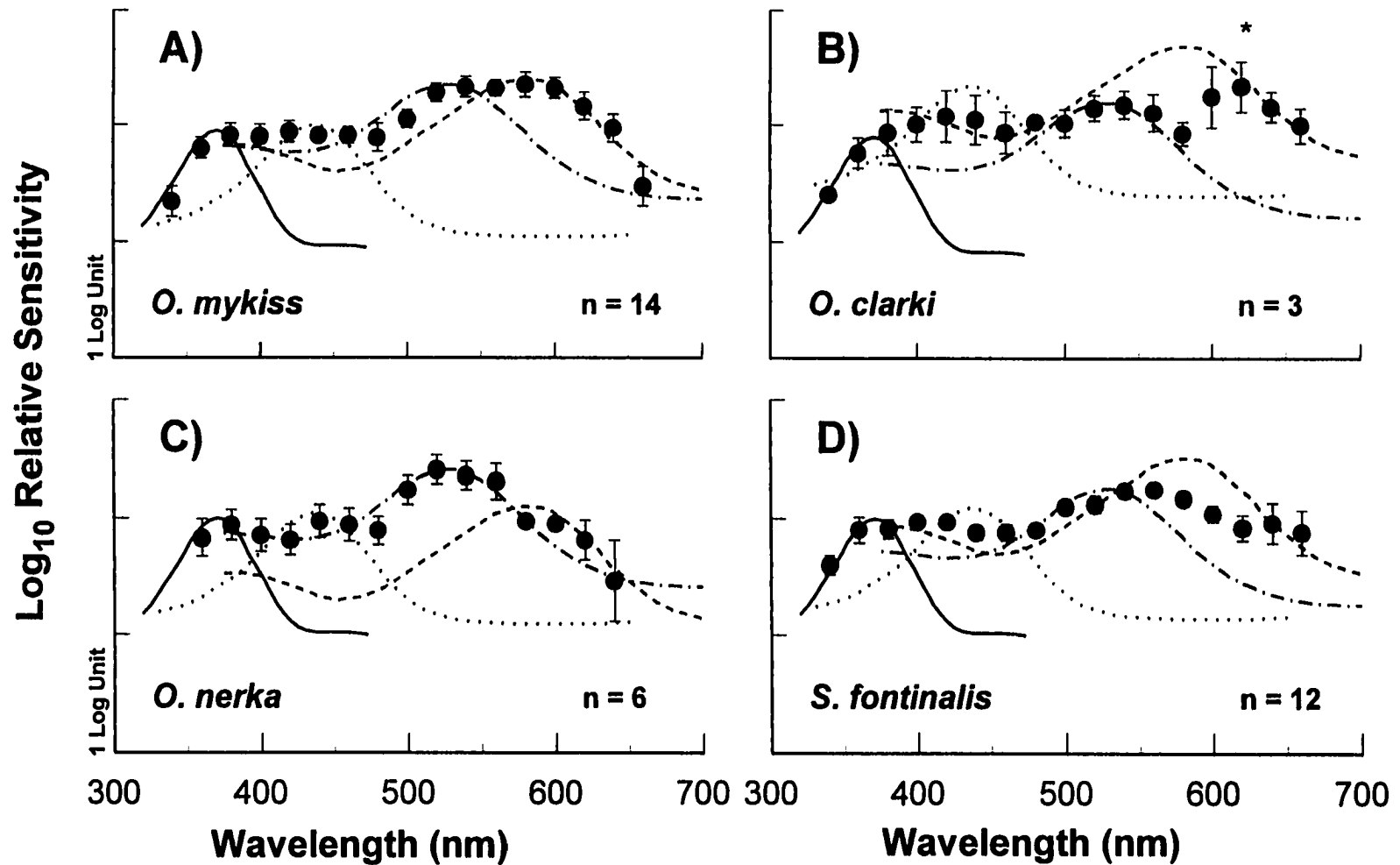


Fig. 13. Comparison of spectral sensitivity of on-responses of: A) *Oncorhynchus mykiss*; B) *O. c. clarki*; C) *O. nerka*; and D) *Salvelinus fontinalis*. Fish were adapted to a white-light tungsten background. Values represent means of standardised and normalised data (± 1 SE). Lines represent rainbow trout MSP absorbance curves (Hawryshyn and Hárosi 1994) corrected for wavelength-specific light absorption by ocular media (Legend as in Fig. 12B). Asterisk indicates anomalous far-red sensitivity.

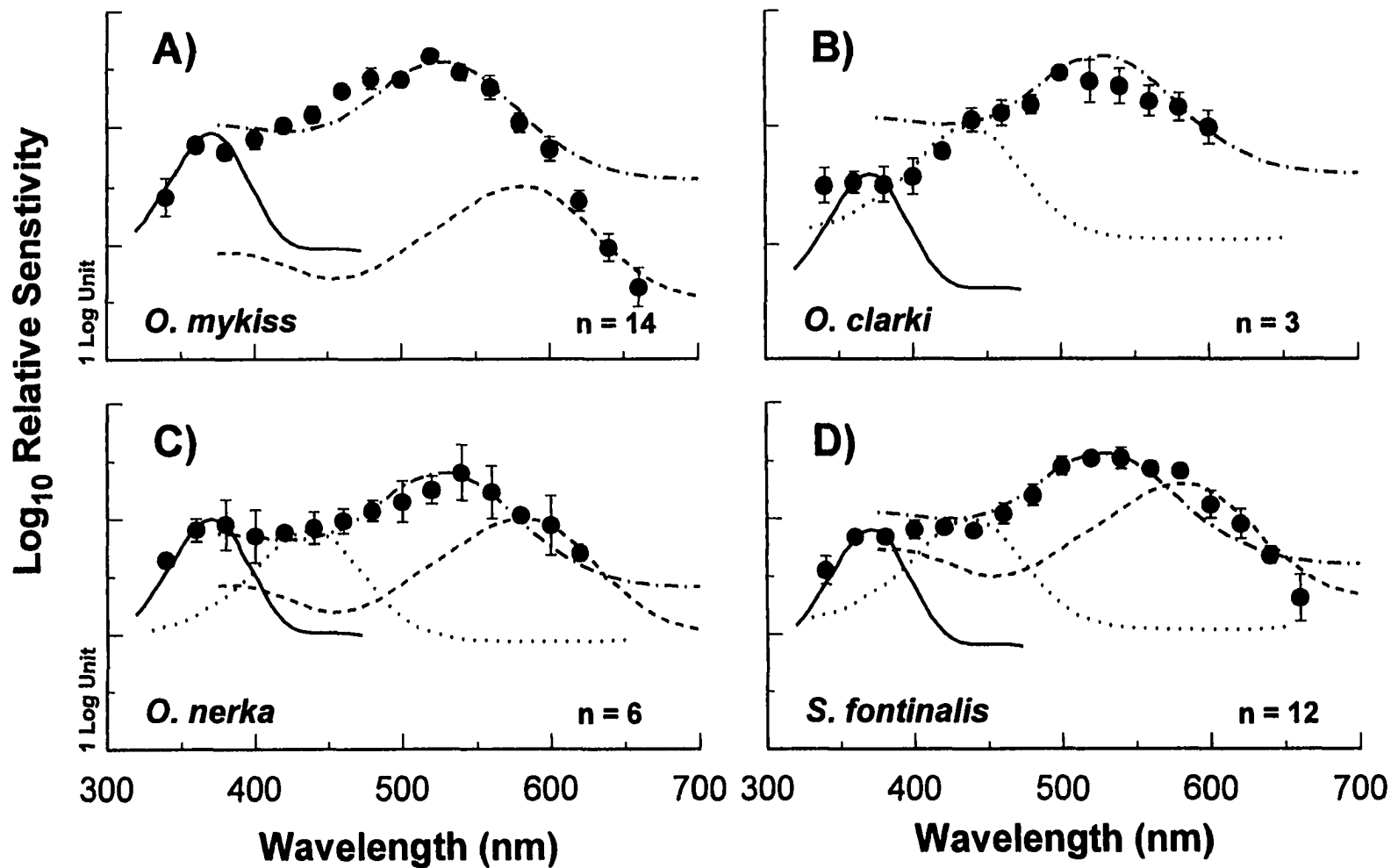


Fig. 14. Comparison of spectral sensitivity of off-responses of: A) *Oncorhynchus mykiss*; B) *O. c. clarki*; C) *O. nerka*; and D) *Salvelinus fontinalis*. Fish were adapted to a white-light tungsten background. Values represent means of standardised and normalised data (± 1 SE). Lines represent rainbow trout MSP absorbance curves (Hawryshyn and Hárosi 1994) corrected for wavelength-specific light absorption by ocular media (Legend as in Fig. 12B).

differed significantly as a function of wavelength (ANOVAR; $F_{0.05,12} = 11.44$, $P < 0.01$ and $F_{0.05,12} = 27.20$, $P < 0.001$, respectively).

For on-responses, the maximal differences in spectral sensitivity observed among test wavelengths was about 1 log unit, although two major plateaux of sensitivity were apparent (Fig. 13A). Based on the overlay of MSP-templates, one of these plateaux was dominated by the L- and M-cone mechanisms, while the other plateau was dominated by the S- and UV-cone mechanisms. The relative sensitivity was a similar amplitude from 380 nm in the near-UV to 480 nm in the blue region of the spectrum. In contrast, for off-responses in *O. mykiss*, the M-cone mechanism appeared to dominate (Fig. 14A). Under white-light adapting conditions, UV sensitivity was potentially present as indicated by the UV MSP-template in the UV region of the test wavelengths for both on- and off-responses (Figs. 13,14). This hypothesis is addressed further under the specific conditions of the UV-isolation background.

Interspecific Comparisons

Under the same white-light adaptation conditions, spectral sensitivities of the on-responses for cutthroat, kokanee, and brook char were generally similar to that of *O. mykiss*, and all species had visual capability across the visible spectrum and into the near-UV (Fig. 13A-D). In general, differences in sensitivity among wavelengths were not greater than 1 log unit for any of the species. Sensitivity in the green to red region of the spectrum (~500 nm to 640 nm) was consistently greatest.

On and off-responses of fish species differed significantly ($F_{0.05,2} = 3.59$, $P = 0.04$). For the spectral sensitivity of on-responses, there was a statistical interaction between species and wavelength ($F_{0.05,26} = 3.10$, $P = 0.001$; $F_{0.05, 52} = 2.49$, $P < 0.001$) that precluded a direct statistical examination of the main effects. Inspection of the graphs for the on-responses, however, reveals that the on-responses differ primarily in the region from 520 to 640 nm. In particular, the on-responses of *O. mykiss* and *O. c. clarki* both appeared to be dominated by a L-cone (red-sensitive) mechanism, and to a lesser extent, the M-cone mechanism (Fig. 13A, B). In comparison to *O. mykiss*, one difference was apparent in the red region of the spectrum of *O. c. clarki* (Fig. 12B). With alignment of the MSP-template to the longwave portion of the data, the maximum difference between the observed sensitivity in the L-cone mechanism compared to the MSP-absorption template for the L-cone was one log unit of sensitivity. Notably, this dip in sensitivity was near where peak sensitivity would be predicted by the L-cone MSP-template. A peak at 620 nm, in the red region of the spectrum, suggests the presence of a far-red mechanism. However, the shape is also concordant with the far shoulder of the L-cone template (Fig. 13B). In spite of the lack of fit by the L-cone MSP-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. 14B).

In contrast, the on-responses for *O. nerka* appeared to be dominated by an M-cone (green sensitive) mechanism, with little sensitivity provided by the L-

cone mechanism (Fig. 13C). Brook char spectral sensitivity had contribution from both M- and L- cone mechanisms (Fig. 13D). In general, however, the differences in spectral sensitivities among wavelengths for brook char were small (i.e., low spectral sensitivity profile). Variability in sensitivity among replicates was greatest near the two extremes of the test wavelengths. Kokanee and cutthroat, however, showed more variability among replicates over much of the spectrum compared to *O. mykiss* and *S. fontinalis*, possibly because of a smaller sample size.

Overall, spectral sensitivities of the on- and off-responses were significantly different ($F_{0.05,59} = 9.26$, $P = 0.003$). Similarly, the sensitivity of both on- and off-responses varied significantly as a function of wavelength ($F_{0.05,13} = 18.29$, $P < 0.001$ and $F_{0.05,13} = 18.22$, $P < 0.001$, respectively).

In general, all species displayed a broad-banded sensitivity for the off-response with maxima in the 500-600 nm region of the spectrum (Fig. 14A-D). Unlike the on-responses, off-responses showed no species differences ($F_{0.05,4} = 2.54$, $P = 0.102$), and several features were consistent among species. Specifically, the λ_{max} of the off-responses in *O. mykiss*, *O. nerka*, and *S. fontinalis* was between 520 nm and 540 nm, whereas the λ_{max} for the *O. c. clarki* was ~500 nm. Comparison of the observed spectral sensitivity of *O. c. clarki* with the MSP-template suggests that their sensitivity is decreased ~0.25 log unit in the region of 520 nm to 560 nm (Fig. 14B).

Off-responses also appeared to have a second, smaller maximum in the near-UV region. This second peak was centred at 360 to 380 nm (Fig. 14A-D).

Spectral Sensitivity under UV-Isolating Background Conditions

Rainbow Trout versus Steelhead

Chromatic adaptation of the S-, M-, and L-cone mechanisms with a yellow-orange background (UV-cone isolating condition) resulted in a detectable peak in the on-responses of the UV region of the spectrum for both rainbow trout and steelhead (Fig. 15A), providing evidence for the presence of UV-cones. There appeared to be no differences between rainbow trout and steelhead in their spectral sensitivity under UV-isolating conditions based on a graphical analysis.

O. mykiss displayed an on-response UV sensitivity peak at 380 nm. The maximum sensitivity, however, was offset from the λ_{max} of the UV-cone MSP-template (Fig. 15A). The S-cone appeared to contribute little to the on-response of the UV-isolated fish. In contrast, a large peak sensitivity also was observed in the red region of the spectrum. Like the UV peak, maximum sensitivity of this mechanism was offset from the λ_{max} obtained from the L-cone MSP-template of rainbow trout. With a yellow-orange background (UV-isolation), the sensitivity of the off-response to varying wavelengths of light was dominated by the M-mechanism in both rainbow trout and steelhead (Fig. 16A). No other cone mechanisms appeared to contribute substantially to the spectral sensitivity of the off-responses under UV-isolating conditions.

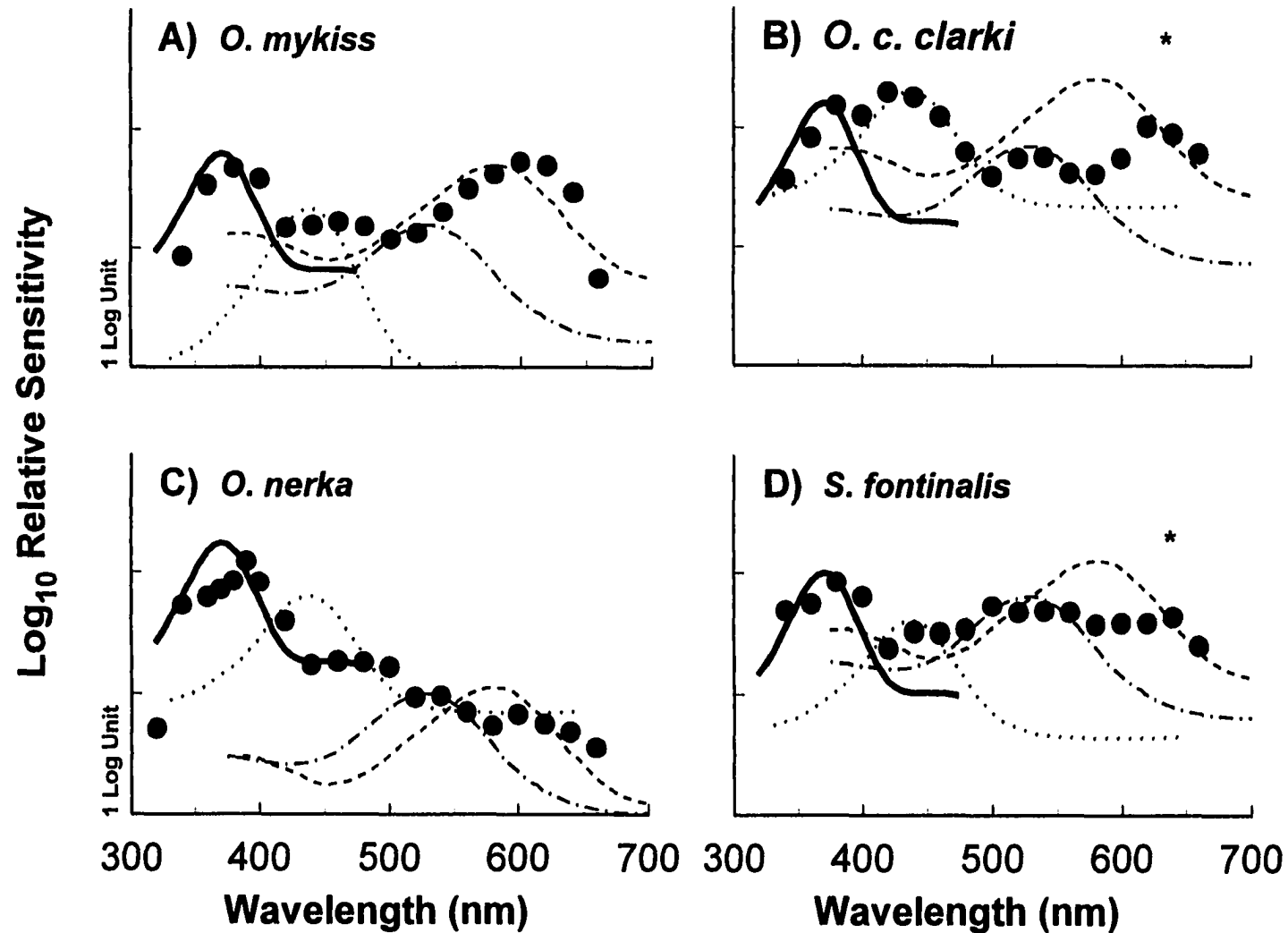


Fig. 15. Comparison of spectral sensitivity of on-responses under UV-isolating conditions for: A) *Oncorhynchus mykiss*; B) *O. c. clarki*; C) *O. nerka*; and D) *Salvelinus fontinalis*. Values represent means of standardised and normalised data (± 1 SE). Lines represent rainbow trout MSP-absorbance curves (Hawryshyn and Hárosi 1994) corrected for wavelength-specific light absorption by ocular media (Legend as in Fig. 12B). Asterisks indicate anomalous far-red sensitivity.

Interspecific Comparisons

As with *O. mykiss*, UV sensitivity was also present in the on-responses of *O. c. clarki*, *O. nerka*, and *S. fontinalis* (Fig. 15B-D), indicating the presence of UV-cones. In contrast to *O. mykiss*, there was a substantial contribution of the on-responses of the S-cone to spectral sensitivity in *O. c. clarki* (Fig. 15B). In addition, the sensitivity of cutthroat trout in the UV and violet region of the spectrum corresponded well with λ_{max} of the UV- and S-cones of the MSP-template, respectively (Fig. 15B). Cutthroat trout also showed a peak in sensitivity in the far-red region of the spectrum (noted by an asterisk). The MSP-template for the L-cone mechanism was aligned to the red end of the sensitivity data. The MSP-template overestimated sensitivity in all but the far-red region of the spectrum (620-660 nm) (Fig. 15B).

Relative to *O. mykiss*, the on-responses of the UV mechanism in *O. nerka* were much higher than the contribution from other cone mechanisms (Fig. 15C). The match of the spectral sensitivity for the UV region of the spectrum was relatively poor except on the descending limb of the MSP-template. Peak sensitivity of the on-response was at 390 nm, in contrast to the λ_{max} of the UV MSP-template at 365 nm.

UV sensitivity also was detected in the on-responses of *S. fontinalis* with a UV-isolating background (Fig. 15D). The fit of the UV MSP-template was relatively good with λ_{max} corresponding to peak sensitivity. Based on overlay of the M-cone and L-cone MSP-templates, these mechanisms also appeared to

contribute to spectral sensitivity. However, like *O. c. clarki*, the sensitivity in the red region of the spectrum was lower than that predicted by the MSP-template for the L-cone. Similarly, a small peak in the far red region of the spectrum also was observed.

As with *O. mykiss* under UV-isolating conditions, the spectral sensitivity of the off-responses in *O. c. clarki*, *O. nerka*, and *S. fontinalis* also was dominated by the M-cone mechanism (Fig. 16B-D). In addition, overlaying an L-cone template suggested that this mechanism also may make a contribution to off-sensitivity under these conditions for *O. c. clarki*, *O. nerka*, and *S. fontinalis*. This contribution appeared to be primarily in the wavelengths beyond 600 nm for at least *O. nerka* and *S. fontinalis*. In addition, when the L-cone MSP-template was aligned to the far-red region of the off-response data, it provided a descriptive fit of the off-sensitivity in the UV region of the spectrum (Fig. 16B-D). In contrast, the UV-region of the M-cone MSP-template overestimated the observed spectral sensitivity.

Polarisation Sensitivity

On-responses

The on-responses of *O. mykiss*, *O. c. clarki*, *O. nerka*, and *S. fontinalis* to the orientation of the polariser was a W-shaped function (Fig. 17A-D). Overall, on- and off-responses were found to be significantly different (ANOVAR; $F_{0.05} = 8.78$, $P < 0.05$). Overall sensitivity of on-responses varied significantly with angle

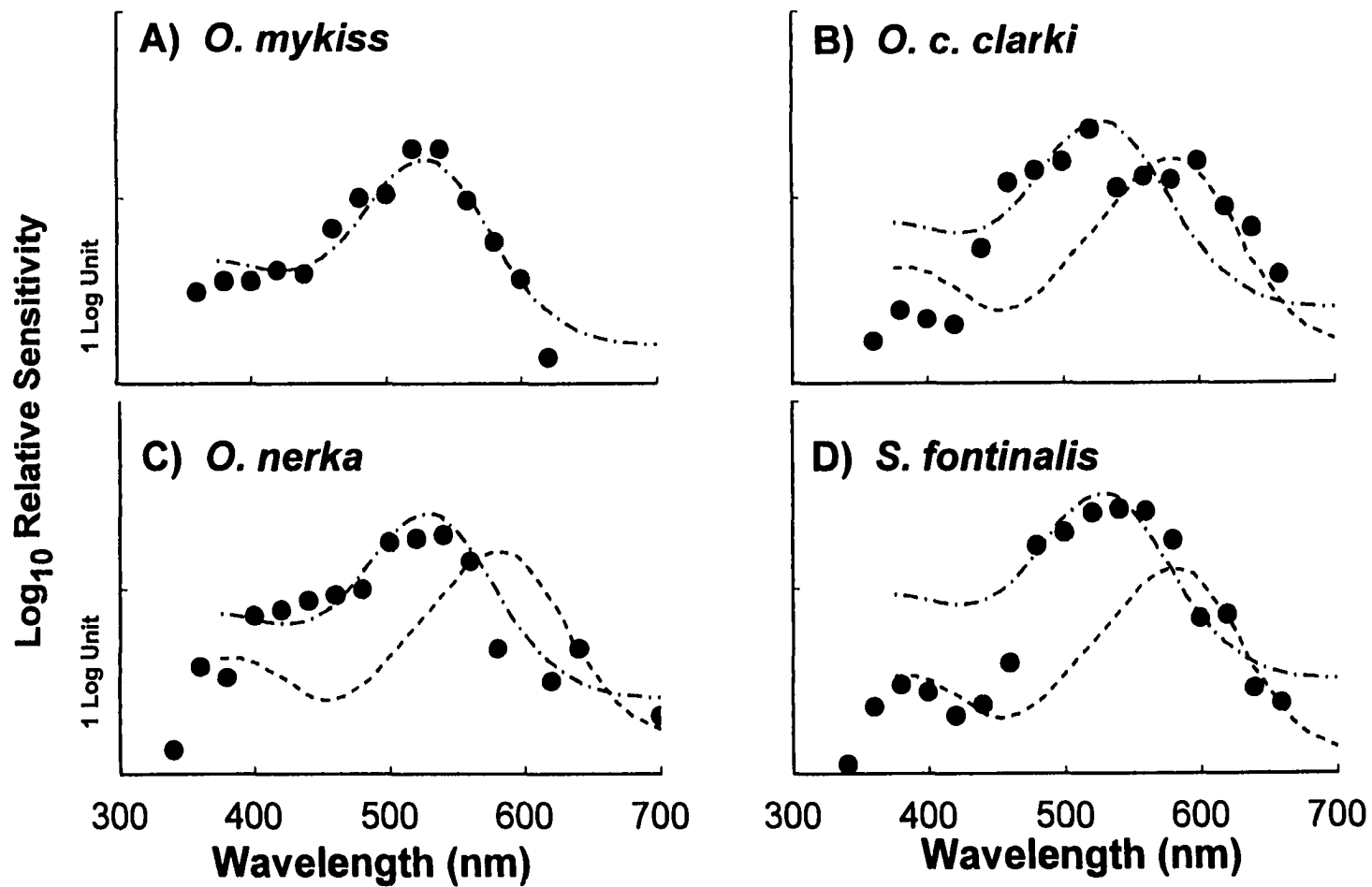


Fig. 16. Comparison of spectral sensitivity of off-responses under UV-isolating conditions for: A) *Oncorhynchus mykiss*; B) *O. c. clarki*; C) *O. nerka*; and D) *Salvelinus fontinalis*. Values represent means of standardised and normalised data (± 1 SE). Lines represent rainbow trout MSP-absorbance curves (Hawryshyn and Hárosi 1994) corrected for wavelength-specific light absorption by ocular media (Legend as in Fig. 12B).

(within subjects effects) (ANOVAR, $F_{0.05,6} = 5.31$, $P < 0.001$). For all species, sensitivity of the on-response to the UV polarised-light stimulus varied significantly with the angle of the stimulus (Table 3). However, measurements of polarisation sensitivity relative to the angular orientation of a plane-polarised UV stimulus indicated no differences among species for the on-responses (ANOVAR, $F_{0.05,2} = 1.63$, $P = 0.222$). In addition, a test of the interactive significance 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 angle of the polarised-light stimulus of all species examined had a W-shaped response with $\phi = 0^\circ, 90^\circ, 180^\circ$, consistent with the model outlined in Equation [5]. However, the angular correlation coefficient (R^2) ranged from 0.53 with *O. c. clarki* to 0.98 in *O. mykiss* (Fig. 16, Table 4). All periodic regressions of the on-responses were nonetheless significant (Table 4, Fig. 17). The peak-width (δ) at the mesor, however, varied from a minimum of 53.6° in *O. nerka* to a maximum of 68.6° in *O. c. clarki*.

Off-responses

In contrast to the on-responses, the off-responses resulted in a bell-shaped function (Fig. 18A-D). As with on-responses, sensitivity of off-responses varied significantly with polariser orientation for all species (Table 3). Observed off-responses in all species were unimodal with $\phi = 90^\circ$ and δ ranging from a minimum of 64.3° in *O. nerka* to a maximum of 87.9° in *O. c. clarki* (Table 4).

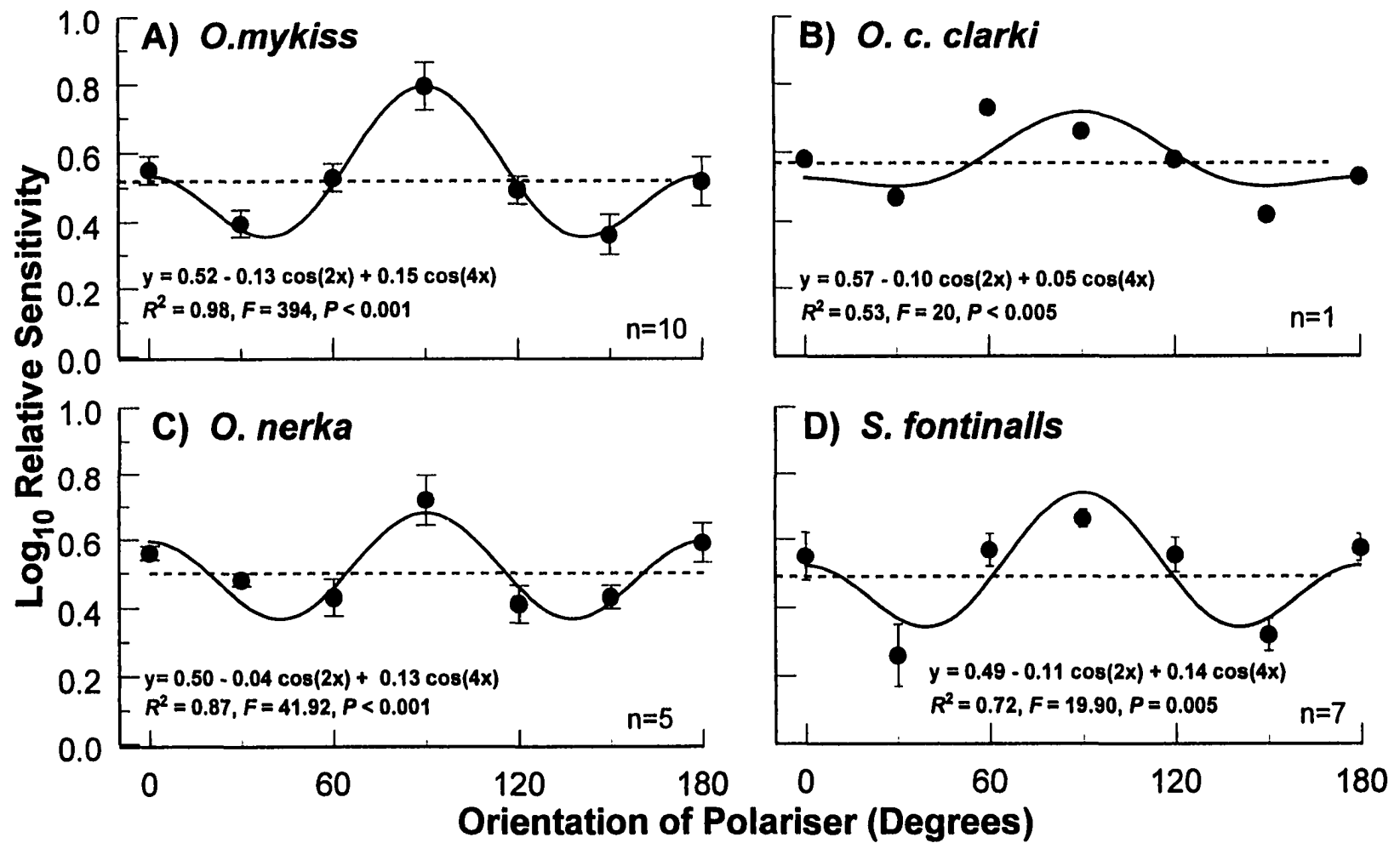


Fig. 17. Polarisation sensitivity of on-responses to plane-polarised UV light for: A) *Oncorhynchus mykiss*; B) *O. c. clarki*; C) *O. nerka*; and D) *Salvelinus fontinalis*. Values represent means \pm 1 SE.

Table 3. Summary statistics of ANOVA with repeated measures tests for differences in sensitivity as a function of angular position of a linear polariser.

Species	GCRs	n	F	P
<i>O. mykiss</i>	on	10	5.78	<0.0001
	off	10	11.32	<0.0001
<i>O. nerka</i>	on	5	5.10	0.002
	off	5	5.12	0.003
<i>S. fontinalis</i>	on	7	7.15	<0.001
	off	7	3.54	<0.030

Table 4. Parameter estimates and periodic regression statistics for the relationship of relative sensitivity versus orientation of linear polariser (Polaroid HNP'B).

Species	GCRs	Period (°)	Acrophase (°)	Amplitude		d (°)	R^2	F	P
				A ₁	A ₂				
<i>O. mykiss</i>	on	90	0, 90, 180	-0.13	0.15	60.0	0.98	394	<0.001
	off	180	90	-0.22		67.5	0.96	108	<0.001
<i>O. clarki</i>	on	90	0, 90, 180	-0.10	0.05	68.6	0.53	20.0	<0.005
	off	180	90	-0.35		87.9	0.87	41.1	<0.001
<i>O. nerka</i>	on	90	0, 90, 180	-0.04	0.13	53.6	0.87	41.9	<0.001
	off	180	90	0.23		64.3	0.85	34.0	<0.001
<i>S. fontinalis</i>	on	90	0, 90, 180	-0.11	0.14	58.9	0.72	19.9	0.005
	off	180	90	-0.13		70.7	0.62	9.1	<0.05

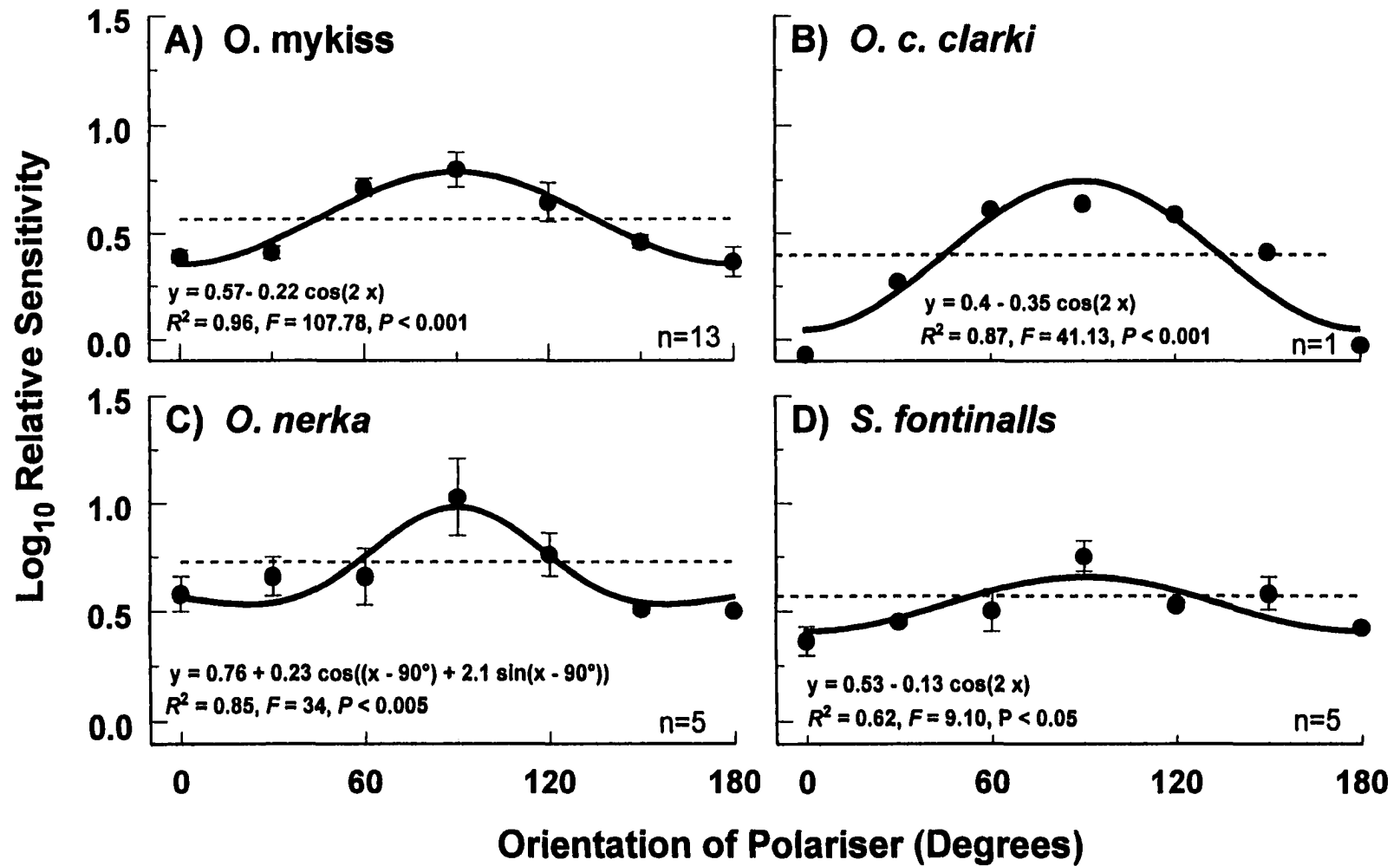


Fig. 18. Polarisation sensitivity of off-responses to plane-polarised UV light for: A) *Oncorhynchus mykiss*; B) *O. c. clarki*; C) *O. nerka*; and D) *Salvelinus fontinalis*. Values represent means \pm 1 SE.

As a result, the relationship of sensitivity as a function of the angle of the polariser in rainbow trout, cutthroat trout, and brook char, was described by Equation [7], a simple cosine function (Fig. 18A,B,D; Table 4). In contrast, the data for *O. nerka* was more sharply peaked than a cosine function. An alternative model suggested to attain a more descriptive fit for sharply peaked data (Batschelet 1981) was:

$$[9] \quad S = M + A \cos(\omega \text{ Angle} - \phi + v \sin(\omega \text{ Angle} - \phi))$$

where M = mesor, A = amplitude, $\omega = 2\pi/\tau$ where τ = period of the function, ϕ = acrophase (the angle associated with maximal sensitivity), and v = coefficient of skewing (Batschelet 1981). This resulting equation provided a descriptive and significant fit for *O. nerka* (Fig. 18C, Table 4).

DISCUSSION

In addition to extending our current knowledge on the phyletic distribution of the UV photoreceptor in the salmonid Genus *Oncorhynchus*, this research also provides the first evidence of an independent UV-sensitive mechanism in the Genus *Salvelinus*. Little work has been conducted on visual physiology of *Salvelinus* spp., in spite of the work conducted on the retinal morphology and development of Arctic char (*S. alpinus*) (Vigh-Teichmann *et al.* 1991). Vigh-Teichmann *et al.* (1991) demonstrated that the photoreceptor mosaic of Arctic char is similar to that observed in other salmonines (Lyll 1957; Ahlbert 1976;

Kunz 1987; Browman and Hawryshyn 1992). Similarities included the presence of corner cones (sometimes termed accessory corner cones) that have been associated with UV sensitivity in *O. mykiss* (Browman and Hawryshyn 1992; Beaudet *et al.* 1993; Hawryshyn and Hárosi 1994). Thus, the presence of UV sensitivity in *S. fontinalis* was not unexpected. However, the physiological evidence of a UV-cone mechanism does help to demonstrate that UV sensitivity is a common feature (an ancestral character or plesiomorphism) present in all clades of the subfamily Salmoninae. In addition, the UV mechanism in all of the fishes in the current study appeared to contribute to the on-response but not to the off-response, as previously noted by Beaudet *et al.* (1993). Even under photic conditions that isolated the UV mechanism, UV contribution to the off-response could not be demonstrated conclusively.

While all fish examined appeared to have photosensitivity consistent with the photopigment MSP-templates, differences were observed in the relative sensitivity of individual cone mechanisms among species and forms of fish. These differences may represent species-specific characteristics that have evolved under the same selective pressures. This is evidenced by the observation that, although while 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 clearly different. As juvenile fishes, *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). Detection of biologically relevant stimuli in the photic environment of kokanee must therefore place different demands on their visual system. In spite of this, in the present study it appears that the photopigments of salmonids, based on the fit of the MSP-template, do not differ; rather, the relative sensitivity to different portions of the light spectrum has been adjusted. A lack of differences in the absorption spectra of rod photopigments also has been observed (Bridges and Yoshikami 1970). It is interesting that photopic λ_{max} wavelengths vary considerably among salmonid species (Table 5). Much variability has been observed in these fish even when using similar techniques. Hence, it is important to stress documentation of the culture conditions during rearing of the fish. This may shed light on factors influencing sensitivity.

Sampling bias also potentially might contribute to the differences among fish species and forms in relative sensitivity. When present, this bias is due to the recording electrode possibly collecting a fraction of ganglion cell axonal output. However, the recording electrode in the present study was very large relative to the diameter of the optic nerve. In addition, sampling replication in the white-light regime tests would have helped to control for this potential problem. Spectral sensitivity of the on-response with a tungsten-background approximating water conditions in a shallow, clearwater system, was dominated by the sensitivity of the L-cone mechanism in *O. mykiss*. Daw (1968) and

Table 5. Comparison of photopic λ_{max} values obtained from electrophysiological (GCR), microspectrophotometric measurements (MSP), and behavioural observations of salmonids.

Species	λ_{max} cones GCR		λ_{max} cones MSP		λ_{max} cones Behaviour	
<i>O. mykiss</i>	370-380 ¹	390 ²	400 ³	365 ⁴	NE ⁵	360 ⁶
	440	420	453	434	440	430
	540	510(520)	530	531	535	540
	580	NE	598	576	630	620
<i>O. nerka</i>	370-380 ¹	380 ⁷				
	440	420				
	520	520				
	580-600	625				
<i>Salmo trutta</i>		355 ⁸				
		441				
		535				
		600				
<i>Salvelinus fontinalis</i>	360-380 ¹					
	420					
	540					
	560					

¹Present study; ²Beaudet *et al.* (1993); ³Kusmic *et al.* (1993); ⁴Hawryshyn and Hárosi (1994); ⁵Douglas (1983); ⁶Hawryshyn *et al.* (1989); ⁷Novales Flamarique and Hawryshyn (1996); ⁸Bowmaker and Kunz (1987); NE= not examined.

Coughlin and Hawryshyn (1994b) also have previously noted this L-cone domination. However, the wavelength of this peak sensitivity in the orange-red end of the spectrum did not match previous observations for *O. mykiss* by either Coughlin and Hawryshyn (1994b) or Hawryshyn *et al.* (1989). Two separate factors could account for this apparent discrepancy: 1) MSP-template development; and 2) rearing conditions of the fish.

First, at the time when these previous studies were conducted, the MSP-absorption curves of *O. mykiss* were only beginning to become available (Kusmic *et al.* 1993, Hawryshyn and Hárosi 1994). Thus, an eighth-order polynomial nomogram (Bernard 1987, Browman and Hawryshyn 1992, Beaudet *et al.* 1993) was aligned to what was interpreted to be the λ_{max} of the data. Actual MSP-absorption curves are now available and correcting them for ocular media transmission has given us a guideline for placement of MSP-templates that was not previously available. For example, in the present study, examination of the on-response sensitivity of *O. c. clarki* revealed a feature of particular interest, a narrow peak observed in the 600-660 nm region of the spectrum (Fig. 13B). This peak appeared to be the contribution of an L-cone with a peak sensitivity of 620 nm. However, the MSP-template of the L-cone as obtained from Hawryshyn and Hárosi (1994) suggested that this λ_{max} may actually be the contribution of an L-cone with a sensitivity near that of *O. mykiss*. The shoulders of the spectral sensitivity peak in this region were too narrow to be from a 620 nm cone based on the size and shape of the cone. In fact, the

descending (long-wavelength) limb of the MSP-template for a 585 nm L-cone was consistent with the observed sensitivity in this region. This region of the L-cone is thought to be consistent in shape among taxa and is thus suitable for the alignment of templates (Lamb 1995). Such “pseudo” peaks in the red region of the spectrum have been described for humans and macaques far from the λ_{max} of any known primate retinal-pigment (Sperling and Harwerth 1971). A similar phenomenon, however, has not been described previously in salmonids. With alignment of the MSP-template, a similar conclusion on the nature of the photoreceptor contributing to the observed spectral sensitivity is reached. Specifically, a factor, such as lateral inhibition, is decreasing the L-cone mechanism sensitivity over part of the range of this mechanism. In *O. c. clarki*, this was apparent as 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 L-cone mechanism sensitivity. Re-examination of spectral sensitivity curves from Coughlin and Hawryshyn (1994b; their Figs. 2, Fig. 3A-C), where the fish were cultured under the same conditions as used in the present study, suggested that the sensitivity in the region from 500-660 nm could be subject to the same phenomenon. Under this scenario, the bichromatic unit (their Fig. 3A) would become monochromatic, the trichromatic unit (their Fig. 3B) would become bichromatic, and their tetrachromatic unit (their Fig. 3C) 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 suppression 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.

A second 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. This was corroborated by Browman and Hawryshyn (1992, 1994), where they used fish cultured under the same conditions as the present study but measured sensitivity with the same technique used in Hawryshyn *et al.* (1989). They also determined that the λ_{max} of the UV-cone mechanism of their experimental control fish (rainbow trout) was near 360 nm. Such shifts are known to occur seasonally for some species of fishes (Beatty 1966, Loew and Dartnall 1976, Beatty 1984, Whitmore and Bowmaker 1989). It is not known if such shifts persist under controlled-photoperiod conditions. However, fish in the holding facility used in the present study did maintain their seasonal reproductive cycles in spite of a controlled photoperiod (personal observation). This suggests an endogenous rhythm to this cycle or seasonal fluctuation of another potential zeitgeber such as temperature (see Tsin and Beatty 1977).

A second 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. These conditions approximate the background conditions of a fish viewing downwelling illumination in the neritic zone or epilimnion. The dominance of the L-cone (red) on-responses and the M-cone (green) off-responses suggests that, under these conditions, these wavelengths are particularly useful for the detection of objects in the water column. This dominance of the on-/off-response system in the visual system of fish has been described previously by Daw (1968), Easter (1975), Beauchamp and Rowe (1977), and Coughlin and Hawryshyn (1994b). Several explanations can be made for this phenomenon. First, it may have evolved as a result of the fact that these fish typically do not live in a red-light rich environment but instead live in an environment where red-light is rapidly attenuated (Tyler and Smith 1970, Dartnall 1975, Novales Flamarique *et al.* 1992). This is certainly the case as depth increases. A higher sensitivity of the L- or red-sensitive cone could thus serve as a form of physiological compensation. Second, the transparency of water to red and green light is much less variable than at wavelengths in the blue or violet portion of the spectrum (Lythgoe and Northmore 1973, Lythgoe 1975b). For example, dissolved organic matter (e.g. humic and fulvic acids) absorb strongly in the blue and violet regions and yet are transparent in the red (Lythgoe 1975b). Third, such a physiological compensation may be important as 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 red sensitivity may thus be an example of offset

sensitivity to enhance contrast (Lythgoe 1966, 1968, 1975a, 1979, Easter 1975). Such offset sensitivity would detect more red photons in the light reflected by an object in the water column relative to the background of the water surrounding that object (Lythgoe 1966, 1968, 1979). In addition, this high red sensitivity also may be exploited by the almost exclusively red nuptial colouration of many salmonine species. Typically, these species spawn in the shallow-water of streams and lake shores where little red light is attenuated. Thus, a red object would be very conspicuous to a fish with high spectral sensitivity in this region of the spectrum. To enhance visual contrast further, in many reproductive salmonids the lower mandible and other previously-unpigmented areas also become heavily pigmented with melanin. One interesting exception is the lake char (*S. namaycush*). Unlike its relatives, *S. namaycush* develops a black body stripe during the breeding season (Scott and Crossman 1973). Because this species spawns at night, this black body stripe most probably serves to increase contrast with the more lightly pigmented areas on the body. This species spawns after dark in lakes (and rarely streams) at depths ranging from 36.6 m to less than a metre (Scott and Crossman 1973). Similarly, whitefish of the genus *Coregonus* (in the sister subfamily Coregoninae) reproduce in lakes from depths of 75 m to depths less than 8 m (Koelz 1929, van Oosten 1939, Scott and Crossman 1973). Like lake char, *Coregonus spp.* typically lack red colouration during spawning. In contrast, the stream-dwelling whitefish (*Prosopium cylindraceum*) is a brightly coloured fish that develops orange pectoral fins during

the spawning period in a manner reminiscent of most salmonines (Scott and Crossman 1973).

The off-response of all species examined with both the UV and white background was dominated by the M- or green-sensitive mechanism. Beaudet *et al.* (1993) and McDonald and Hawryshyn (1995) observed similar results under shortwave/UV-isolating conditions. The offset of the wavelength of the λ_{max} of the on- and off- channels from one another provides us with some insight into the difference in their roles during photopic conditions. In a blue water (oligotrophic) or green water (eutrophic) system, a target that does not match the background because it reflects light at a different wavelength would be detected as an on-response, given that the animal has a detector capable of discriminating this reflected light from the background light. Similarly, the image of an object that does not reflect light, such as a silhouette that moves across the retinal field, should be darker than the background and would be detected as an off-response. Wheeler (1979a) 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 under a white-light background. This would suggest that in the relatively more monochromatic limnetic environment of the kokanee, colour contrast of objects may not be as important as it is in streams or the neritic zone.

The widespread distribution of the UV photoreceptor in salmonids has been corroborated recently by Hisatomi *et al.* (1996). Their study indicated a

widespread distribution of amino acid sequences in salmonids, with similar properties to the UV photopigment in goldfish. Based on molecular data, Hisatomi *et al.* (1996) stated that similar sequences have been identified in: 1) the Ostariophysi, Family Cyprinidae [goldfish and zebra danio (*Danio rerio*)]; 2) a Protacanthopterygian, Family Salmonidae [chum salmon (*O. keta*)], mistakenly ascribed to the Clupeiformes (herrings) by Hisatomi *et al.* (1996); and 3) an Acanthopterygian, Family Oryziidae [the medaka (*Oryzias sp.*)]. Hisatomi *et al.* (1996) mistakenly called this fish a killifish (Family Cyprinodontidae) of the top-minnow family (which in fact are in the Family Atherinidae). There are important reasons to identify Hisatomi *et al.*'s (1996) clerical errors in taxonomy of the fish he studied. First, such errors lead to confusion by other researchers when doing comparative studies. Second and more importantly, the taxonomic corrections lead to the suggestion that the gene coding for UV pigments is present in several distantly-related groups of teleost fishes. This is significant because it suggests that UV photoreceptors are an ancestral feature of the entire Euteleostei. Because this group contains approximately 21,000 species and is the most speciose group of vertebrates (Nelson 1994), this would mean the number of species with UV sensitivity is potentially very large. A cogent question that arises from the observation that UV photoreceptors are found in all tetrapod classes, in addition to teleosts (Chapter I), is: Are these UV receptors homologous? Armed with the knowledge that teleosts are only distantly related to the line of evolution that gave rise to tetrapods, is it conceivable that UV-

sensitivity could be a feature of their common ancestor? This warrants extending investigations of UV sensitivity and molecular biology to other classes of fishes and other vertebrates in general to address questions of the origins and evolution of the UV photoreceptors. Finally, if UV photoreceptors are homoplasies amongst teleosts and tetrapods, what conditions gave rise to their convergent evolution?

It has been suggested that birds and mammals, in addition to the regular three classes of photoreceptor cells (S-, M-, and L-cones), have either a violet or a UV photoreceptor class of cells (Jacobs 1992), but not both. Therefore, we must ask ourselves what selective pressures and evolutionary events have caused fish to evolve a fourth class of photoreceptor in fish and reptiles? Some evidence suggests that salmonids are naturally tetraploid (Behnke 1992) as opposed to diploid as in most vertebrates. Such a doubling of ploidy level may have been the event that provided the new genetic material that allowed a fourth photoreceptor class to evolve. However, if the UV photoreceptor is widespread, then it most probably precedes the origin of the Salmoninae.

The selective pressures that gave rise to the UV photoreceptor are at present a matter of speculation. Two studies, Loew *et al.* (1993) and Browman *et al.* (1994) provided strong evidence that UV sensitivity increases prey detectability. Two additional studies, in particular, have provided physiological evidence that UV sensitivity contributes to colour vision through opponent interactions (Hárosi and Hashimoto 1983, Coughlin and Hawryshyn 1994b).

These findings are corroborated by behavioural evidence of Neumeyer (1985). This would be consistent with the idea that UV sensitivity extends the range of vision. The reason why a fish should evolve a wide spectral 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 photic conditions (Lythgoe and Northmore 1973, Munz and McFarland 1977). Following Levine and MacNichol's (1979) system for ecological and behavioural classification of fishes, the salmonids in this study would conform to Groups I, II, and III (three of four groups). The visual environment of salmonids, while not as complex as coral reef environments, nonetheless exposes the retina of a salmonid to a number of different spectral backgrounds (Novales Flamarique *et al.* 1992, Novales Flamarique and Hawryshyn 1993). This euryphotic sensitivity is mirrored in the visual systems of other neritic and epipelagic fishes, as well as many aquatic invertebrates, hymenoptera, and birds (Table 6). For an in-depth comparison of arthropods see Chittka (1996). One common feature of all of these organisms is that they must extract visual information from a three-dimensional spatial environment. Not surprisingly, other more two-dimensionally oriented species, such as the grasshopper (*Phaeoba* sp.) (Kong *et al.* 1980), the waterstrider (*Gerris lacustris*) (Bartsch 1995), and the demersal Atlantic cod (*Gadus morhua*) (Bowmaker 1990), appear to have more narrow bandwidths of spectral sensitivity (stenophotic sensitivity) (Table 6). Furthermore, it is interesting that some fish lose some classes of photoreceptors when they settle

Table 6. A comparison of the λ_{max} of UV-, S-, M-, and L-cone photoreceptors and the peak-to-peak bandwidth (nm) of sensitivity for various members of the animal kingdom.

Order	UV	S	M	L	Bandwidth
Anseriformes ¹	420	452	502	570	120
Cladocera ²	348-356	434-440	521-525	592-611	284
Cypridontiformes ³	N/A	409	463	573	164
Gadiformes ⁴	N/A	446	517	N/A	71
Hemiptera ⁵	N/A	440-470	500-530	N/A	90
Hymenoptera ⁶	328	464	540	592	264
Hymenoptera ⁷	344	436	544	N/A	200
Orthoptera ⁸	N/A	N/A	525+545	N/A	20
Passeriformes ⁹	355	454	499	568	213
Primata ¹⁰	N/A	437	535	N/A	98
Primata ¹¹	N/A	437	533	564	127
Rodentia ¹²	360	N/A	493	N/A	133
Salmoniformes ¹³	365	440	540	585	220
Stomatopoda ¹⁴	397	416-434	492-538	N/A	141

¹*Anas platyrhynchos* (Jane and Bowmaker 1988); ²*Daphnia magna* (Smith and Macagno 1990); ³*Anableps anableps* (Avery and Bowmaker 1982); ⁴*Gadus morhua* (Bowmaker 1990); ⁵*Gerris lacustris* (Bartsch 1995); ⁶*Tenthredo campestris* (Peitsch et al. 1992); ⁷*Apis mellifera* (Peitsch et al. 1992); ⁸*Phaeoba* sp. (Kong et al. 1980); ⁹*Leothrix lutea* (Maier and Bowmaker 1993); ¹⁰*Lemur catta* (Jacobs and Deegan 1993); ¹¹*Homo sapiens* (Brown and Wald 1963); ¹²*Merimones unguiculatus* (Jacobs et al. 1991); ¹³*O. mykiss* (Hawryshyn and Hárosi 1994); and ¹⁴Cronin et al. (1993).

to a demersal existence (Boehlert 1978, Shand 1993). Thus, one could speculate that euryphotic photopic sensitivity arose when fish moved from a two-dimensional to a three-dimensional world, as a result of the importance of maintaining visual contrast across a variety of spectral backgrounds.

Interestingly, Old World primates, with a primary adaptation to a three-dimensional arboreal environment, are trichromats with good colour vision (Jacobs and Deegan 1993). They also have a relatively wide maximum peak-to-peak bandwidth compared to most other mammals (Jacobs and Deegan 1993).

As an argument against this hypothesis, diurnal, arboreal Prosimians, such as lemurs from Madagascar (Jacobs and Deegan 1993), and Platyrrhine monkeys from the New World, are dichromats with only two cone pigments (Jacobs and Neitz 1987).

It is particularly interesting that there are relatively small differences in the visual sensitivity among a variety of juvenile salmonids. Bridges and Yoshikami (1970), in an extensive study, noted a lack of differences in salmonid rod photopigments. These species are closely related with the ancestral lineages leading to the Pacific salmon and those leading to the Pacific trouts diverging by the end of the Miocene Epoch (~5 mya). This is consistent with both the geographic distribution and phyletic history of these species (Behnke 1992, Stearley and Smith 1993). Coastal rainbow trout, steelhead, and coastal cutthroat trout are closely related, having separated by the late Pliocene (Behnke 1992). In fact, to date, no distinction between coastal rainbow and steelhead

forms is formally recognised (Don Campton jr., 1997, personal communication). Additionally, a large degree of natural introgression has occurred between *O. mykiss* and *O. clarki clarki* (Campton and Utter 1985). Such introgression events further reduce the genetic and phenotypic differences between these already closely related species. Given that much of the current salmonid range represents recolonisation from refugia of the last glacial period of 70,000 to 10,000 years ago, it would be expected that individuals within a species are closely related.

A second factor contributing to the non-specificity of the visual system in salmonids is undoubtedly their wide geographic range and the diversity of environments they occupy. Most species have ranges extending thousands of kilometres (e.g., coastal cutthroat trout are believed to have originated in the Columbia River basin and have expanded their range from California to Prince William Sound, Alaska) (Hart 1973, Scott and Crossman 1973, Behnke 1992). Endemic freshwater environments of salmonids range from ultra-oligotrophic to eutrophic or highly productive lakes and streams, although there is some degree of correlation between species and habitat (Scott and Crossman 1973, Behnke 1992, also see Groot and Margolis 1991). As well, environmental conditions affecting water bodies are not static and undergo seasonal changes, such as increases in turbidity in spring, changing tannin content, and algal blooms in the summer. Flexibility in the visual system should therefore be important. Additionally, different life-history stages of some salmonids, such as steelhead

and sockeye salmon, live in vastly different optical environments (e.g., natal streams, lakes, estuaries, and open ocean). Salmonids therefore should be considered to have a generalist visual system. The present study supports this view.

Polarisation Sensitivity

The similarity of spectral sensitivity of the visual system among the salmonids examined also was mirrored in the overall similarity of their polarisation sensitivity. The overall shape of the on- and off-responses to near-UV light under white-light adapting conditions has been described previously by Parkyn and Hawryshyn (1993) (Chapter II), Coughlin and Hawryshyn (1994a), and Novalés Flamarique and Hawryshyn (1996). In spite of this overall similarity, some differences in the off-responses among the species should be noted. In particular, δ was greater for *O. mykiss*, *O. c. clarki*, and *S. fontinalis* than it was for *O. nerka*. Such differences in the response function could arise as a function of inhibition of sensitivity by opponent polarisation sensitivity mechanisms. Thus, the amplitude and δ of a polarisation sensitivity curve will undoubtedly be influenced by the adaptation state of the polarisation sensitive mechanisms. Alternatively, there could be intrinsic differences in the polarisation sensitive photoreceptors among the species. Based on evidence from spectral sensitivity, at least, it appears that these species share common classes of photoreceptors.

Some differences also were observed in polarisation sensitivity in the present study relative to the literature. From Fig. 13 it was evident that spectral sensitivity of the off-response is dominated by the M-cone and L-cone mechanisms. The off-sensitivity to polarised light also is dominated by the M-cone and L-cone mechanisms, which are preferentially-sensitive to horizontally-polarised light at least in the axons of optic nerve ganglion cells (Chapter II). Coughlin and Hawryshyn's (1995) finding of little polarisation sensitivity in the on-response of the M-cone mechanism (1 of 3 single-unit recordings) or L-cone mechanism (1 of 9 single-unit recordings), as recorded from the *torus semicircularis*, is perplexing in light of their observation that horizontal-polarisation sensitivity is present in the on-response of the optic nerve using multi-unit recording (a corroboration of Parkyn and Hawryshyn 1993), as well as in off-response (Coughlin and Hawryshyn 1994b, Chapter II). This suggests one of several possibilities. First, differences existed between adaptation conditions of this study and Coughlin and Hawryshyn (1995). In particular, the description of polarisation sensitivity in the on-responses of M- and L-cones in Parkyn and Hawryshyn (1993) and Chapter II was under chromatic adaptation conditions to physiologically isolate for these respective mechanisms. Examination of spectral sensitivity in Fig. 12 and Fig. 14 suggested that under white-light or yellow-orange background conditions the M-cone does not contribute greatly to on-response spectral sensitivity. Second, Coughlin and Hawryshyn (1994b) examined only 9 units with L-cone mechanism input in the *torus semicircularis*,

so perhaps sampling bias also may contribute to these differences. A third possibility is that on- and off-responses of the M-cone mechanism may be processed in an area of the brain that was not sampled. Because horizontal-polarisation sensitivity is present in the on-responses in the optic nerve and not the *torus semicircularis* (Parkyn and Hawryshyn 1993, Coughlin and Hawryshyn 1994b), these signals may proceed 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 no evidence in fish to suggest that different populations of photoreceptors are subserving spectral and polarisation sensitivity, as they do in stomatopods (Marshall 1988) 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 at all but is merely a by-product of the mechanism that gives rise to polarisation sensitivity in the L-cone.

SUMMARY

Spectral and polarisation sensitivity were compared among rainbow trout, steelhead, cutthroat trout, kokanee, and brook char raised under similar conditions using multi-unit recording from the optic nerve. Visual pigment templates from rainbow trout, obtained from Hawryshyn and Hárosi's (1994) study, were corrected for ocular media absorption and overlaid on the resultant curves to aid in comparisons among the species. Under white-light background

conditions, the fishes had similar ranges of spectral sensitivity and could detect light from 340 nm to 660 nm. Some differences in sensitivity were observed among species. In particular, the retinal channel responsible for the detection of increments of light (on-responses) was dominated by the long-wavelength (L-) cone (red sensitive) in most species. However, in kokanee, the landlocked form of sockeye salmon, the middle-wavelength (M-) cone (green sensitive) was dominant. These differences may relate to the photic environments from which these fish originated. Using chromatic adaptation, the study also demonstrated the presence of an independent UV-cone mechanism in brook char. This was the first physiological evidence of near-UV sensitivity in the genus *Salvelinus*. Therefore, UV sensitivity is a feature common to all of the major clades of the Subfamily Salmoninae.

Polarisation sensitivity also was examined among rainbow trout, steelhead trout, cutthroat trout, kokanee, and brook char and was modelled using periodic regression analysis. Comparisons suggest that all of these species have a two-channel polarisation detection system. This system has contributions from both the horizontally-polarised light sensitive mechanism and the vertically-sensitive mechanism for the on-response. In contrast, the off-response appeared to have a contribution from only the horizontally-sensitive mechanism. Differences between the results of the present study and other studies are discussed in light of the adaptation state of the fish and our growing body of knowledge on the basis of polarisation sensitivity in fishes.

CHAPTER IV

EFFECTS OF THE TUBERCULOSTATIC DRUG ETHAMBUTOL ON SPECTRAL AND POLARISATION SENSITIVITY OF RAINBOW TROUT

INTRODUCTION

Several studies on humans have examined red-green colour blindness induced by the tuberculostatic drug Ethambutol (Pau and Wahl 1972, Trusciewicz 1975, Zrenner and Krüger 1981). It has also been shown in goldfish, both with behavioural studies and physiological recording from horizontal cells in the retina, that Ethambutol decreases sensitivity in the L-cone mechanism and to some extent the M-cone mechanism (Van Dijk and Spekreijse 1983, Spekreijse *et al.* 1991, Kohler *et al.* 1992, Wietsma *et al.* 1995). When using physiological recording techniques, the L-cone mechanism typically dominates spectral sensitivity of the on-response in both goldfish and rainbow trout under white-light background conditions (Daw 1968, Wheeler 1979a, Douglas and Hawryshyn 1990, DeMarco and Powers 1991, Coughlin and Hawryshyn 1994b, Chapter III). In addition, it contributes to polarisation sensitivity in salmonids, such as rainbow trout (Parkyn and Hawryshyn 1993, Coughlin and Hawryshyn 1994a, Chapter III).

Ethambutol is believed to act upon horizontal cells in the retina (Van Dijk and Spekreijse 1983, Spekreijse *et al.* 1991). Additionally, spectral sensitivity

differences have been recorded from goldfish ganglion cells following exposure to Ethambutol. At present, however, the effects of Ethambutol on UV-cone mechanism and polarisation sensitivity of fishes are unknown. Examination of the effects of Ethambutol on the visual system of trout may therefore provide insight into physiology of the trout visual system. The objective of the present study was to use, for the first time, multi-unit recording from the optic nerve to characterise the effects of Ethambutol on spectral sensitivity. In addition, the effects on trout are unknown and one special feature of multi-unit response methodology is that it allows the characterisation of the effects of the drug on both the on- and off-response channels mediating vision. These channels monitor increments and decrements of light and appear to be dominated by different cone mechanisms (Wheeler 1982, 1987, Beaudet *et al.* 1993, Parkyn and Hawryshyn 1993, Chapter III). Using this methodology then, this study will experimentally test the effects of Ethambutol on the on- and off-response channels contributing to spectral and polarisation sensitivity.

METHODS

Juvenile rainbow trout (3.4 ± 0.7 g ($\bar{x} \pm 1SE$)) were obtained from the Vancouver Island trout hatchery, Duncan, B.C. Twelve, 2-L aquaria with a flow-through water source were maintained at 15° C. Six of these aquaria were

randomly assigned to controls and the remaining six tanks were assigned to the treatment group. One fish was placed in each aquarium.

The treatment group were fed pellets containing Ethambutol Dihydrochloride ($C_{10}H_{24}N_2O_2 \cdot 2HCl$) (Sigma Chemical Company, St. Louis MO). To formulate this feed, 300 mg powdered Ethambutol Dihydrochloride was mixed with 100 g of finely ground Biodiet Grower[®] fish food pellets (Warrington, Oregon). Water was added to reconstitute the mixture as a paste. The resultant paste was extruded as strings from a syringe onto wax paper and air dried. The strings were cut with a razor blade to produce pellets ~2 mm in diameter and ~3 mm in length and stored in a sealed container in a refrigerator. An identical procedure was carried out to produce the control pellets except that Ethambutol was not added. All fish (both control and treatment groups) were maintained on the control-feed ration for one week prior to commencement of the feeding trials. Fish were fed by carefully placing one pellet on the surface of the water. When this pellet was consumed a second pellet was provided. Fish were fed twice daily. Consumption of two treatment pellets per day resulted in the treatment fish being administered $900 \text{ mg Ethambutol} \cdot \text{kg}^{-1} \text{ body weight} \cdot \text{d}^{-1}$ or approximately $3.0 \text{ mg Ethambutol} \cdot \text{d}^{-1}$ per fish. Following consumption of the treatment pellets, all fish were fed to satiation with control-feed pellets. For the first week of administration of the treatment pellets, the fish did not readily consume the Ethambutol pellets. Several pellets were offered to the treatment fish but were not consumed immediately. These pellets were allowed to sit in the

tank until completion of a particular feeding session, after which they were removed. By the second week, the treatment fish were eating the Ethambutol pellets. The duration of the experiment was therefore extended an additional week to ensure that the fish had been receiving a constant dose of Ethambutol for a minimum of 4 wks. Mass and length were compared between treatment and control groups at the termination of the feeding trials to determine whether growth rates differed between the two groups (One-way ANOVA). Such differences might indicate reduced health in one group, which could potentially affect the experimental results. Statistical significance was indicated by $P \leq 0.05$.

Spectral and polarisation sensitivity were determined against a white-light adapting background (Chapter II) by recording multi-unit responses from the optic nerve (GCRs). Details of the methodology are presented in Chapter II and Chapter III, Parkyn and Hawryshyn (1993), and Beaudet *et al.* (1993). Spectral sensitivity of Ethambutol-treated fish and controls were compared using an Analysis of Variance with Repeated Measures (ANOVAR) (SPSS v. 6.0) (Huhyn and Mandeville 1979, Norušis and SPSS Inc. 1993, Zar 1996). Following characterisation of spectral sensitivity, three of the control fish and three of the Ethambutol-treated fish were examined to determine their polarisation sensitivity. The small sample size of this latter experiment precluded statistical analysis.

RESULTS

Both groups of fish grew during the feeding trials of the experiment. In addition, growth did not differ between the control and treatment fish, in terms of mass (Ethambutol Group: 4.4 ± 0.8 g, $n=6$ [$\bar{x} \pm 1SE$]; Control Group: 4.0 ± 1.2 g, $n=6$); One-way ANOVA, $F_{0.05(1), 1.11} = 0.29$, $P = 0.60$) and total length of fish (Ethambutol Group: 74.6 ± 5.2 mm; Control Group: 78.8 ± 4.9 mm; One-way ANOVA, $F_{0.05(1), 1.11} = 1.73$, $P = 0.23$).

Spectral Sensitivity

Both on- and off-responses were present in multi-unit responses of the optic nerve following treatment with Ethambutol (Fig. 19, 20). Relative to the control fish, however, Ethambutol altered spectral sensitivity of the on-response in *O. mykiss* (Fig. 18). Specifically, overall spectral sensitivity of on-responses for Ethambutol-treated fish were significantly lower than control fish (ANOVAR, $F_{0.05(1), 1.11} = 15.52$, $P < 0.003$) (Fig. 18). Sensitivity varied significantly as a function of wavelength ($F_{0.05(1), 16, 144} = 16.99$, $P < 0.001$), A significant remainder (treatment x wavelength interaction) ($F_{0.05(1), 16, 144} = 2.17$, $P = 0.008$), however, precluded a statistical examination of wavelength as a main effect. Graphically, this interaction was represented by a concomitant decrease in spectral sensitivity of Ethambutol-treated fish at the two ends of the fish's visual spectrum

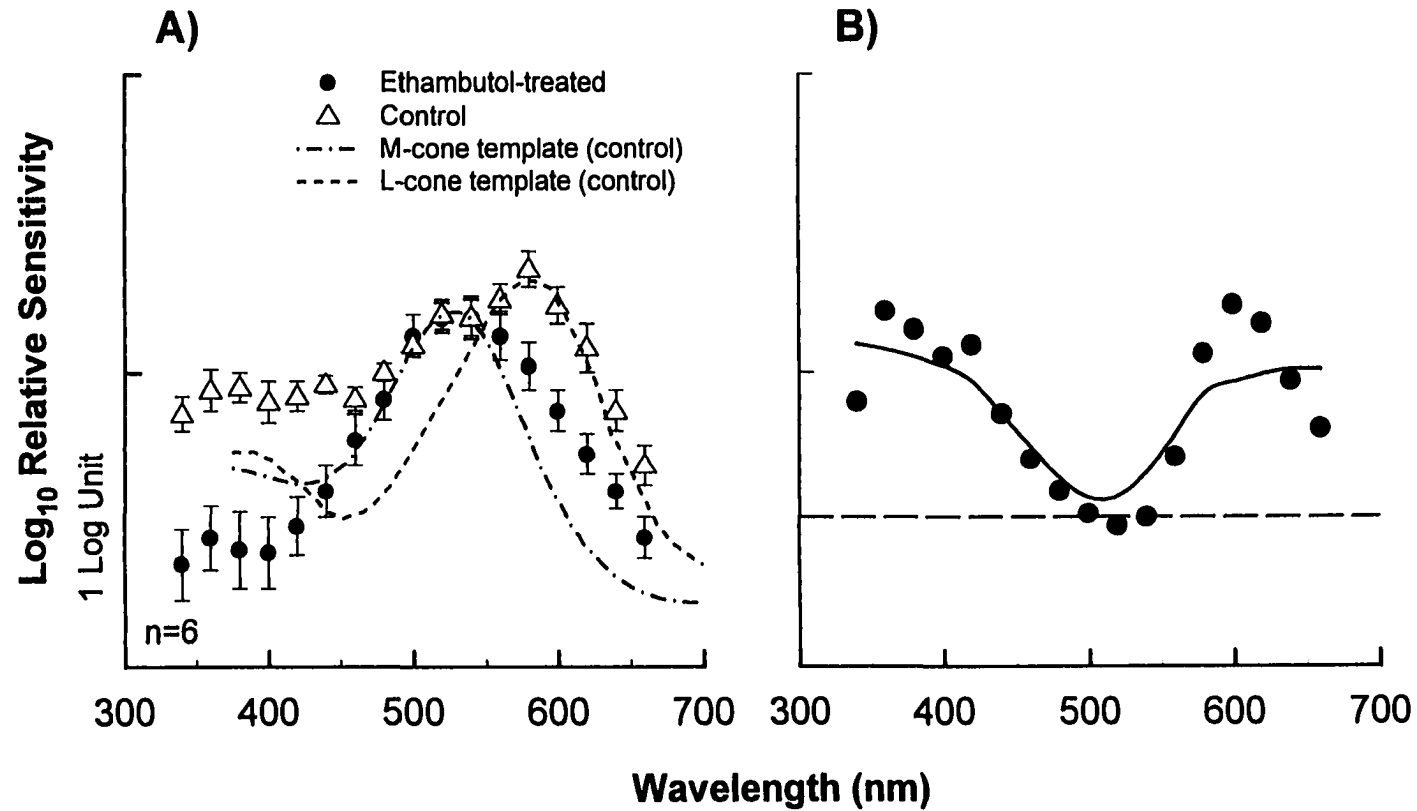


Figure 19. A) Relative spectral sensitivity of on-responses obtained from multi-unit recordings in the optic nerve for Ethambutol-treated fish and control fish; and B) Difference in spectral sensitivity (Control - Treatment). Background adaptation conditions were a dim white-light (Tungsten-Halogen) background (see Parkyn and Hawryshyn 1993). Values represent means \pm 1 SE.

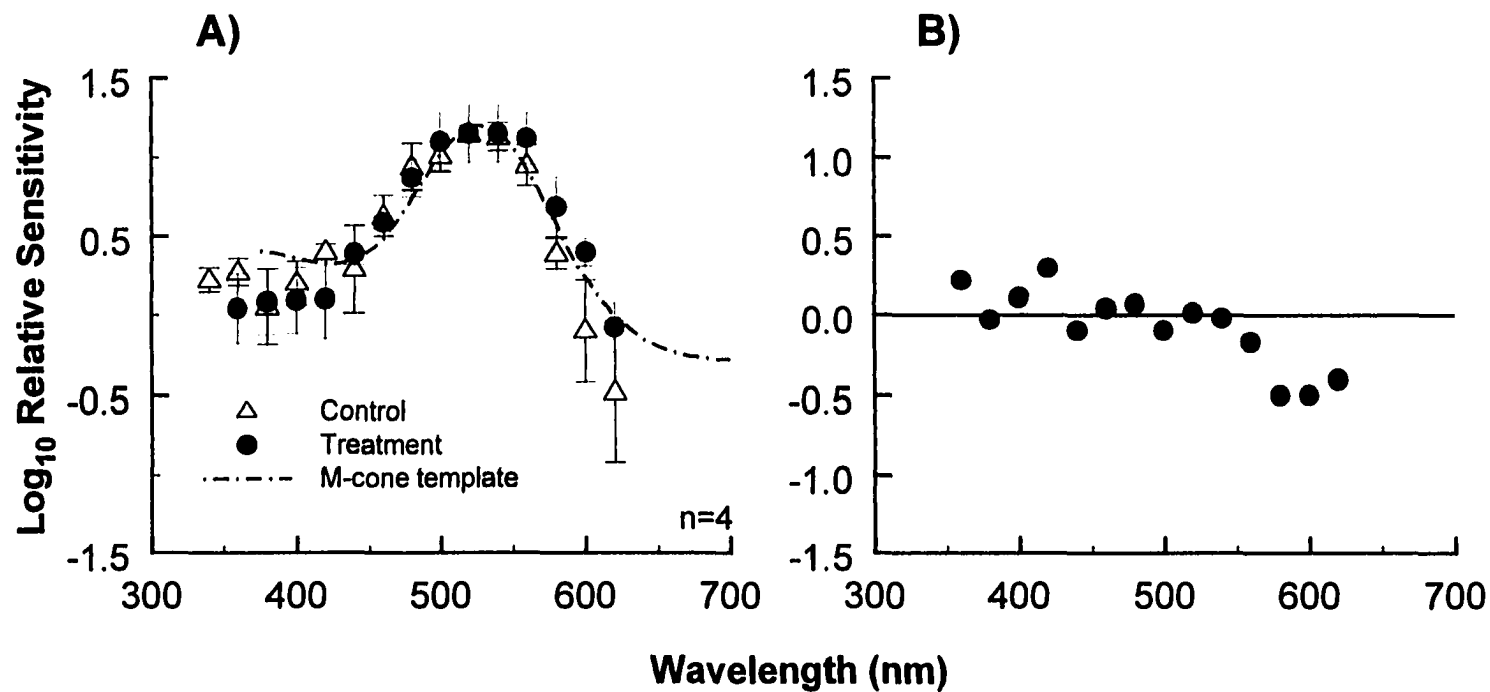


Figure 20. A) Relative spectral sensitivity of off-responses obtained from multi-unit recordings in the optic nerve for Ethambutol-treated fish and control fish; and B) Difference in spectral sensitivity (Control - Treatment). Background adaptation conditions were a dim white-light (Tungsten-Halogen) background (see Parkyn and Hawryshyn 1993). Values represent means ± 1 SE.

(Fig. 18A). This difference was most evident in the 600-640 nm (red region) and the 340-440 nm (near-UV/Blue region) (Fig. 19B).

In contrast to control fish, no differences were detected between the on-response and the off-response of the Ethambutol-treated fish (Fig. 19A, 20A). Both the on- and off-responses of the Ethambutol-treated fish were dominated by the M-cone mechanism (Fig. 19A, 20A). In addition, the spectral sensitivity of the off-responses did not differ between treatment and control fish (ANOVAR, $F_{0.05, 1.7} = 0.10$, $P = 0.75$) (Fig. 20B).

Polarisation Sensitivity

As with spectral sensitivity, polarisation sensitivity of the on-response was also affected by Ethambutol. Relative to control fish (Fig. 21B), no vertical polarisation sensitivity (0°, 90°, 180°) was evident in the on-response of treatment fish (Fig. 21A). In contrast, the off-response was dominated by a horizontally sensitive mechanism for both the Ethambutol-treated fish and the control fish (Fig. 21A, 21B).

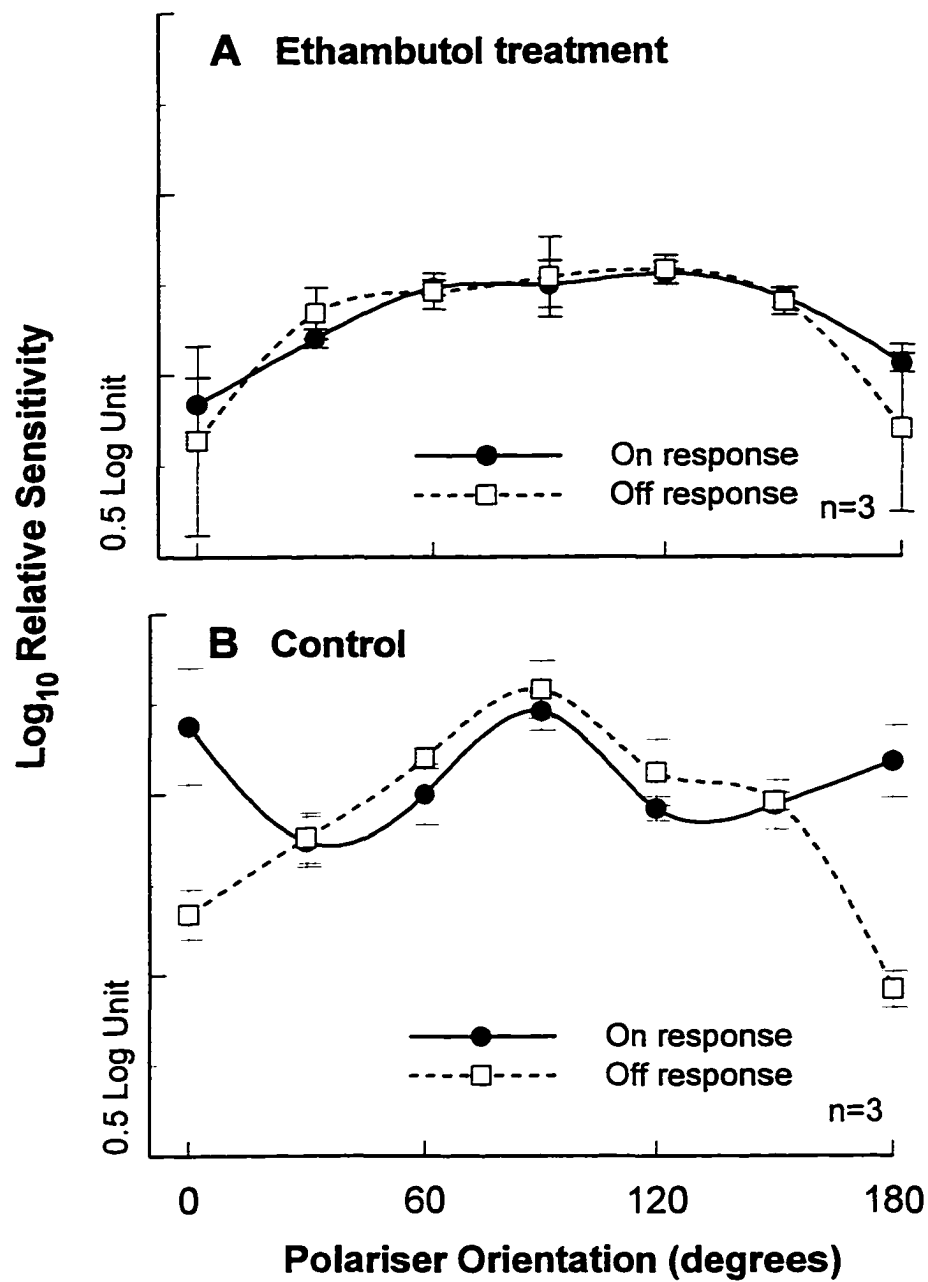


Figure 21. Polarisation sensitivity of on- and off-responses to a polarised light stimulus ($\lambda = 360$ nm) recorded using multi-unit responses from the optic nerve of: A) Ethambutol-treated fish; and B) Control fish. Background adaptation condition was a dim white-light (Tungsten-Halogen) background. Values represent means ± 1 SE.

DISCUSSION

Spectral Sensitivity

Ethambutol and other pharmacological agents, such as APB (DL-2-amino-4-phosphonobutyric acid), appear to affect colour vision pathways and spectral sensitivity (Spekreijse *et al.* 1991, DeMarco and Powers 1994, Bilotta *et al.* 1995, Wietsma *et al.* 1995). Ethambutol was first believed to act upon horizontal cells in the retina, perhaps through an inhibition of spinule formation (Van Dijk and Spekreijse 1983, Spekreijse *et al.* 1991). This would result in the retina remaining in something similar to a dark-adapted state. However, more recent work has indicated that the long-term effect on horizontal cell spinule formation is not significant, rather the rate of spinule formation is lowered, while the final number is unaffected (Kohler *et al.* 1992, Wietsma *et al.* 1995). This renders the effect on horizontal cells as transitory, occurring for about 15 min following an Ethambutol injection (Wietsma *et al.* 1995). In addition, Wietsma *et al.* (1995) also found that it did not affect spectral sensitivity of mono-, bi-, or tri-phasic horizontal cells; thus, the effect must be occurring at a point past the horizontal cells. Wietsma *et al.* (1995) have now proposed that Ethambutol affects the inner plexiform layer. Previous examination of the effect of Ethambutol on spectral sensitivity in goldfish concentrated on the drug's effect on sensitivity in the red, green, and violet range of the spectrum. The present study provides the first evidence that sensitivity of the UV-cone mechanism is also decreased by

Ethambutol. In addition, some decrease in S-cone sensitivity was observed. Spekreijse *et al.* (1991) stated that Ethambutol does not affect sensitivity in the UV range of the spectrum but, in fact, they did not examine the effects of Ethambutol in the UV region of the spectrum. Rather, their test fish were trained at 404 nm to discriminate colours in the violet and blue region of the spectrum with no comparisons in the UV region of the spectrum. Under such a scenario, a fish would not need a UV photoreceptor to discriminate in the violet region, because the S-cone and the M-cone are less affected by Ethambutol. Differences in violet-blue sensitivity in the present study may be a result of differences in the methodologies used to examine sensitivity or, alternatively, differences in the visual systems of goldfish and rainbow trout. As in previous studies on goldfish by Van Dijk and Spekreijse (1983) and Spekreijse *et al.* (1991), the Ethambutol-induced decreases in L-cone sensitivity also were observed in the present study. Since the β -band of the L-cone mechanism appears to contribute to on-response spectral sensitivity in the UV region of the spectrum (Chapter III), one cannot rule out that the observed decreases in the UV portion of the spectrum result from decreases in the contribution from this cone mechanism. However, rainbow trout of similar sizes to those used in this study do have UV cones that contribute to on-response spectral sensitivity (Hawryshyn *et al.* 1989, Beaudet *et al.* 1993, and Chapter III). The low sensitivity in this region of the spectrum results from the action of Ethambutol or opponent inhibition by the M-cone mechanism cannot be ascertained without a

study of chromatic adaptation to attempt to physiologically isolate the UV mechanism.

Overall, on-responses from the optic nerve were altered by Ethambutol whereas off-responses were not affected. The similarity of the on- and off-responses of the treatment fish suggests that the M-cone mechanism dominates both spectral channels in rainbow trout under such conditions. This was corroborated by the similarity of both the on- and off-spectral sensitivity curves, as visualised using MSP-templates of rainbow trout photopigment absorption curves that have been corrected for ocular media losses of light (Hawryshyn *et al.* 1989, Hawryshyn and Hárosi 1994, Chapter III). In this respect, Ethambutol appears to mimic the effects of M-cone isolation using chromatic adaptation. In contrast to trout, the off-response in goldfish is dominated by an L-cone mechanism (Wheeler 1979a, DeMarco and Powers 1991). Yet, in both species, the L-cone sensitivity is affected. Thus, while the mode of action of Ethambutol is not entirely clear, further research is required to address the question of what features of the M-cone mechanism render it relatively unaffected by Ethambutol.

Polarisation Sensitivity

The observation that both polarisation and spectral sensitivity were affected by Ethambutol gives credence to the idea that, at least at the level of the retina, these two visual functions are sharing much of the same neural hardware.

With Ethambutol treatment, an entire opponent mechanism was removed from polarisation sensitivity (Fig. 21). This gives further support to the idea that opponent mechanisms or cone pathways have specific classes of receptors associated with them (Marc and Lam 1981, DeMarco and Powers 1994). In fact, the observed polarisation sensitivity of the Ethambutol-treated fish to only horizontally-polarised light was reminiscent of large fish that have undergone an ontogenetic loss of the UV photoreceptor, or fish that have undergone chromatic adaptation to isolate the horizontal polarisation-sensitive mechanism (Parkyn and Hawryshyn 1993, Coughlin and Hawryshyn 1994a). Because the L-cone contribution to spectral sensitivity was reduced, the single polarisation sensitive channel was contributed by the M-cone mechanism for both the on- and the off-response. Coughlin and Hawryshyn (1994a) found that the M-cone mechanism does not appear to contribute to polarisation sensitivity in the *torus semicircularis* of *O. mykiss*. However, the M-cone has been demonstrated to contribute to horizontal polarisation sensitivity of both the on- and off-responses in multi-unit recordings from the optic nerve (Parkyn and Hawryshyn 1993, Novales Flamarique 1997), at least under conditions of chromatic adaptation. Given that Ethambutol mimics the effects of chromatic adaptation, it may be a useful tool for examining the nature of the role of M-cone polarisation sensitivity in the processing of polarised light cues in rainbow trout and other salmonids.

SUMMARY

Juvenile rainbow trout (3.4 ± 0.7 g) were fed $900 \text{ mg}\cdot\text{kg}^{-1}$ body mass $\cdot\text{day}^{-1}$ of the tuberculostatic drug Ethambutol to examine its effects on their visual system. Fish were fed daily for one month and then spectral sensitivity and polarisation sensitivity of Ethambutol-treated fish were compared to control fish. At the end of the feeding trials, the masses of both experimental and control fish were similar and both groups of fish had gained weight. Using multi-unit recording from the optic nerve, spectral sensitivity of the on-response of control fish was dominated by the L-cone mechanism under white-light conditions. In contrast, spectral sensitivity of the on-response of the Ethambutol-treated fish was dominated by the M-cone mechanism. Examination of polarisation sensitivity also indicated that only the horizontal class of polarisation sensitive mechanisms contributed to polarisation sensitivity for both the on- and off-channels of ganglion cells. The UV mediated, vertical-sensitive channel was absent. These two lines of evidence suggest that Ethambutol also reduces sensitivity of the UV-cone mechanism, in addition to the L-cone mechanism.

CHAPTER V

ORIENTATION OF SALMONIDS TO LINEARLY-POLARISED LIGHT: LABORATORY AND FIELD COMPARISONS

INTRODUCTION

Studies of the sensory basis of directed movements, such as migration, are a common topic in the zoological literature. Speculation on the mechanisms mediating migration in fishes has been documented since at least Buckland (1880), over a century ago. The first scientific study of this phenomenon was undertaken by Craigie (1926) on migrating Fraser River sockeye salmon. Since that time many studies have been conducted on the mechanisms of migration. In spite of this, there is much we do not understand concerning the roles and relative importance of different sensory modalities available to fishes for migratory behaviour. Trout, char, and salmon are of particular interest because of both their economic value and their high degree of philopatry or spawning-site fidelity (in excess of 85%) during reproduction (Quinn 1990). This philopatry appears to be a general feature of the subfamily Salmoninae, regardless of life history strategy or degree of diadromy (Quinn 1990). Sensory mechanisms and cues implicated to mediate migratory behaviour in fish include rheotaxis (Harden-Jones 1968, Quinn and Groot 1984a, Arnold and Cook 1984), chemotaxis (Buckland 1880, Craigie 1926, Hasler and Wisby 1951, Fontaine and Vibert

1952, Wisby and Hasler 1954, McInerney 1964, Kleerekoper 1969, Johnson and Hasler 1980, Hasler and Scholz 1983, Nevitt *et al.* 1994), sound (Kleerekoper and Chagnon 1954), thermal gradients (Ekman 1932, Neave 1964, Groot and Quinn 1987), magnetic sense (Quinn and Brannon 1982, Taylor 1986, Klimley 1993), electrolocation (Heiligenberg 1974, 1977, Metzner and Viete 1996), and vision-based cues such as sun-compass orientation, including solar azimuth and polarised-light (Aronson 1951, Hasler *et al.* 1958, Braemer 1960, Winn *et al.* 1964, Dill 1965, 1971, Groot 1965, Hasler 1971, Forward *et al.* 1972, Forward and Waterman 1973, Waterman and Forward 1972, Kleerekoper *et al.* 1973, Churmasov and Stepanov 1977, Kawamura *et al.* 1981, Levin and Belmonte 1988, Hawryshyn and Bolger 1990, Hawryshyn *et al.* 1990).

With the discovery that several species of fish could orient to linearly - polarised light¹ (menotaxis), it was proposed that they also may use polarised light for orientation during migration (Groot 1965, Dill 1965, 1971, Forward *et al.* 1972, Forward and Waterman 1973, Hawryshyn and Bolger 1990, Hawryshyn *et al.* 1990). The utility of polarised light as a cue for menotaxis has been demonstrated for invertebrates (see Chapter I), as well as for amphibians (Taylor and Adler 1973) and birds (Able 1982, 1989, Moore and Phillips 1988, Phillips and Moore 1992). However, for a contrasting view point on polarisation sensitivity in birds, see Kramer (1950), Montgomery and Heinemann (1952),

¹ No evidence of biological utility has been ascribed to circular-polarised light by animals. However, it can be detected by trained, human observers (Gerharz 1982).

Coemans *et al.* (1990), and Vos Hzn *et al.* (1995).

Although physiological characterisation of polarised-light sensitivity in salmonine fishes has been presented elsewhere (Chapters II and III, Parkyn and Hawryshyn 1993, Coughlin and Hawryshyn 1995, Novales Flamarique and Hawryshyn 1997a), little recent work has been directed at examining roles of polarisation sensitivity in behaviour of these fishes. To date, three extensive investigations of the relationship of polarisation sensitivity to migration in salmonids have been undertaken using behavioural methodologies: 1) Groot (1965); 2) Dill (1965, 1971); and 3) Hawryshyn *et al.* (1990) and Hawryshyn and Bolger (1990). However, each of these studies was restricted to the behavioural performance of a single species and there has been a general lack of comparative studies.

Recently, the importance of the involvement of UV light in the perception of polarised light by juvenile salmonids (i.e., parr) has been recognised (Hawryshyn and McFarland 1987, Hawryshyn *et al.* 1990, Hawryshyn 1992, Hawryshyn and Parkyn 1993, Coughlin and Hawryshyn 1995, Novales Flamarique and Hawryshyn 1997a). This role of UV light was not known at the time that previous field-based studies of polarised-light orientation in fishes were conducted (e.g., Groot 1965, Forward and Waterman 1973), although Kramer (1950) believed that UV light may be an important factor in the orientation of birds.

The objectives of the present study were twofold: 1) to examine the behavioural responses of juvenile salmonine fishes (rainbow trout, steelhead, and brook char) to determine if menotaxic responses occur under controlled laboratory conditions, using an operant behaviour methodology. This also involved a comparison of differences in menotaxic responses among species because steelhead and brook char have not previously been examined. In addition, the model species, rainbow trout, was examined as to whether or not menotaxis is an innate or learned response and 2) to determine the importance of polarised light to the orientation of juvenile salmonine fishes (steelhead and sockeye salmon) in the field under semi-natural conditions. As well, the accuracy, and thus the utility, of this cue was compared between the laboratory and the field experiments. In addition, conditions under which polarisation sensitivity might reasonably serve as a cue for orientation were assessed.

METHODS

Training under Laboratory Conditions

Juvenile rainbow trout and steelhead (the anadromous form of rainbow trout) were obtained from the Fraser Valley Trout Hatchery and the Vancouver Island Trout Hatchery, respectively, and were held in the fish culture facility at the University of Victoria for a minimum of 3 weeks. Juvenile brook char were obtained from stock that was spawned in the fish-holding facility of the University

of Victoria (see Table 2 for species origins). Fish were trained to orient either perpendicular or parallel to the axis of a linear polariser using a methodology modelled after Hawryshyn and Bolger (1990). Training of the fish involved the use of a 1 m x 1 m L-shaped training tank constructed from clear, UV-transmissive Plexiglas (OP-1, Cyro Plastics), within a larger 2.5 m diameter tank (Fig. 22). The training tank was constructed out of Plexiglas to allow the fish to become familiar with the larger surrounding tank where subsequent testing was conducted. The training tank was filled with water to a depth of 15 cm. The water in the training tank was changed between each fish-training episode. This was done both to keep the water temperature relatively constant between training bouts and to reduce the effects of olfactory cues on behaviour of replicates (C. Groot, personal communication).

Observations were conducted on juvenile rainbow trout (mean mass ($\bar{x} \pm 1SE$) = 4.8 ± 0.3 g) to determine the effectiveness of the training protocol. Fish were deprived of food for 3 days prior to the first training session. A single fish was placed in a clear plastic cylinder at the vertex of the right angle of the tank. After a 5-minute acclimation period, the cylinder was lifted away from the training tank by an attached monofilament line. Fish then were observed and the frequency with which they selected either one of the two end channels of the training tank was recorded for a complete training session. A fish was considered to have selected a channel once it remained in one arm of the training tank for more than 30 sec, or if the fish swam to the end of the channel.

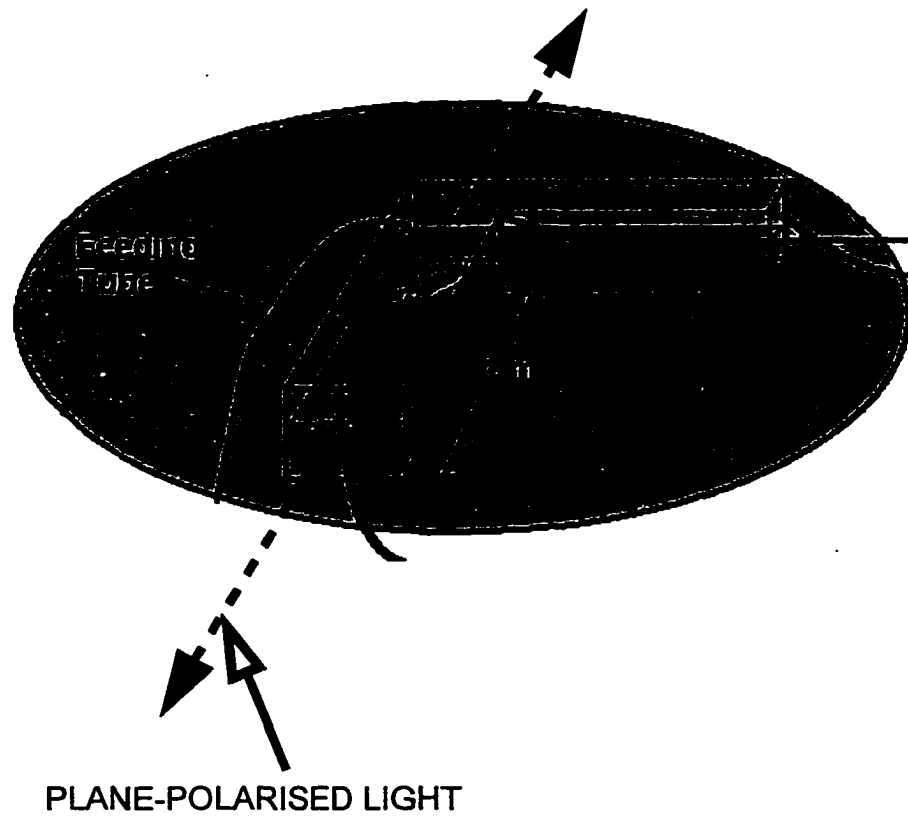


Fig. 22. Plexiglas training tank used in laboratory experiments illustrating positions of Tygon® tubing used for delivery of food rewards (Biodiet® pellets). Polarised light field was generated using a 1000 W Quartz Halogen Tungsten Lamp and HNP'B® Polariser.

When this occurred, the fish received a food pellet (Biodiet Grower[®], Warrington Oregon) through one of four Tygon[®] tubes located at the two ends of the tank and on opposite walls of the training tank at its vertex (Fig. 22). The food pellets were delivered by sending a short pulse of air down the tube, causing the pellet to land on the surface of the water near the end of the tank. When the fish swam back to the vertex of the tank, a second pellet was rewarded. Fish that did not begin to respond to training by actively foraging were eliminated from the trials after three training sessions and thus were not included in the training calculations. Unresponsive fish typically would remain at the release site for the duration of the trial and would not respond even if a food reward was presented. Fish that did perform a correct behaviour received rewards for each correct response for up to 10 pellets in a single training session. After three successful training sessions on a fixed-ratio reinforcement schedule, a variable-ratio or intermittent reinforcement schedule was initiated (Mazur 1986). Fish then were randomly rewarded with a single pellet, after performing 1 to 6 correct behaviour responses, for a total of up to 10 pellets. Fish were maintained on this training schedule for an additional seven training sessions. Following successful completion of the training schedule, the fish were tested.

The Polarised-light Source

The light source, a voltage-regulated DC 1000 W tungsten-halogen lamp (Oriol[®]) with UV-A transmissive optics, was suspended from the ceiling above the

centre of the tank. Infra-red radiation was removed from the light envelope by a water-cooled, UV-transmissive filter (Oriol[®]) fitted to the end of the projection lens assembly. The projection lens of the lamp was adjusted to maximise the spread of the light beam to a 50-cm diameter. The beam of light was projected upon a 50-cm disk of UV-transmissive Plexiglas (Cyro Plastics OP-I[®]). The Plexiglas was frosted on both sides and was used as a diffuser to remove inherent polarisation in the optical system. Interaction of inherent polarisation in the optical system and the polarising filter can result in the formation of potential brightness cues (Coemans *et al.* 1990, Parkyn and Hawryshyn 1993).

Therefore, the diffuser was tested for polarised-light transmission using two polarisers (HNP'B, Polaroid[®]) and a Photodyne radiometer (Optikon[®]). This was done by placing a piece of polariser on either side of the Plexiglas, one as a polariser, and one as an analyser. The light source was used to illuminate the polariser. Measurements of the intensity of transmitted light were made when the axes of the polarisers were crossed and when they were perpendicular relative to each other. Percent polarisation was calculated following Hawryshyn and Bolger (1990) [Equation 2].

To ensure that depolarisation by the Plexiglas was not wavelength dependent, polarisation transmission of four bandwidths of polarised light was examined using interference filters (Table 7). The light was depolarised almost completely and % polarisation did not differ as a function of wavelength (Kruskal-Wallis test, $\chi_{3df}^2 = 6.1$, $P = 0.11$).

Table 7. Percent polarisation transmission of linearly-polarised light projected through the Plexiglas diffuser, as measured using a photometer and an analyser (Polaroid HNP'B). Wavelengths correspond to 380, 460, and 540 nm narrowband interference filters, and a 600 nm longpass interference filter in combination with a 650 nm shortpass filter for the red region of the spectrum.

Wavelength (nm)	Polarised light (with Diffuser) % (± 1 SE)	Polarised light (no Diffuser) % (± 1 SE)
380	0.57 (0.4)	93.6 (1.7)
460	0.68 (0.2)	94.4 (1.2)
540	0.62 (0.3)	95.4 (2.6)
600 LP+650 SP	0.33 (0.7)	97.1 (1.2)

A 100-mm diameter HNP'B[®] (Polaroid Corp.) linear-polarising filter was used to polarise the light presented to the fish. A bi-weekly check of the polarising filter with a monochromatic light source ensured that at least 85% of the light was polarised for a series of wavelengths ranging from 360 nm to 700 nm. The filter was discarded and replaced if it failed to meet these criteria.

The orientation of the polariser was changed randomly between both training and testing trials (Zar 1996). During training, the position of the training tank was adjusted accordingly to maintain the same relationship between the orientation of the tank and the orientation of the polarised-light cue. This procedure was used to help obviate the potential for entrainment to brightness cues and landmark orientation (Waterman 1972). To reduce the fish's detection of stray light arising from the sides of the lamp housing and the computer analysis station, a frame was constructed around the tank and black plastic sheeting was hung. To reduce such problems further, the experiments were conducted in a light-tight room and the tank surface was coated with a flat-white paint. A pseudo-colour digital-image of the tank surface was made using a video camera and a Matrox[®] (Montréal) PIP 512 Digital Image Processor image-processing board and was used to identify potential bright spots in the tank. The optical system was then adjusted to minimise such differences. A radiometer (Optikon[®]) with a radiance collector (10° acceptance angle) subsequently was used to measure light intensity across the tank (Hawryshyn *et al.* 1990). The

intensity of light decreased approximately 1 log unit from the centre of the tank to its periphery.

Testing under Laboratory Conditions

Video-tracking Fish Movements

Fish were released into the large circular testing tank from the same clear plastic cylinder that was used during training. Fish were allowed to acclimate in the cylinder for 3 minutes prior to testing. Position of the fish during experimental trials was recorded on VHS video tape from a position 4 m above the fish, using a wide angle lens on a standard video camera (Sony) equipped with a wide angle lens (Optex®). The video tape was then pre-processed by background subtraction using an Image-1® Digital Image Processing System (Universal Imaging Corp. West Chester, PA). Using this technique, a pre-trial video frame (fish absent) was subtracted from subsequent video-image frames from the testing sequence on the tape (fish present). Thus, the background was largely removed from the video image. This procedure enhanced the contrast between the fish and the background, therefore increasing the probability that the image processor would detect and accurately determine the x,y position of the fish. The enhanced digital frames were copied to video tape and analysed using the F-chase program (Fig. 23) with a Matrox (Montréal) PIP® 512 Digital Image Processor. The F-chase program essentially provided instructions to the image processor on how to locate the position of dark pixels (the fish) against a light

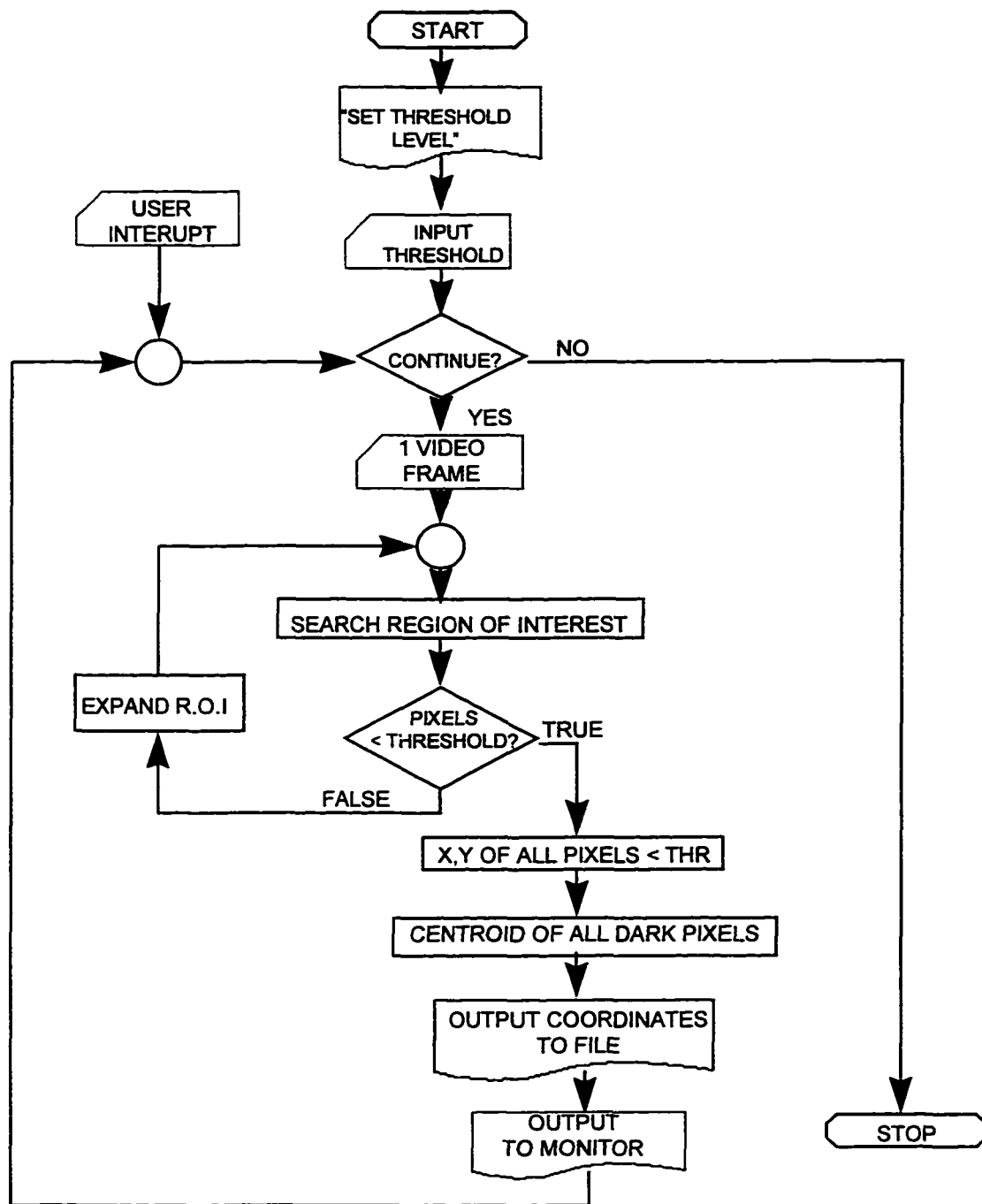


Fig. 23. Flow diagram of the F-chase tracking algorithm to determine the x,y co-ordinates of a fish released in the test tank.

background (the test tank floor) in each video frame. A pre-defined region of interest (ROI) at the centre of the tank was examined for dark pixels. This reduced the number of pixels in the video frame being analysed. The result was an increase in the speed at which the computer could track the position of the fish. If dark pixels were not located in the ROI, the computer expanded the search area. This procedure was repeated until the fish was located in the video frame. The number of dark pixels was determined, and the centroid of the cluster of pixels (analogous to the centre of mass) was determined. The x,y co-ordinates of the centroid of the dark pixels (corresponding to the fish's instantaneous position) were then plotted on the computer screen. A time stamp and x,y co-ordinates of the centroid also were written to a computer file for off-line analysis. The next video frame then was examined. The sampling rate of this program was one frame of video per 1/30th of a second.

Calculation of the Direction of Orientation

Following determination of the x and y co-ordinates of the fish after release, the mean direction of orientation ($\bar{\phi}_s$) was calculated. To determine this value, x,y co-ordinates (in pixels) of an individual fish's position were converted to polar co-ordinates (ϕ , radius), following Tierney (1975) and Batschelet (1981). The rate of data collection was high; upwards of 9000 data points were collected over the course of a trial for a fish that responded slowly. Some of these data points represented x,y co-ordinates for a constant position, (e.g., when the fish

sat near the release site for a few minutes). Such repeated data points had the potential to unduly weight or bias the determination of $\bar{\phi}_s$ if included in the analysis. Thus, these redundant positions were excluded from the analysis and $\bar{\phi}_s$ was determined from only those data points indicating a change in position. $\bar{\phi}_s$ was then determined following Batschelet (1981) and Zar (1996):

$$[10] \quad \bar{\phi}_s = \begin{cases} \arctan \bar{y} / \bar{x} & \text{if } \bar{x} > 0 \\ \pi + \arctan \bar{y} / \bar{x} & \text{if } \bar{x} < 0 \end{cases}$$

$$\text{where:} \quad \bar{x} = \frac{1}{n} \left(\sum_{i=1}^n \cos \phi_n \right) \quad \text{and} \quad \bar{y} = \frac{1}{n} \left(\sum_{i=1}^n \sin \phi_n \right)$$

The mean vector of orientation (r_v), which is an index of the directional tendency of an individual, was calculated following Batschelet (1981) and Zar (1996):

$$[11] \quad r_v = \sqrt{(\bar{x})^2 + (\bar{y})^2}$$

From r_v , the circular standard deviation (s) also was calculated (Zar 1996):

$$[12] \quad s = \sqrt{2(1 - r_v)}$$

The polar co-ordinates, ϕ and r , representing the instantaneous positions of the fish as it moved in the tank, were plotted as polar scatter plots (Axum 5.0, Mathsoft). The axis (radius) of the polar plot represented the distance from the

centre of the tank for individual fish. The angular values represented the bearing of the fish during its movements from the release area.

Testing and Statistical Analyses

In the first set of experiments, 50 juvenile rainbow trout (parr) were tested to determine if they had an innate response to plane-polarised light. These fish ranged in size from 3.2 g to 9.8 g ($\bar{x} \pm 1SE$, $6.8 \text{ g} \pm 1.4 \text{ g}$). They fish had not previously been trained to orient to plane-polarised light and thus were naïve fish. The movements of individual fish were recorded on video until they reached the periphery of the tank or until 5 minutes had elapsed from the time of their release. Fish that did not move during the trials were substituted by another fish and not included in calculations of orientation.

In the second set of experiments, rainbow trout parr of the same size were trained to orient relative to either a perpendicular or a parallel oriented e-vector using the training apparatus (Fig. 22). The relative orientation of the fish trained to orient to different angles of plane-polarised light was compared to assess the utility of this cue as a compass mechanism. Different groups of fish would have to respond at a different angle relative to the cue to maintain a directional response. An additional test was performed to determine if the orientation responses of fish trained to orient to the plane of polarised light differed significantly from a uniform distribution in the absence of polarised light. Ten fish trained to orient parallel to the plane of polarised light were tested with a UV-

transmissive Plexiglas diffuser in place of the polarising filter. A rejection of the null hypothesis of uniform distribution would suggest that the fish were using an orientation cue other than polarised light, e.g., brightness cues from the light source.

Following determination of $\bar{\phi}_s$ for each fish, the angular orientation responses were standardised relative to zero radians within the two test groups of parallel- versus perpendicular-trained fish. This was done to allow comparison of trials, because the orientation of the polariser was randomised among different experimental replicates to ensure that responses were not a function of avoidance or attraction to some external cue. Grand means, $\bar{\phi}_g$, were then determined for both parallel-trained and perpendicular-trained fish using the values for $\bar{\phi}_s$, in a procedure analogous to Equation [9] (Batschelet 1979, 1981, Zar 1996):

$$[13] \quad \bar{\phi}_g = \begin{cases} \arctan \bar{Y} / \bar{X} & \text{if } \bar{X} > 0 \\ \pi + \arctan \bar{Y} / \bar{X} & \text{if } \bar{X} < 0 \end{cases}$$

where: $\bar{X} = \frac{1}{n} \left(\sum_{i=1}^n \cos \bar{\phi}_n \right)$ and $\bar{Y} = \frac{1}{n} \left(\sum_{i=1}^n \sin \bar{\phi}_n \right)$

Similarly, \bar{r}_v and s_g were calculated in an analogous manner to Equations [11] and [12], respectively, using \bar{X} and \bar{Y} :

$$[14] \quad \bar{r}_v = \sqrt{(\bar{X})^2 + (\bar{Y})^2}$$

and

$$[15] \quad s_g = \sqrt{2(1 - \bar{r}_v)}$$

A V-test (Batschelet 1981) was applied to test the H_0 that the mean orientation response of the fish was distributed uniformly versus the H_A that the responses were non-uniform (i.e., clumped) around an *a priori* specified angle (the orientation of the polariser). When the data were perceived to be axial (bi-directional), a doubling of angles procedure was performed (Durand and Greenwood 1958, Groot 1965, Emlen 1969, Batschelet 1981). In one experiment, following application of the V-test to test distributions of rainbow trout, a non-parametric Watson's U^2 test (Zar 1996) was used to address the H_0 that fish trained perpendicularly to the plane of polarised light had a similar distribution to fish trained parallel to the plane of polarised light. The angular data from the perpendicular-trained fish were transformed by subtracting $\pi/2$ from the angles. This standardised the data to facilitate comparisons among training regimes. All statistical tests in the present study were performed with a significance level of $\alpha = 0.05$.

Orientation responses of trained, juvenile steelhead (~3-5 g) and brook char (~7-10 g) also were determined and compared with rainbow trout. A V-test was used to examine the distribution of steelhead and brook char, as per rainbow trout. The resulting test statistics were compared to a critical value obtained from Zar (1996) The multi-sample Wheeler and Watson test (Mardia

1972, Zar 1996) then was used to test for differences in orientation responses among rainbow trout, steelhead, and brook char.

Ten rainbow trout trained to orient parallel to the plane of polarised light in the controlled laboratory experiments also were tested outside to determine their orientation responses under natural lighting conditions. The testing tank was of identical construction to the tank in the light-controlled conditions. This test was used to determine if fish trained under a simple polarised-light field in the laboratory could orient using the plane of polarised light outdoors, even in the presence of many potentially confounding polarisation and skylight cues. However, spectral cues were reduced somewhat because a fish at the centre of the testing tank, near the bottom, could minimally view the sky down to 38°, whereas a fish at the edge of the tank could view the opposite region of the sky down to 22°. A forest, however, obscured the horizon in the south and west regions of the sky, such that the minimum viewable angle was 29° for a fish at the polar opposite of the tank. Horizon and sunset cues therefore were reduced. An E-vector axis-finder (Edmund Scientific, Barrington, N.J.) was used to locate the region of the sky with maximum e-vector. A radiometer (Optikon[®]) with a radiance collector (10° acceptance angle) was used to measure integrated solar energy from 200 nm-1200 nm. These values were used to calculate the % polarised light in the region with maximally polarised light. Per cent polarisation was determined using Equation [2]. Testing was conducted at sunset, May 24 and May 27, 1994, to provide the fish with a natural polarised light cue in

approximately the same position, Zenith, as in the laboratory. Responses of fish were recorded on video tape for analysis with the F-chase program. Unlike experiments in the laboratory, the polarised light field had the potential to change in both position and relative percentage among replicates and over the course of this experiment. Each fish's response therefore was plotted as the difference between the fish's position and the orientation of plane-polarised light at Zenith.

Training under Semi-natural Conditions

Experiments were conducted in New Lake, at Mountain Trout Sales, Sooke, British Columbia. The site was selected for several reasons. Located on a large plateau on the south side of a mountain, it provided a good view of the sky to the east, south, and west. Water clarity was high in the fall and winter, which made viewing the fish easier. Because of permit restrictions on the lakes associated with the fish farm, *O. mykiss* and *O. nerka* were the only species that could be examined. However, this site was ideal because it had restricted access, and thus the potential for accidental perturbation or vandalism was greatly reduced.

Twenty-five fish were held in each of three circular net-pens (Fig. 24A). The fish in the present study were placed in their net-pens on October 1, 1995. Two of the net-pens held steelhead, and a third held sockeye. Each net-pen was attached to a floating ring that was anchored in three places to permit accurate positioning of the net-pens and to prevent shifting of the feeder position

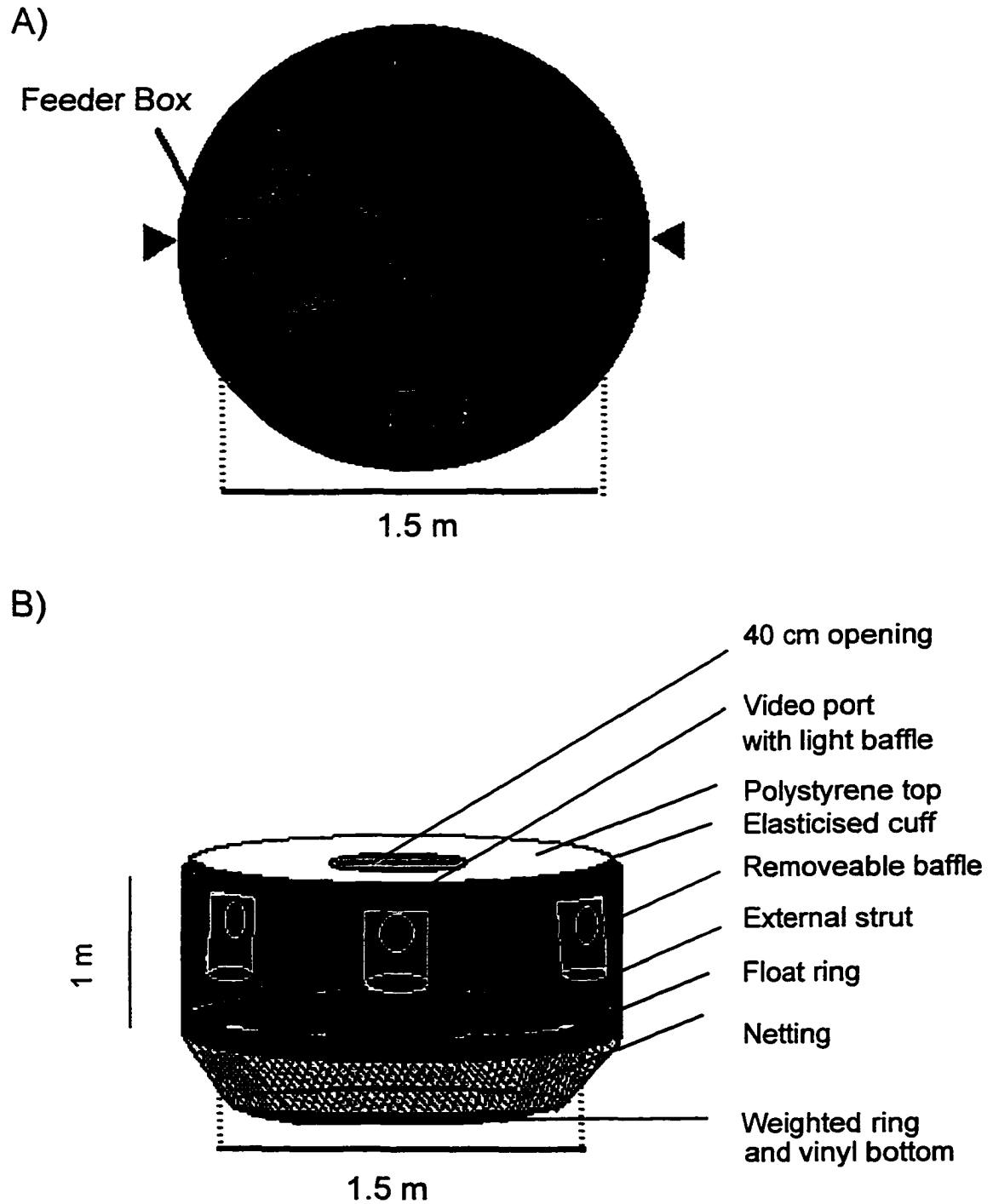


Figure 24. A) Net-pen used to train fish under semi-natural (field) conditions. An automatic feeder in one box was aligned along a compass bearing, indicated by triangles; and B) test-pen used for experiments at Sooke, B.C. Pens were tethered to the lake bottom to prevent them from shifting position.

with the wind. The bottom of each net-pen was lined with a white vinyl material to increase contrast. This allowed fish to be viewed from a distance. Predator nets were placed over each net-pen to prevent avian predation. Each predator net was constructed of large mesh (5 cm stretched-mesh) monofilament gillnet. Predator nets were removed 15 min prior to any video recording.

The position of each net-pen in the lake was displaced by about 50 m twice weekly to reduce the possibility that the fish could use landmarks along the lake shore to orient. Additionally, individual net-pens were rotated at least three times a week by moving a battery operated automatic feeder (Nutrimatic[®]) to one of four boxes on the top ring of the net-pen. The box containing the feeder corresponded to a number selected from a random numbers table (Zar 1984). This was done to prevent the fish from orienting to landmarks. The box with the feeder was then aligned to the particular compass bearing for each net-pen, using a Silva Ranger compass. Fish were fed Biodiet[®] Grower pellets (Warrington, Oregon) at both dawn and dusk. Feeding times were adjusted weekly to fall within the time period of dawn and dusk (civil twilight) in the Victoria region, as obtained from Environment Canada. Each net-pen was cleaned prior to its rotation to remove uneaten food and to keep the white vinyl-bottom and walls of the net-pen free of detritus. Fish were trained under these feeding conditions for a minimum of 2 months.

At the time of initiation of the experiments, *O. mykiss* and *O. nerka* weighed 2.8 ± 0.3 g ($\bar{x} \pm 1SE$) and 3.4 ± 0.4 g, respectively. Fish positions

were assessed a minimum of three times per week during most of the study, and a minimum of two times each week during periods of heavy snowfall and ice (because of access limitations). For purposes of comparison, eight days of data are presented, during which recordings were made from both mid-day and at sunset between December 11, 1995, and February 3, 1996. The analysis of the data was restricted to this period to control for potential seasonal effects. The data set was further restricted by examining only those periods with either 100% cloud cover or a clear sky at the time of both the mid-day and evening recording sessions. Video recordings were made at each net-pen for 5 min during each of the two time periods. Observations were made using a video camera on a raised tripod placed on a small knoll to the north of the net-pens, using a zoom lens equipped with a polarising filter to reduce glare. Weather conditions were recorded and maximum % polarisation of the celestial hemisphere was noted.

Video recordings for a particular sampling period (i.e., under sunny or cloudy conditions at mid-day or before feeding in the evening) were analysed by obtaining 10 subsamples of the positions of the 25 fish in a particular net-pen at times (with corresponding frames) selected using a random numbers table (Zar 1984). A particular frame was included in the calculations of the mean position of the group of fish if a minimum of 15 out of the 25 fish could be detected in the video frame. If this was not the case, another random number was selected to choose the next video frame. These subsamples were used to determine the mean angular orientation of the sample ($\bar{\phi}_s$) because sockeye, in particular, were

observed to swim in a circular fashion around the tank. With the sockeye, therefore, if the sampling regime had been at a fixed-time interval then the sampled data could have reflected the periodicity of the circling behaviour, biasing the calculation of $\bar{\phi}_s$. Subsampling over the entire prefeeding video-recording session should therefore provide a more representative sample of the position of fish.

To determine the mean angular responses of the fish, still frames of the video image were examined using a Panasonic® 8950 VHS player. An Image-1® Digital Video Image Processing System was employed when it was difficult to determine the position of the fish in the video frames. This usually occurred for smaller fish. Background subtraction and contrast enhancement were the two methods employed to increase visibility of the fish. Fish position was scored within 24 bins of 15°, and position was noted (i.e. inside versus the outside half of the net-pen). A χ^2 test was used to determine if fish in the inside half of the tank were distributed in a similar manner to fish in the outside half of the tank (Batschelet 1981, Zar 1996). This was done because it was more difficult to score the position of the fish if it was near the centre of the tank. If the distributions were found to be similar then the data from the two halves were combined. If the distributions were not similar (i.e. $P \leq 0.05$), then the data were treated separately by doubling the bin size of the inside circle to 30° prior to determination of the mean. The ϕ and r_v were then calculated for each subsample of fish in the net-pens (Batschelet 1981). The mean direction of the

sample ($\bar{\phi}_s$) and its mean vector (\bar{r}_v) then were calculated based on the subsamples. The grand mean direction of orientation, $\bar{\phi}_g$, then was determined from the sample means using a modification of Equation [12] for second-order analysis (Zar 1996) that weighted the mean by the length of \bar{r}_v :

$$[16] \quad \bar{\phi}_g = \begin{cases} \arctan \bar{Y} / \bar{X} & \text{if } \bar{X} > 0 \\ \pi + \arctan \bar{Y} / \bar{X} & \text{if } \bar{X} < 0 \end{cases}$$

where: $\bar{X} = \frac{1}{n} \left(\sum_{i=1}^n \bar{r}_n \cos \phi_n \right)$ and $\bar{Y} = \frac{1}{n} \left(\sum_{i=1}^n \bar{r}_n \sin \phi_n \right)$

It was important to weight the mean by \bar{r}_v in this instance because r_v reflected the degree of similarity of orientation responses within a single set of 10 subsamples. The mean vector of orientation, \bar{r}_v , was calculated using Equation [14].

Statistical comparisons were not made within the training tanks because the subsamples lacked statistical independence and because the total number of statistical replicates was small. Rather, these data initially were collected to: 1) determine if the fish were entraining on the compass bearing of the box containing the automatic feeder; and 2) determine if this entrainment varied with time of sampling or between sunny or cloudy conditions. This is relevant for the next portion of the study, in which orientation of the fish was tested under semi-natural conditions, with control of the presence or absence of environmental cues.

Testing Conditions under Semi-natural Conditions

The Testing Apparatus

A second type of net-pen (the test-pen) provided control of individual light cues and was used to assess orientation response of trained fish (Fig. 24B). This test-pen was identical to the training net-pens except that it lacked the feeder boxes. In the training net-pens it was possible to infer what cues the fish were using to orient only on the basis of meteorological conditions. Therefore, a test-pen was used to control for the visual cues available to the fish. A lid made of white polystyrene (4 cm thickness) was used to limit the amount of sky available to the fish. Testing the role of polarised light versus celestial-brightness cues on the orientation of fish was made possible by the addition or removal of: 1) a polystyrene cover; 2) a diffuser; or 3) a diffuser plus a polariser, placed over an aperture in the polystyrene lid (Fig. 24B). Six external struts were used to support a removable urethane-coated black, cloth wall. This barrier was used to prevent the fish from directly viewing the horizon or the sky immediately above the horizon in some of the experiments. A black, elasticised cloth cuff was used to prevent light from leaking through any spaces between the lid and the top of the cloth walls. The walls had video viewing ports with light traps to prevent light from leaking in from the port from which video recordings were made. The viewing port from which taping occurred was randomised among trials (Zar 1984) to reduce the potential effect of the position of the video camera on the

behaviour of the fish. The video-camera was equipped with an ultra-wide-angle lens (Optex[®]) and was set in macro-mode to enable it to view the entire tank. The camera was mounted on a tripod for stability. The fish were tested in small groups ($n = 5$), because in preliminary trials with another group of fish, it was observed that solitary fish had a tendency to hide along the periphery of the tank, whereas fish in a small group did not (Personal observation). In these trials, five groups of five fish were placed individually in the test-pen. This provided five replicate trials from each training net-pen (for a total of 25 fish). The experiments were conducted on January 29 and February 1-3, 1996. Thus, factors such as changes in daylength among experiments were not of concern.

Testing of Orientation Responses

Experimental manipulations used to test the orientation responses of fish are summarised in Table 8. In the first testing scenario, the fish were placed in the test-pen without any walls or without deprivation of any light cues. This scenario was akin to the training net-pen without the four feeder boxes. The fish were tested on both a sunny clear day and on a cloudy overcast day. In the second scenario, fish were deprived of all light cues, including polarised light, spectral, and solar cues (Table 8). This was done by placing polystyrene over the aperture in the top of the polystyrene lid of the test-pen and draping the tank with the black cloth (Fig. 24B). In the third experiment, the 40-cm diameter aperture in the polystyrene lid was covered by a disk of frosted, UV-transmissive

Table 8. Experimental scenarios for the testing pen at Sooke, British Columbia, Canada. Refer to text for details of tests.

Testing Scenario	Polariser	Spectral Cues at Zenith	Solar Cues (Brightness)
1	open sky	present	present
2	absent	absent	absent
3	diffuser	present	absent
4	absent	absent	present
5 (40 cm aperture)	Zenith	present	absent
6 (10 cm aperture)	Zenith	present	absent
7	HNP'B	present	absent
8 (polariser shifted 90°)	HNP'B	present	present

Plexiglas OP-1® (CYRO Plastics). The characteristics of this disk were previously described in Table 7. This experiment tested the orientation responses of the fish to spectral and brightness cues at Zenith in the absence of both polarised-light cues and a direct view of the region of the horizon (where the sun had set). In this experiment, a fish at the water surface could view a diffused region of the sky of $\sim 23^\circ$, whereas a fish at the bottom of the tank could view an angle of 19° . Under the fourth scenario, the fish was provided with only a view of the horizon where the sun had set and thus had access to chromatic and horizon e-vector cues. This was done by removing the black cloth barrier surrounding the testing net-pen and placing polystyrene over the aperture of the polystyrene lid of the test-pen. In the fifth experiment, orientation was examined when the fish could view Zenith only through the 40 cm aperture in the polystyrene. The black cloth skirt was used to remove cues from the horizon. The fish could detect polarised light, if present, but little of the brightness or spectral cues associated with the horizon. Under the sixth scenario, a 10-cm disk in the centre of the UV-transmissive Plexiglas plate was removed and a 5-cm high piece of double-frosted Plexiglas pipe (10 cm diameter) was attached. This pipe served as a radiance collector to prevent viewing of the sky outside the diameter of the hole in the Plexiglas. This limited the view of the celestial hemisphere to a 6° angle of acceptance for a fish viewing Zenith from the surface of the tank and a 5° angle of acceptance for a fish sitting at the bottom of the tank. This allowed the testing of orientation to a very limited patch of sky at Zenith (with other solar cues not

present). For the seventh testing scenario, a UV-transmissive polariser (HNP'B, Polaroid) was coupled to the diffuser material to provide linearly-polarised light without any interaction from inherent celestial-polarised light. This polariser was aligned with the band of maximum polarised light in the sky.

Finally, an eighth testing scenario was performed to determine which cue dominates when a fish is challenged with solar cues on the position of the sun and conflicting information on the angle of maximally-polarised light in the sky. In this case the polariser was rotated 90° to the observed maximum e-vector at Zenith and the black skirt was removed. Such a scenario would never be encountered by the fish in nature [see Wehner (1997) for charts of the distribution of polarised light in the sky at different elevations of the sun]. Essentially, when the sun is near the horizon, the distribution of maximum e-vector (under cloudless conditions) is a band across the sky passing over the observer, 90° to the axis defined by the solar and anti solar-meridian. Thus, the fish is provided with a conflict. Under such circumstances, does the fish demonstrate a preferential use of one cue for orientation over another? For this scenario, the fish were acclimated in the testing pen for 5 minutes prior to video recording. A diffuser was used to eliminate any polarised light cues during this acclimation period. The axis of the maximum e-vector was then determined using the e-vector axis finder. Since e-vector was changing with the azimuth of the sun, the position of the polariser had to be adjusted between experiments. For example, the band of maximally-polarised light on January 29, 1996, at

1550h was at a bearing of 108/288°, whereas at 1630h the bearing was 116/296°. At the onset of the test, the diffuser plate was coupled with a HNP'B polariser, which was shifted 90° to the band of maximally-polarised light in the sky.

RESULTS

Orientation Responses of Fish Under Controlled Laboratory Conditions

Acquisition of the Plane-polarised Cue

During the training protocol the total number of responses of fish doubled from the first training session to the second (Fig. 25A). During the first and second sessions, however, the fish did not associate the orientation of the e-vector with the location of food rewards, as evidenced by an equal percentage of correct and incorrect responses (Fig. 25B). Following the second training session, both the mean number of correct and incorrect responses decreased slightly, although the rate of correct and incorrect responses began to diverge (Fig. 25A). Following initiation of an intermittent reinforcement schedule at the fifth training session, the mean number of correct responses increased and reached a constant level by session eight (Fig. 25A). The percentage of correct responses also remained relatively constant from the fifth session on, ranging between 70% and 80% (Fig. 25B). However, the mean number of incorrect responses declined slightly after the intermittent reinforcement schedule was

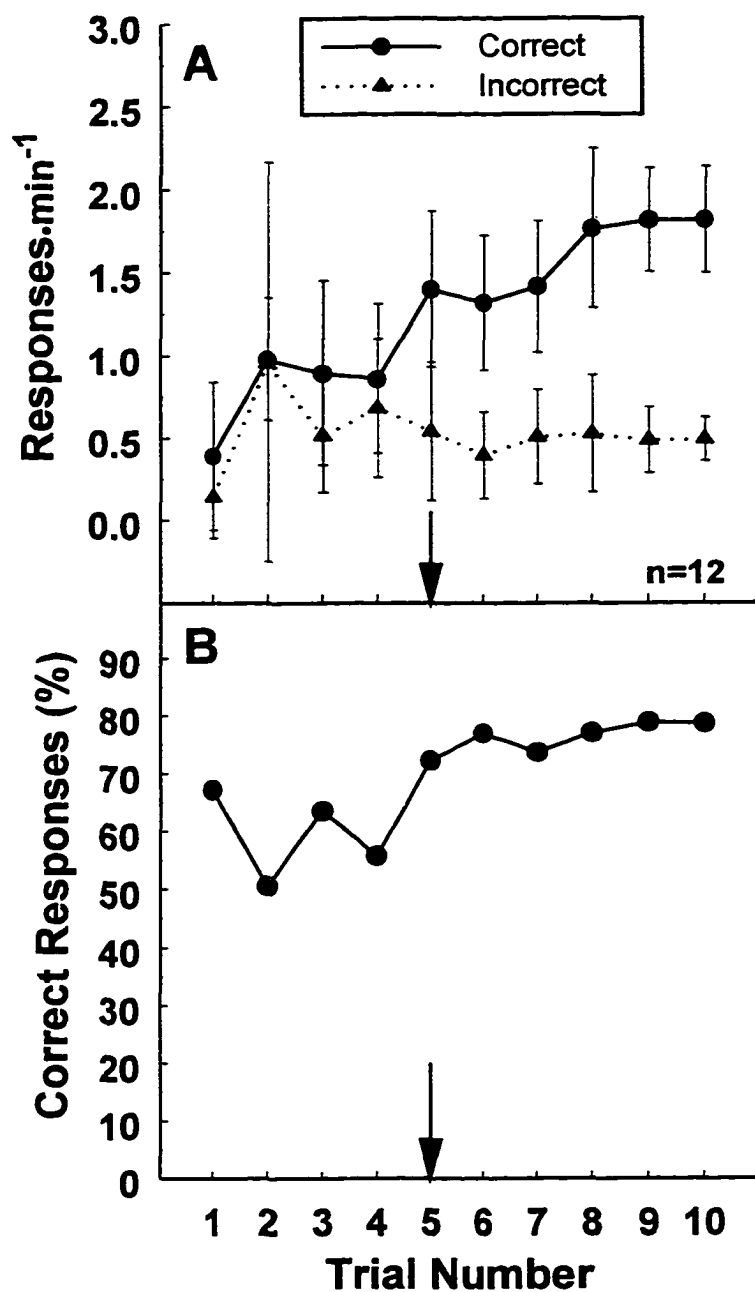


Fig. 25. Responses of juvenile rainbow trout during entrainment to a linearly-polarised light field, as a function of number of trials: A) Number of responses per minute ($\pm 1SE$); and B) Percentage of correct responses. Arrow indicates start of variable ratio reinforcement schedule.

instituted, and then remained relatively constant for the remainder of the training sessions (Fig. 25A).

Orientation of Naïve Juvenile Rainbow Trout under Laboratory Conditions

The movements of untrained (naïve) fish from the release area toward the periphery of the tank varied among individual fish from being undirected (Fig. 26 A,B,C) to movements that were relatively directed (Fig. 26B). The grand mean angle of orientation ($\bar{\phi}_g$) of untrained fish was 1.96 ± 1.17 radians, $n=50$ ($112.3^\circ \pm 67.0^\circ$) ($\bar{x} \pm 1SE$), while $\bar{r}_v = 0.32$ (Fig. 27). The distribution of the angular responses of naïve fish did not differ significantly from a uniform distribution (V-test: $P > 0.05$). Thus, as a group, these juvenile rainbow trout had no common, innate orientation response. An additional 13 fish did not move from the release area in the centre of the tank during the trials and thus were excluded from the analysis.

Testing of Fish Trained Under a Linearly-polarised Light Field

In contrast to the naïve fish, trained rainbow trout typically demonstrated more rapid and direct movements toward the edge of the testing tank following release for both parallel-trained fish (Fig. 28A-D) and perpendicular-trained fish (Fig. 29A-D). The vector, r_v , varied from near 0.48 (undirected) to 0.98 (very directed). Many fish, regardless of whether being trained perpendicular or parallel to the plane of polarised light, swam in a rectilinear manner of varying amplitude (e.g., Figs. 28A and 29A). Fish trained to orient either parallel (Fig.

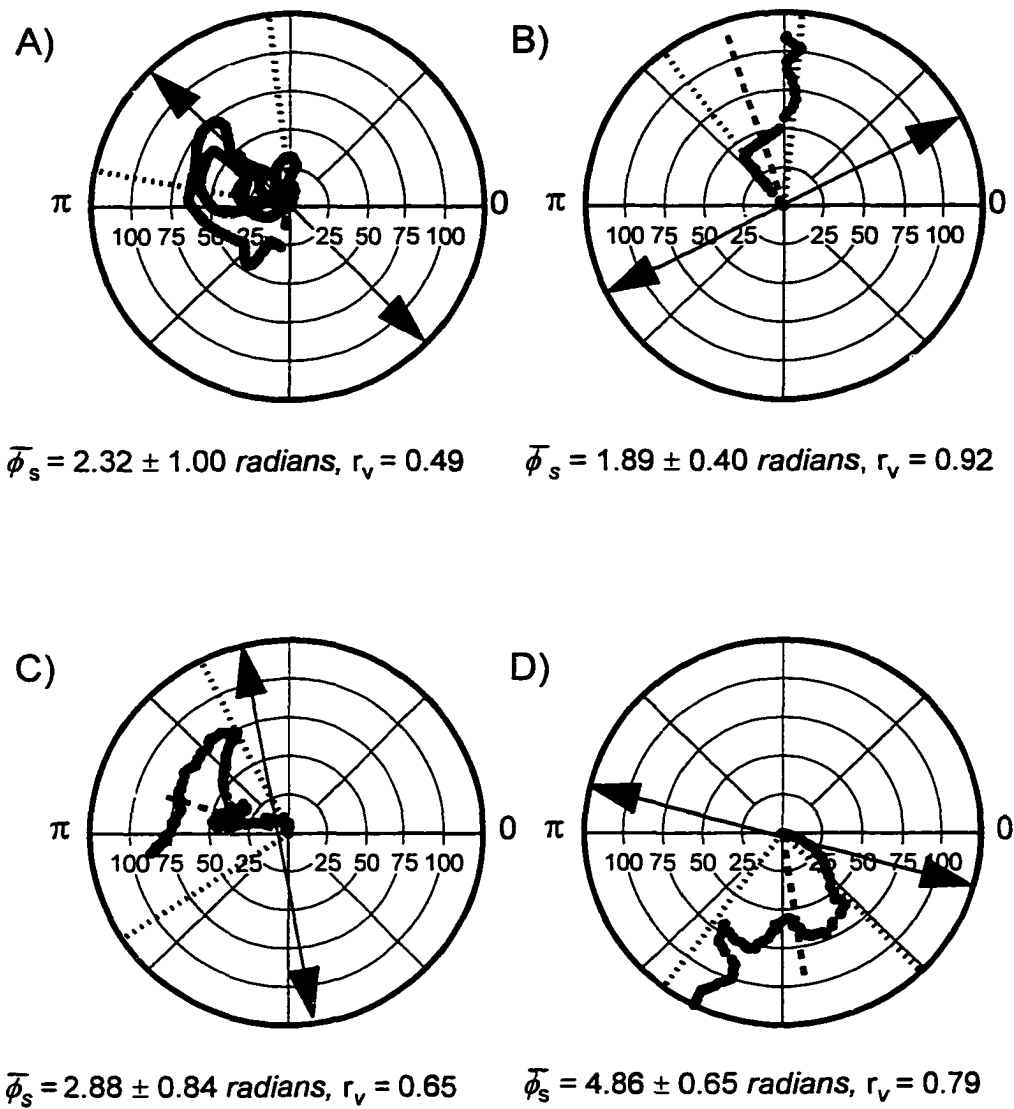
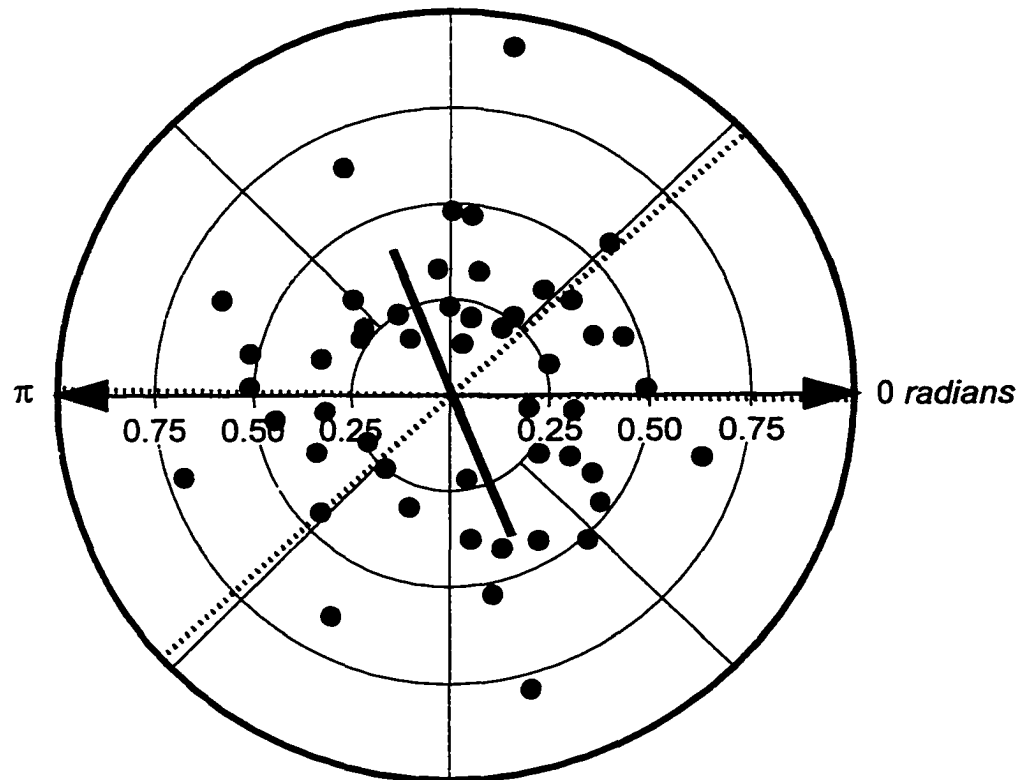


Fig. 26. A-D. Individual tracking records of four naïve (untrained) rainbow trout recorded using the F-chase program. Heavy dashed line represents $\bar{\phi}_s$, while dotted lines represents the circular standard deviation. The radial distance of the heavy solid line is proportional to r_v . Double-headed arrow indicates orientation of polarised-light stimulus. Axis distance equals distance from centre of tank (cm).



$$\bar{\phi}_g = 1.96 \pm 1.17 \text{ radians}, \quad r_v = 0.32$$

$$\text{V-test: } u = -2.23, P > 0.05 \quad n = 50$$

Fig. 27. Orientation responses of individual naïve rainbow trout (●) to a plane-polarised stimulus. Individual points represent $\bar{\phi}_s$, the mean angular orientation of individual fish. The heavy solid line represents $\bar{\phi}_g$, the grand mean of all the samples. The dotted lines represent the circular standard deviation from the mean. The radial distance from the centre represents \bar{r}_v , the mean vector of directionality, on a scale of zero to one.

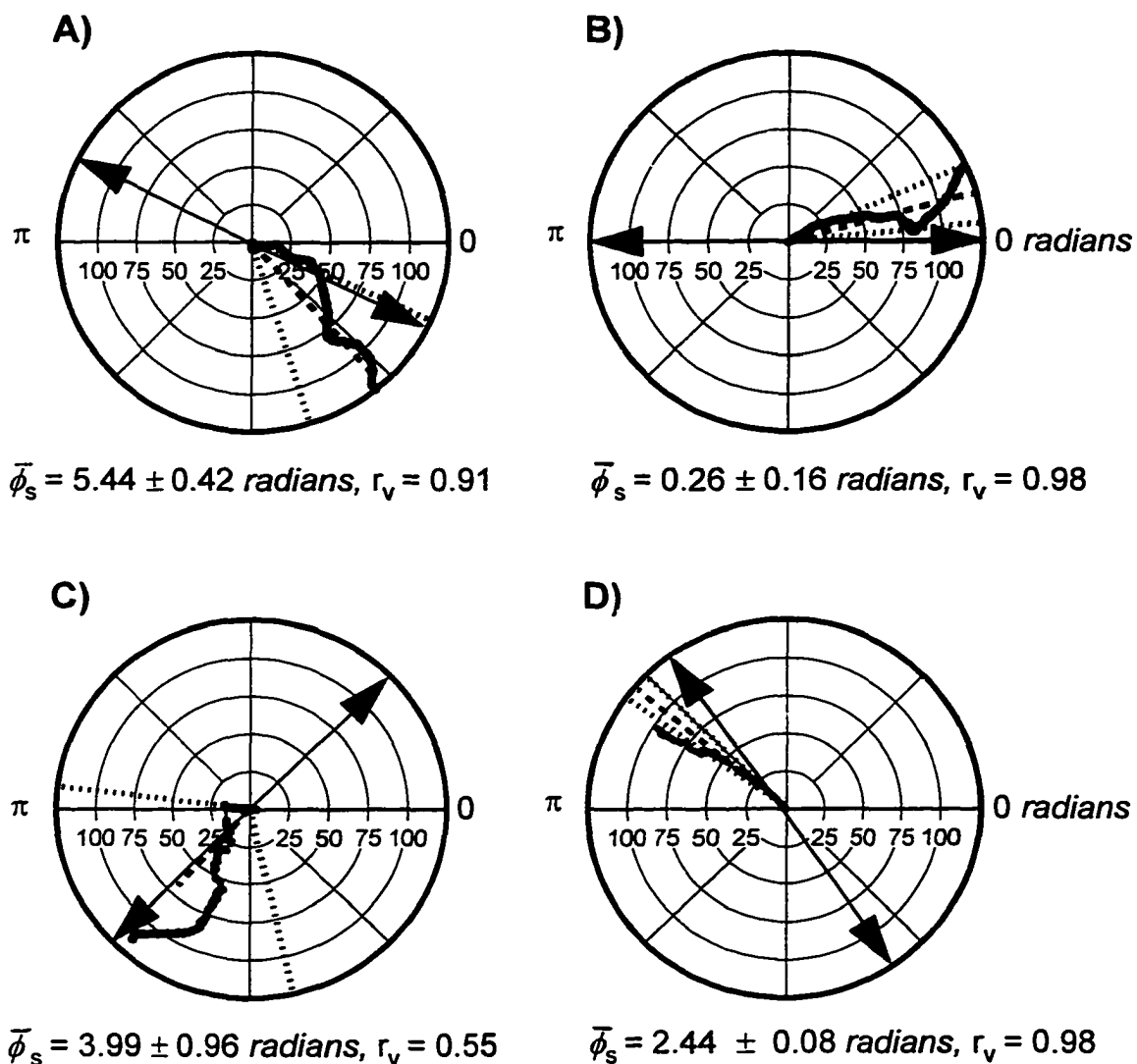


Fig. 28. A-D. Tracking records of orientation responses of individual rainbow trout entrained to orient parallel to the plane of polarised light, as tracked using the F-chase program. Heavy dashed line represents $\bar{\phi}_s$, while dotted lines represent circular standard deviation. Double-headed arrow indicates orientation of polarised-light stimulus. The radial distance is proportional to r_v . Axis distance equals distance from centre of tank (cm).

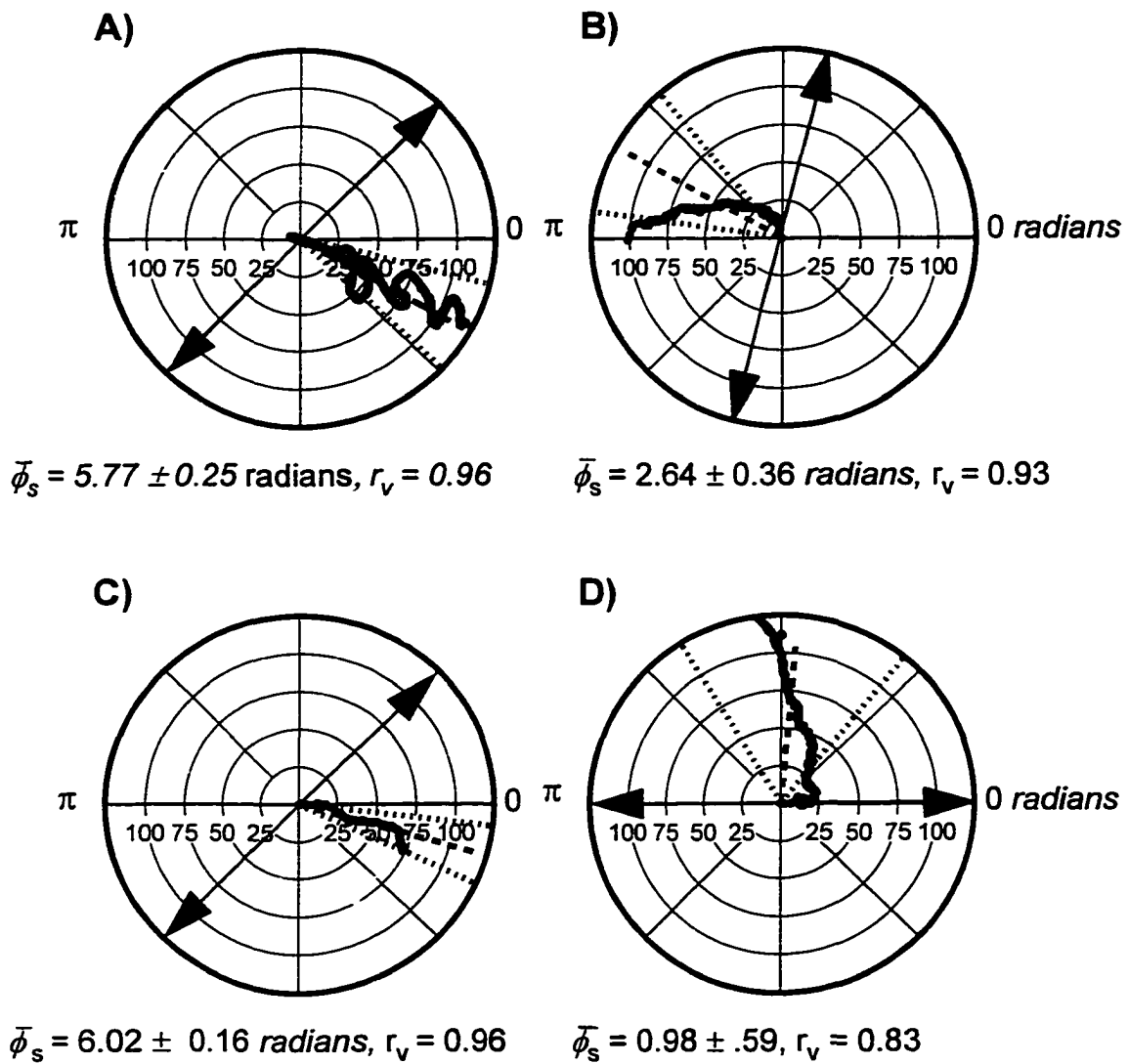


Fig. 29. A-D. Tracking records of orientation responses of individual rainbow trout entrained to orient perpendicular to the plane of polarised light, as tracked using the F-chase program. Heavy dashed line represents $\bar{\phi}_s$, while dotted lines represent circular standard deviation. The radial distance of the heavy solid line is proportional to r_v . Double-headed arrow indicates orientation of polarised-light stimulus. Axis distance equals distance from centre of tank (cm).

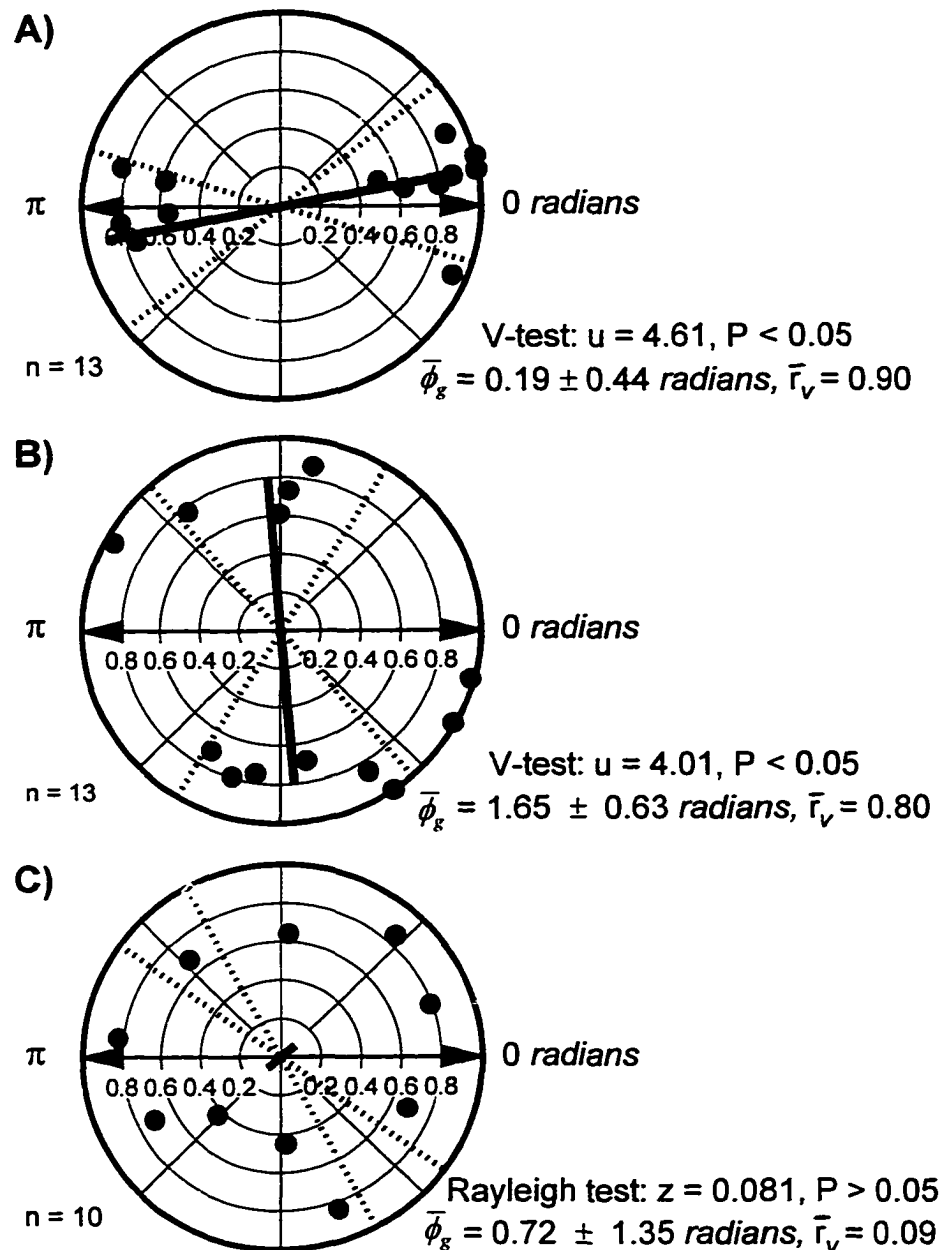


Fig. 30. Orientation responses of individual rainbow trout (●) trained to orient: A) parallel to the plane of polarised light; B) perpendicular to the plane of polarised light; and C) parallel to plane of polarised light with a diffuser present. Heavy solid line represents $\bar{\phi}_g$, while dotted lines represent s_g . Individual points represent $\bar{\phi}_s$, the mean angular orientation of individual fish. Double-headed arrow indicates orientation of polarised-light stimulus. Axis distance equals \bar{r}_v , the vector of directionality, on a scale of zero to one.

30A) or perpendicular (Fig. 30B) to the plane of polarised light responded with a significantly non-uniform distribution, with responses clumped around either the parallel ($V_{\text{test}}: u=4.61, P < 0.05, n=13$) or the perpendicular axis of the polariser ($V_{\text{test}}: u= 4.01, P < 0.05, n=13$). In addition, the orientation responses of fish trained to respond perpendicularly to the plane of polarised light were not distributed differently than the orientation responses of fish trained to orient parallel to the plane of polarised light (Watson's U^2 -test: $n_1 = 13, n_2 = 13, u = 0.155, P > 0.05$). Thus, the distributions of the two groups of trained fish were both axial (bi-directional) and the distribution of their orientation responses were similar (i.e., they oriented equally well regardless of whether they were perpendicular- or parallel-trained fish). In contrast, juvenile rainbow trout trained to orient parallel to the plane of polarised light responded with an overall uniform distribution when the polariser was replaced by a diffuser (Rayleigh-test: $z = 0.081, P > 0.05$) (Fig. 30C).

Species Comparisons.

Steelhead and brook char also exhibited significant menotaxis (Fig. 31A, B). Individual r_v 's varied between 0.42 and 0.99, with the majority of fish having directional vector responses greater than 0.70. Thus, these fish responded in a directed manner upon release. The \bar{r}_v (0.64) of steelhead was lower than brook char (0.82). It should also be noted that the orientation responses of one steelhead (Fig. 31A) and two brook char (Fig. 31B), which were greater than one

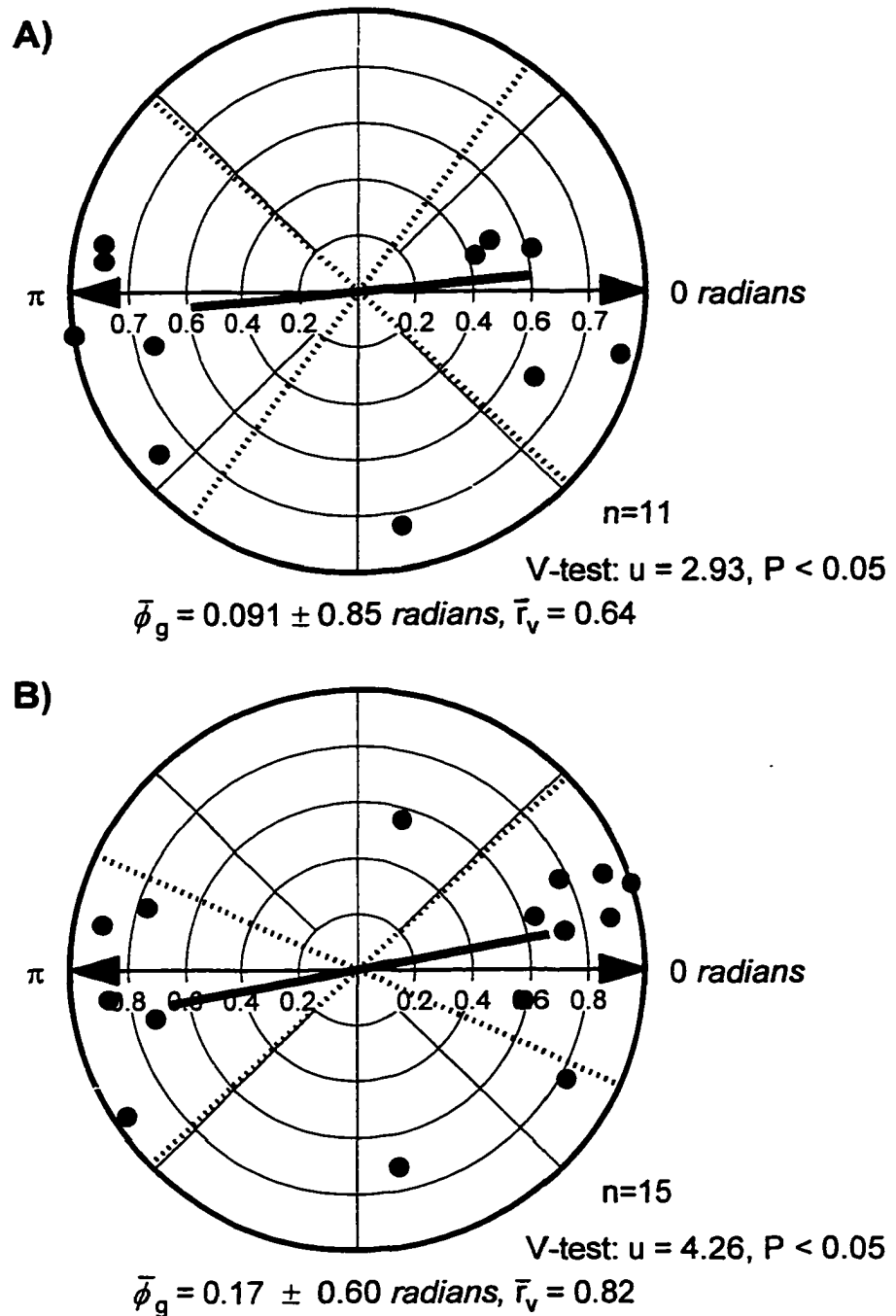
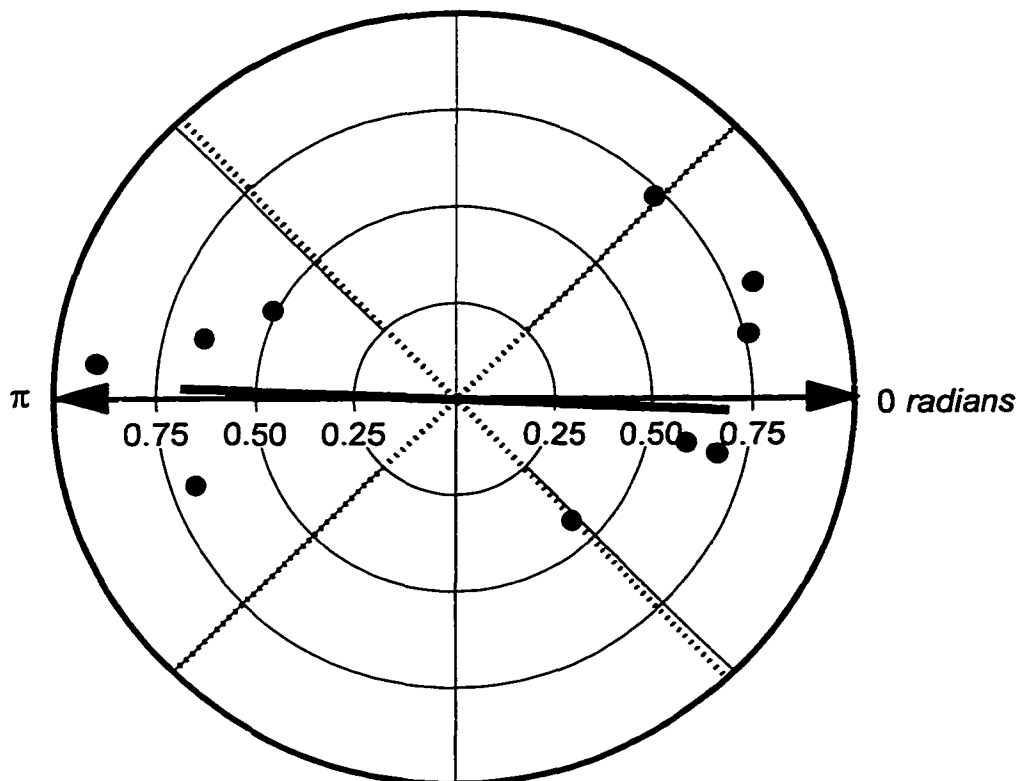


Fig. 31. Orientation responses of: A) steelhead; and B) brook char, trained to orient parallel to the plane of polarised light. Heavy solid line represents $\bar{\phi}_g$, while dotted lines represent s_g . Individual points represent $\bar{\phi}_s$, the mean angular orientation of individual fish. Double-headed arrow indicates orientation of polarised-light stimulus. Axis distance equals r_v on a scale of zero to one.

circular standard deviation from the mean, and were thus outliers. The orientation responses of both steelhead and brook char were significantly different from a uniform distribution and these responses were clumped around the angle corresponding to the position of the polariser (V-tests: $u=2.93$, $P < 0.05$, $u=4.26$, $P < 0.05$ respectively) (Fig. 31 A,B). No significant differences were detected among the orientation responses of rainbow trout, steelhead, and brook char trained to orient parallel to the plane of polarised light (Wheeler and Watson test: $\chi^2_{0.05,4} = 9.488$, $W= 6.067$, $0.1 < P < 0.25$).

Outdoor Tests of Laboratory-trained Fish

Rainbow trout trained in the laboratory to orient parallel to the plane of linearly polarised light were tested under semi-natural conditions while in a tank under natural lighting at sunset (~1945 h P.S.T, May 24th and May 27th 1994) (Fig. 32). The e-vector maximum was located near Zenith along a compass bearing of approximately 131°/311° (including correction for magnetic declination). In addition, the maximum percentage polarised light in the sky during twilight at the onset of testing on May 24th was 59% (Integrated radiance with HNP'B polariser: $\Pi = 14 \mu\text{W}$, $\perp = 3.6 \mu\text{W}$ [10° radiance collector]) and was 67% on May 27th when the tests were completed (integrated radiance with HNP'B polariser: $\Pi = 18 \mu\text{W}$, $\perp = 3.1 \mu\text{W}$). Under semi-natural conditions, the orientation responses of laboratory-trained fish were clumped around the



$$\bar{\phi}_g = 6.24 \pm 0.82 \text{ radians} \quad \bar{r}_v = 0.66$$

$$\text{V-test: } u = 2.74, P < 0.05 \quad n = 10$$

Fig. 32. Orientation responses of rainbow trout entrained in the laboratory to orient parallel to the plane of polarisation, following exposure to a clear celestial hemisphere at sunset. Heavy solid line represents $\bar{\phi}_g$, whereas dotted lines represent s_g . Individual points represent $\bar{\phi}_s$. Double-headed arrow indicates orientation of polarised-light stimulus as determined using an e-vector axis finder. Axis distance equals r_v on a scale of zero to one.

orientation of the maximum e-vector (V-test: $u=2.74$, $P < 0.05$, $n= 10$) (Fig. 32). Eight of ten fish responded within 45° of the axis of the ambient plane of maximally polarised light, whereas the remaining two fish responded within 47° of the axis of polarisation. Individual values for r_v varied between 0.43 and 0.90, with nine of ten fish having an r_v greater than 0.5. This indicated that the fish maintained a relatively constant bearing following release. Therefore, the H_0 that the fish had a uniformly circular distribution was rejected in favour of the H_A that the fish were clumped along a bi-directional vector (Fig. 32).

Orientation Responses of Fish Trained under Semi-natural Conditions

General Observations

General differences in the behaviour of *O. mykiss* and *O. nerka* parr were apparent. Typically, *O. nerka* schooled in a circular motion along the periphery of the net-pen, with occasional pauses and changes in the direction of movement. The average rate of circling of the tank was 2.0 ± 0.6 cycles per minute ($\bar{x} \pm 1SD$, $n = 40$, 5 minute video sessions). Additionally, *O. nerka* schooled with all individuals oriented in roughly the same direction, which is referred to as schooling by some researchers (Pitcher 1986, Wootton 1990). The direction of orientation among individuals therefore corresponded to the direction of movement for the group of sockeye. In contrast, no circling behaviour was observed in *O. mykiss*. Steelhead typically maintained their position by hovering in the water column.

Orientation of Fish in Training Net-pens

In general, the distribution of the fish in the training net-pens from Dec. 11 to Feb. 3, as assessed from the video frames, was not a uniform circular distribution. In total, 480 video frames were sampled, and approximately 12,000 individual fish positions were recorded. Comparison of the vectors of orientation (r_v 's) over eight days of sampling (Fig. 33 A-F), indicated that a total of 33 of 48 of the distributions of fish (groups of 25) had r_v values greater than 0.6. This indicated that the fish distributions were similar among the 10 subsamples of video frames examined for the sampling period, regardless of whether they were steelhead or sockeye.

Distributions of fish on sunny days were typically different from those on overcast days with 100% cloud cover (Figs. 33 A-F). On sunny days, the fish had an axial (bimodal) distribution regardless of the time of day (Figs. 33 A-F). On cloudy days, both axial and unimodal distributions were observed (Figs. 33 A,D,F and Fig. 33 B,C,E, respectively). At mid-day in sunny conditions, one of three of the $\bar{\phi}_g$ coincided with the position of the box containing a feeder (Fig. 33A), whereas the other two $\bar{\phi}_g$ were approximately perpendicular to the position of the feeder (Fig. 33 C,E). On overcast days at mid-day, all $\bar{\phi}_g$ were approximately perpendicular to the position of the feeder (Figs. 33 A,C,E).

A sharp contrast was observed in the $\bar{\phi}_g$ of the distributions of the fish in the evening, near feeding. On clear evenings, all $\bar{\phi}_g$ were similar to the compass orientation of the feeder box (Figs. 33 B,D,F). However, on cloudy evenings the $\bar{\phi}_g$ of the steelhead were distributed approximately perpendicular to the position

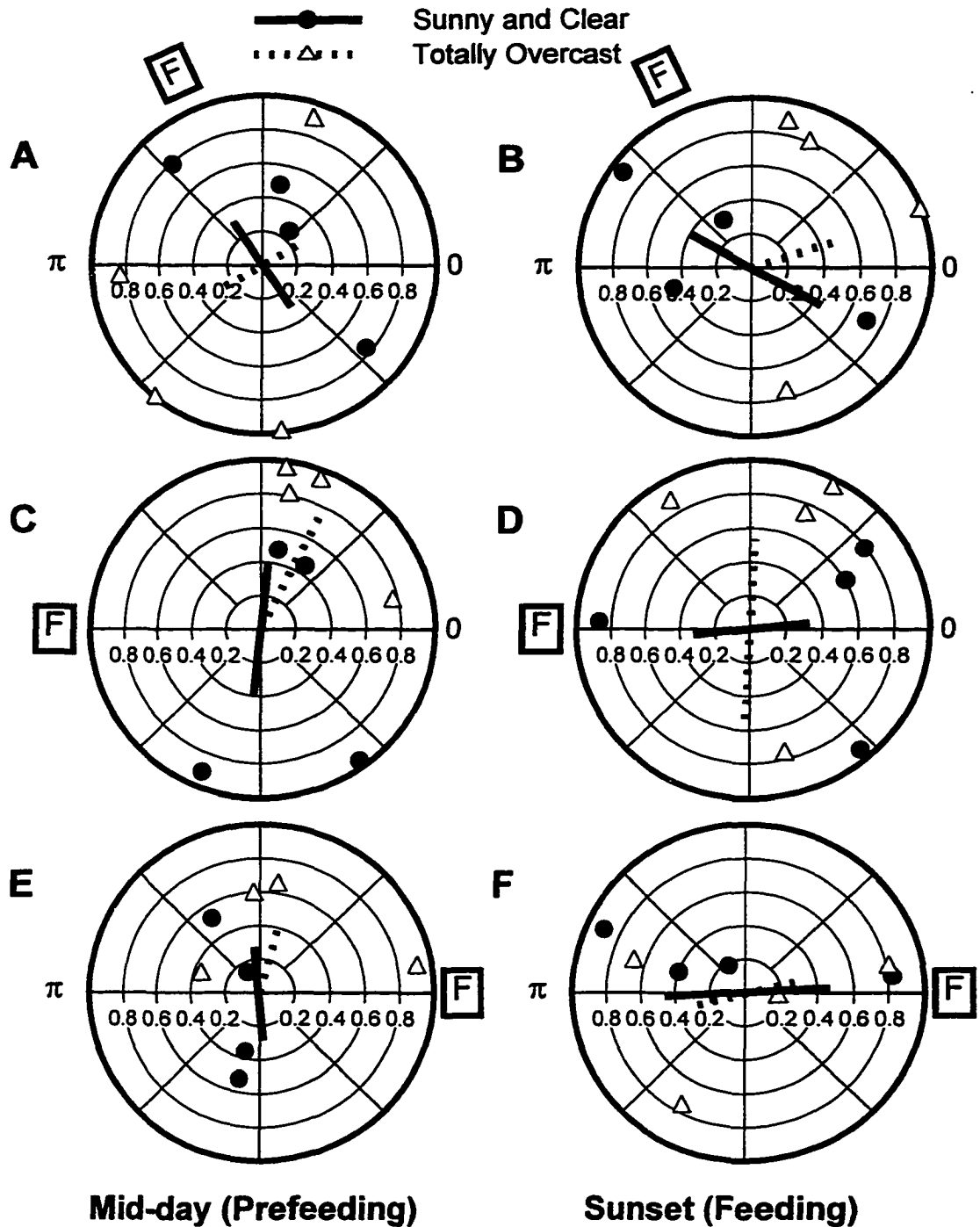


Fig. 33. Orientation responses of *O. mykiss* (A,B,C,D) and *O. nerka* (E,F) in training net-pens at mid-day and sunset on sunny and overcast days. Each symbol represents the $\bar{\phi}_s$ of 10 repeated measures of the instantaneous position of 25 fish over a 5 min period of time, with the radial distance from the centre representing r_v on a scale of zero to one. The heavy solid and dashed lines represent $\bar{\phi}_g$ for sunny and overcast days, respectively. Their lengths represent \bar{r}_v for the data. F = position of the feeder.

of the feeder box (Figs. 33B,D). In contrast, the sockeye appeared to maintain a distribution that was of a similar angle to the orientation of the feeder (Fig. 33F).

Testing during Net-pen Experiments

The percentage of polarised light was significantly greater when tests were performed during clear, sunny periods ($45.1\% \pm 7.4\%$, $n=7$) than during cloudy, overcast periods ($11.67\% \pm 2.5\%$, $n = 4$) (Mann-Whitney 2-tailed test: $W = 10$, $P < 0.008$).

Testing Scenario 1 (Table 8): On sunny days when clouds were absent and the fish had all celestial cues available, steelhead had a statistically significant unimodal distribution (V-test: $u=1.94$, $P < 0.05$) (Fig. 34A). The fish were unimodally-oriented along the bearing corresponding to the compass position of their particular feeder of their training net-pen. In contrast, on cloudy days under the same test-pen conditions, sockeye distribution was not significantly different from a uniform distribution (V-test: $u= 0.44$, $P > 0.05$) (Fig. 34B).

Testing Scenario 2 (Table 8): In the absence of celestial cues (i.e., solar, brightness, spectral, or polarised light), the H_0 that steelhead were uniformly distributed was not rejected (V-test: $u=0.044$, $P > 0.05$) (Fig. 34C).

Testing Scenario 3 (Table 8): When steelhead were provided with only spectral and brightness cues through a diffuser over the 40-cm aperture in the lid, the fish were also unoriented (V-test: $u=0.066$, $P > 0.05$) (Fig. 34D).

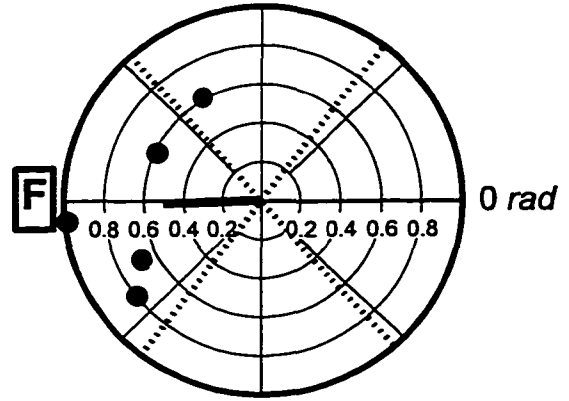
Testing Scenario 4 (Table 8): In contrast to Scenario 3, when steelhead

A) Sunny Conditions

- Scenario 1 (Steelhead)
Polarised Light = 45%
Feb. 2, 1996, twilight pm

$$\bar{\phi}_g = 3.18 \pm 0.94 \text{ radians}, \bar{r}_v = 0.61$$

(V-test: $u=1.94$, $P < 0.05$)

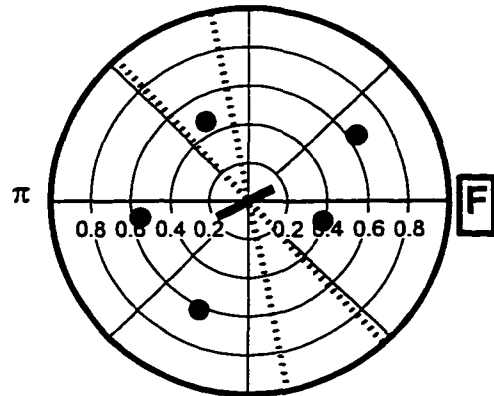


B) Cloudy Conditions

- Scenario 1 (Sockeye)
Polarised Light = 9%
Feb. 3, 1996, 1615h

$$\bar{\phi}_g = 0.47 \pm 1.3 \text{ radians}, \bar{r}_v = 0.16$$

(V-test: $u = 0.44$, $P > 0.05$)



C) No Cues

- Scenario 2 (Steelhead)
Polarised Light <1%
Jan. 29, 1996, 1515h

$$\bar{\phi}_g = 1.69 \pm 1.29 \text{ radians}, \bar{r}_v = 0.17$$

(V-test: $u = 0.066$, $P > 0.05$)

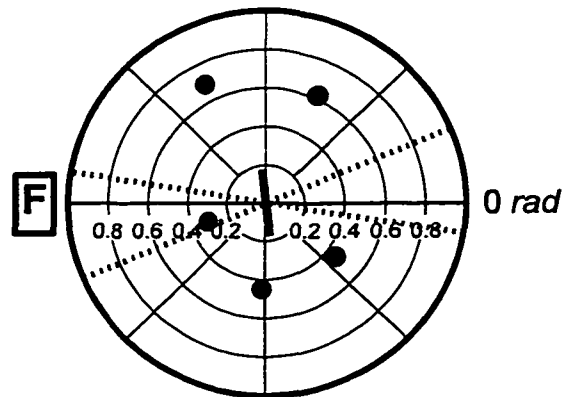


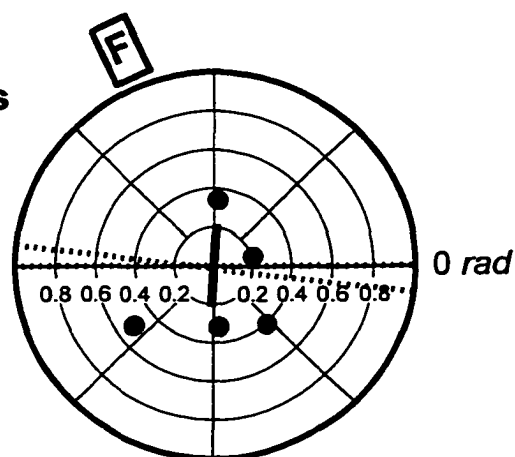
Fig. 34 A-C. Mean orientation responses of 5 groups (●) of 5 fish in the testing pen following the experimental scenarios outlined in Table 8. The heavy solid line indicates $\bar{\phi}_g$, while the length of the line represents \bar{r}_v on a scale of zero to one. Dotted lines represent s_g . F = position of the feeder from training net-pen.

D) Zenith Brightness/Spectral Cues

- Scenario 3 (Steelhead)
- Diffuser present
- Feb. 2, 1996, 1430h

$$\bar{\phi}_g = 1.36 \pm 1.35 \text{ radians}, \bar{r}_v = 0.21$$

(V-test, $u = 0.22$, $P > 0.05$)

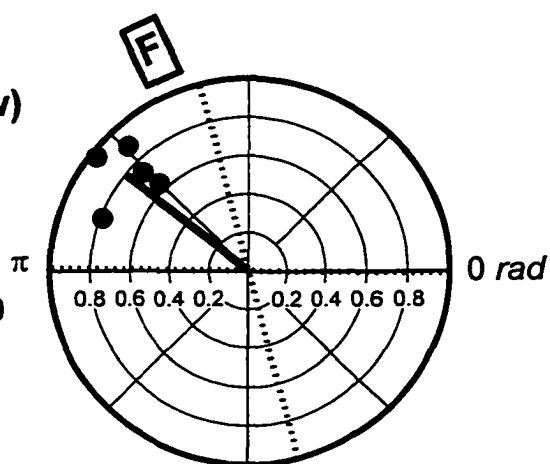


E) Sun at Horizon (no Zenith view)

- Scenario 4 (Steelhead)
- Polarised light = 12%
- Feb. 3, 1996, 1500h

$$\bar{\phi}_g = 2.47 \pm 0.64 \text{ radians}, \bar{r}_v = 0.79$$

(V-test: $u = 1.97$, $P < 0.05$)



F) Zenith Only

- Scenario 5 (Sockeye)
- Polarised light = 51%
- Feb 2, 1996, Twilight am

$$\bar{\phi}_g = 0.11 \pm 0.99 \text{ radians}, \bar{r}_v = 0.56$$

(V-test: $u = 1.74$, $P < 0.05$)

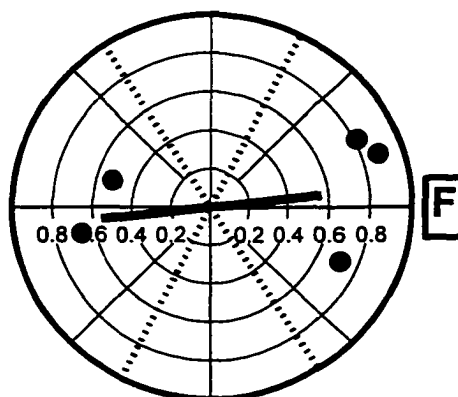


Fig. 34 D-F. Mean orientation responses of 5 groups (●) of 5 fish in the testing pen following the experimental scenarios outlined in Table 8. The heavy solid line indicates $\bar{\phi}_g$, while the length of the line represents \bar{r}_v on a scale of zero to one. Dotted lines represent s_g . F = position of the feeder from training net-pen.

were provided with only a clear view of the setting sun (solar cues), the fish were oriented in a unimodal manner (V-test: $u=1.97$, $P < 0.05$) (Fig. 34E). These fish had a $\bar{\phi}_g$ similar to the compass bearing of their feeder in their training net-pen.

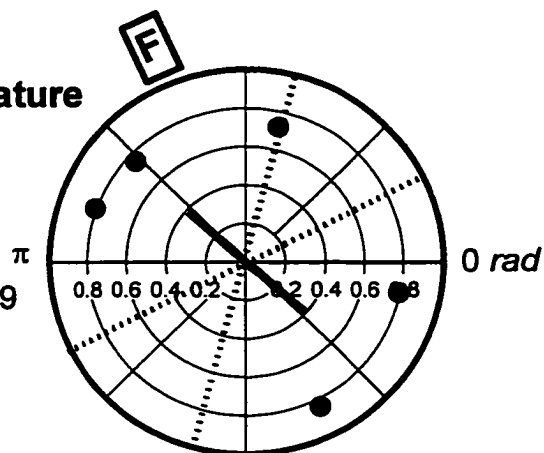
Testing Scenario 5 (Table 8): In this scenario, sockeye were provided with an open view of the Zenith region of the sky through the 40-cm aperture in the test-pen lid, under clear sky conditions. Polarised light in this region of the sky was 51%. Under these conditions, the fish had an axial distribution corresponding to the location of their feeder box in their training net-pen (V-test: $u= 1.74$, $P < 0.05$) (Fig. 33F).

Testing Scenario 6 (Table 8): When the aperture of the lid was reduced to provide the fish with only a 10-cm aperture ($\sim 6^\circ$ arc of sky), the distribution of steelhead was not different from a uniform distribution (V-test: $u=1.1$, $P > 0.05$) (Fig. 33G). It appeared, however, that there was a general trend for the fish to have an axial distribution with a $\bar{\phi}_g$ similar to the compass orientation of their feeder.

Testing Scenario 7 (Table 8): In this scenario, the same small aperture used in Scenario 6 was covered with a UV-transmissive polariser and a diffuser (to remove inherent celestial polarisation). The polariser was aligned with the axis of the maximum percent polarised light in the sky. The inherent polarisation in the sky was low ($\sim 8\%$) because sky conditions were cloudy. With the polariser in place, both steelhead and sockeye oriented along the bearing of their particular feeder in their training net-pen (V-test: $u=2.09$, $P < 0.05$ and $u=1.65$, P

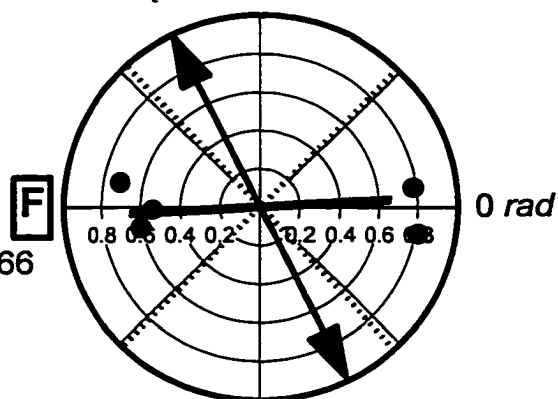
G) Zenith Brightness/Small Aperature

- Scenario 6 (Steelhead)
- Polarised light = 43%
- Jan. 29, 1996, 1600h
- $\phi_g = 2.41 \pm 1.10$ radians, $r_v = 0.39$
- (V-test, $u = 1.1$, $P > 0.05$)



H) HNP'B Polariser

- Scenario 7 (Steelhead)
- Polarised light > 95%
- Feb. 3, 1996, 1535h
- $\phi_g = 3.13 \pm 0.82$ radians, $r_v = 0.66$
- (V-test: $u = 2.09$, $P < 0.05$)



I) HNP'B Polariser

- Scenario 7 (Sockeye)
- Polarised light > 95%
- Feb. 1, 1996, 0900h
- $\phi_g = 0.70 \pm 1.10$ radians, $r_v = 0.44$
- (V-test: $u = 1.65$, $P = 0.05$)

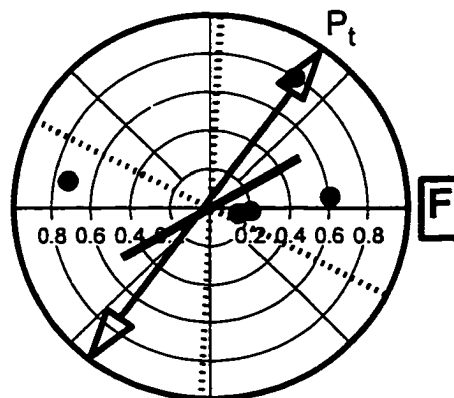


Fig. 34 G-I. Mean orientation responses of 5 groups (●) of 5 fish in the testing pen following the experimental scenario's outlined in Table 8. The heavy solid line indicates $\bar{\phi}_g$, while the length of the line represents \bar{r}_v on a scale of zero to one. Dotted lines represent s_g . F = position of the feeder from training net-pen. P_t represents orientation of UV-linear polariser and the arrow represents orientation of the band of maximally polarised light in the sky prior to the experiment.

= 0.05, respectively) (Fig. 34H,I).

Testing Scenario 8 (Table 8): In the final scenario, fish were allowed to view the horizon at twilight along with the light from the UV-transmissive polariser. The polariser was rotated 90° to the orientation of the polarised light in the sky at Zenith. Under these conditions, steelhead oriented in the direction of the bearing of where their feeder would be in their training net-pen, if shifted by 90° (V-test: $u=1.88$, $P < 0.05$) (Fig. 34J). Notably, one replicate of five fish still appeared to orient on the original bearing of their feeder in their training net-pen (i.e., not shifted 90°) (Fig. 34J). In contrast, sockeye did not orient significantly (V-test: $u=0.19$, $P > 0.05$) (Fig. 34K) in the direction of either: 1) where the feeder was located in the training net-pen; or 2) at 90° ($\pi/2$ radians) where the fish were predicted to orient if their responses were governed by the orientation of the polariser alone. However, the $\bar{\phi}_g$ of the fish was very similar to what one would predict if the fish were orienting solely on the basis of polarised light (Fig. 34K).

DISCUSSION

Acquisition of the Polarised-light Cue

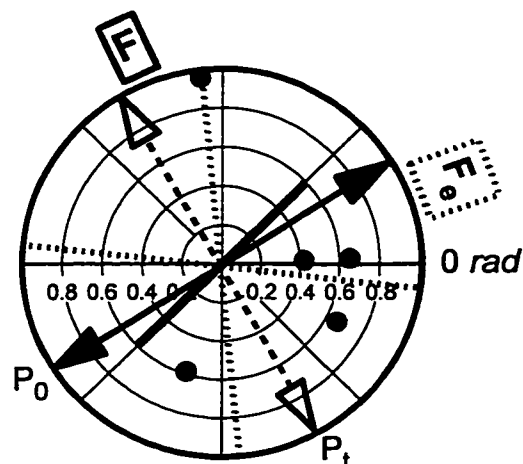
Trout, char, and salmon feed opportunistically (Johnson and Ringler 1980) and thus feed readily in laboratory situations (Adron *et al.* 1973, Hawryshyn and Bolger 1990, Hawryshyn *et al.* 1990, present study). This makes them suitable subjects to train for tasks using operant behaviour modification. For rainbow

J) Horizon + Polariser

- Scenario 8 (Steelhead)
Feb. 1, 1996, 0800h
Polarised light > 95%

$$\phi_g = 0.78 \pm 0.89 \text{ radians}, r_v = 0.60$$

(V-test: $u = 1.88, P < 0.05$)



K) Horizon + Polariser

- Scenario 8 (Sockeye)
Jan. 29, 1996, 1615h
Polarised light > 95%

$$\phi_g = 1.82 \pm 1.23 \text{ radians}, r_v = 0.26$$

(V-test: $u = 0.19, P > 0.05$)

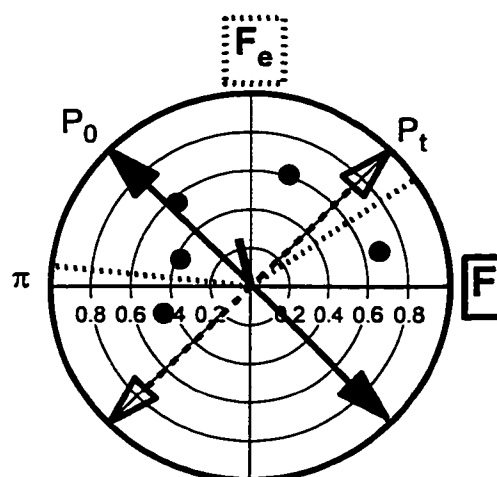


Fig. 34 J-K. Mean orientation responses of 5 groups (●) of 5 fish in the testing pen following the experimental scenario's outlined in Table 8. The heavy solid line indicates $\bar{\phi}_g$, while the length of the line represents \bar{r}_v on a scale of zero to one. Dotted lines represent s_g . F = position of the feeder from training net-pen. P_0 and solid arrow = the orientation of the band of maximally polarised light in the sky and P_t and dashed arrow = orientation of the UV-linear polariser. F_e = expected position of orientation, if fish is orienting to polarised light.

trout used in the present study, the learning curve for acquisition of the correct behavioural response was similar in shape to that observed by Adron *et al.* (1973). The observation that the fish could select the appropriate channel to receive a food-reward demonstrated their ability to orient themselves relative to the angular position of the polarised light cue. In contrast to Adron *et al.* (1973), however, it should be noted that the mean number of correct responses in this protocol did not exceed 79%. All fish swam on occasion into the incorrect channel in the training tank. Rather than interpreting this number to represent an inherent limitation in the ability to fully discriminate the orientation of the cue, I suggest that the intermittent reinforcement schedule may have entrained this behaviour pattern. This may have resulted from the fish not being able to assess whether the food resource had become permanently depleted. Thus, in spite of the observed plateau in the responses, continued training might have caused an extinction or a further decrease of this wandering behaviour. Alternatively, although food was consistently available along a single plane in the training tank, it was not a great expenditure of energy for the fish to check the other plane occasionally. An interesting test of this idea would be to determine the time budget (allocation) of the fish in a training tank that offers several different choices of channels instead of two. The prediction would be that the fish spend a similar amount of time as observed in the present study swimming along the correct plane, and partition the remaining time amongst the other (incorrect) planes. A decrease in the time spent in the correct channel would suggest that

the time allocated to each channel may be dictated by factors such as the time it takes to travel to the end of the incorrect channels and back.

Orientation Responses under Controlled Laboratory Conditions

The lack of a common directional response in untrained, juvenile rainbow trout suggested that these fish did not have an innate orientation response to polarised light during the period of testing. Several interpretations of the lack of innate orientation are possible. First, orientation to polarised light may be a learned response and therefore these juvenile fish have not had the opportunity to acquire a response to polarised light. If a critical period exists for the acquisition of the ability to orient to polarised light, this would necessitate exposure of young fish to natural polarised-light patterns. This could be of relevance for stocking programmes intent on the establishment of self-sustaining populations. Second, the fish may not have responded because of handling stress. However, fish were acclimated to testing conditions prior to release to minimise this problem. In addition, a third interpretation, that the fish were derived from different stocks and thus had different preferential directions, could be ruled out because the rainbow trout were obtained from a single brood of a strain of wild-stock fish (Chapter III). Finally, the lack of a common or unified orientation response in naïve fish may have been a result of the relevant releasers for menotaxic behaviour not being present. Such releasers might include photoperiod, water temperature, and size or stage of development of

fish. Age and physiological condition have long been known to influence the timing and type of behavioural responses of salmonids to their environment (Hoar 1953, 1954). Therefore, at the parr stage of development, an innate directional preference, even if present, may not have been expressed because of the behavioural or physiological state of the fish. Further investigation of laboratory-raised (naïve) fish at different stages of their lives is required to fully address this issue.

In contrast to the responses of naïve fish, the coupling of the polarised light cue with a relevant resource (food) provided behavioural evidence of the potential of several species of salmonids to orient using polarised light. The similarities in the orientation proficiency of fish, irrespective of whether the fish was trained to orient perpendicular or parallel, indicated that trained fish can orient relative to the distribution of maximum e-vector corroborates the findings of Hawryshyn *et al.* (1990). In addition, the lack of an oriented response in trained fish exposed to an unpolarised light source further demonstrated that the fish were orienting relative to the plane of polarised light rather than brightness cues from the light source.

The sinusoidal swimming pattern observed in some of the fish may provide an insight into how fish are orienting to polarised light. These movements may be indicative of the fish making comparisons of differences in the light environment, both along the axis of polarisation and at an angle to it. Such a movement pattern is reminiscent of the flight paths of insects orienting

using chemotaxis (see Waterman 1989). Because the fish were tested in a static-water tank, we can discount the hypothesis that fish were being carried off course by water movements and were performing corrections. Similarly, the fact that water changes were made between testing trials discounted the hypothesis that the fish were following odour gradients from previously tested fish. One alternative, is that these movements may represent exploratory behaviour of these fish as they swim toward the periphery of the tank.

Our knowledge of polarisation sensitivity in salmonine fishes is now extended to include the highly migratory steelhead and the less diadromous brook char, based on the controlled laboratory experiments. Menotaxis previously has been described for some species in the genus *Oncorhynchus* but not for the genus *Salvelinus*. This is also consistent with observations of the physiological similarity of polarisation sensitivity among these species (Chapter III).

Fish trained to orient relative to a single plane of polarised light in the laboratory also could discriminate the band of maximally polarised light at Zenith, in spite of inexperience with the complexity of brightness and spectral cues in natural outdoor conditions. Undoubtedly, near sunset, the polarised-light field is least complicated for an observer sensitive to e-vector (see Horváth and Varjú 1995) . Thus, at this time of day, position of the sun can unambiguously be determined by the distribution of polarised light and brightness cues. Use of brightness cues alone may lead to error in instances of patchy cloud cover. It

remains to be determined how fish trained with this methodology will respond at other times of day when the maximum e-vector is not at Zenith.

Orientation Responses under Semi-natural (Field) Conditions

The observed differences in the swimming activity between steelhead and sockeye are likely indicative of differences in their lifestyle. Juvenile *O. mykiss* can have either a lotic (in streams) or lentic (in lakes) distribution in the freshwater phase of its life history (Behnke 1992). In lotic systems, *O. mykiss* are typically associated with the stream bottom and maintenance of a feeding station, whereas in lakes they usually are found inshore, or in the hypolimnion (deeper waters) when offshore. Steelhead, however, are wide-ranging and are found in the neritic zone (inshore near the bottom) (Ruggerone *et al.* 1990), as well as the pelagic zone offshore (Hart 1973). Excluding time spent in the redd, *Oncorhynchus nerka* live in the hyperlimnion (surface waters) of lakes, for at least the early phases of their life history. The sockeye form of the species later occupies neritic and pelagic regions in the North Pacific Ocean, whereas the kokanee form is an active swimmer that remains in the hyperlimnion of lakes (Taylor and Foote 1991). Even in freshwater, these fish undergo fairly extensive movements (Johnson and Groot 1963, Groot 1965). Groot (1965) also observed that wild sockeye salmon smolts often swim continuously in tank situations. However, in his study, Groot (1965) observed that the orientation responses of sockeye salmon were more directional, with the fish swimming to one edge of

the tank and then turning back on themselves. In contrast, in the present study, the fish swam in a circuit around the tank, pausing near the feeder or 180° to the feeder. Other sockeye in Groot's (1965) study were observed to hold their position and occasionally reverse their orientation without moving from the centre of the tank. This behaviour was similar to what was observed for *O. mykiss* in the present study. The sockeye in the present study frequently reversed their direction of movement, however, they were rarely not swimming (personal observation). In spite of these differences, the sockeye in the training-pen were positioned for a greater proportion of total time along the axis containing the feeder during the evening (Fig. 33F). However, this behaviour did not transfer to the test-pen, where sockeye were observed to hold their position. In the test-pen, therefore, sockeye behaved in a manner more similar to the latter behaviour described by Groot (1965). Unlike sockeye, steelhead in both the training- and testing-pens maintained their positions and did not swim continuously.

It is clear that meteorological conditions can influence the ability of the fish to find the location of the appropriate feeder, a behaviourally relevant resource, as evidenced from the training net-pens. These findings were consistent with those described for *O. nerka* (Groot 1965), as well as other fishes, including perciform fishes such as centrarchids, cichlids, and other salmonids (Hasler *et al.* 1958, Braemer 1960), as well as *Fundulus notti* (Goodyear 1970). One of the most interesting findings was that, under semi-controlled conditions, fish provided with only a view of Zenith oriented to the maximum percentage of

polarised light as low as 51% (Fig. 34F) . A comparable laboratory study found rainbow trout to be able to orient to polarised light as low as 65% (Hawryshyn and Bolger 1990). One possible explanation for the observed differences in the minimal level of polarised light requisite for orientation responses to may be attributable to larger amount of UV polarised light in the celestial hemisphere relative to the tungsten light source used in their study. Alternatively, the fish may be using another sensory modality such as magnetic orientation or spectral and brightness gradients at Zenith . However, orientation responses were not observed in the absence of any cues (Fig. 34C) or when only Zenith brightness and spectral cues were available. Knowledge that fish can orient at an even lower level of polarisation than previously observed increases the appeal of polarisation sensitivity as a mechanism for orientation. Similarly, sockeye and steelhead can orient in the presence of solar cues near the horizon. This orientation response was accurate even in the absence of appreciable amounts of polarised light (e.g., Fig. 34E). As well, the mean responses were unimodal as in some other studies (Taylor 1987, Levin and Belmonte 1988). Bimodal responses however, were observed for orientation to polarised light in the laboratory and in the field (Hawryshyn and Bolger 1990).

Under natural conditions, fish typically can depend on many features of their environment to serve as cues for behavioural responses. For free-swimming, stream- and lake-dwelling fishes, landmark orientation on underwater and shoreline features may provide an additional cue for orientation, when cues

such as the position of the sun or maximal plane of polarised light are not available. By moving the net-pens around the lake and rotating the net-pens, the ability of fish to landmark was reduced undoubtedly. The observation that sockeye were able to orient along the compass bearing containing the feeder on cloudy evenings for 3 of 4 days (Fig. 34F) was interesting. Several explanations are possible for these observations. First, the sensitivity of sockeye to polarised light may be greater than that of rainbow trout. This explanation is improbable because the polarisation sensitivity of *O. nerka* appears very similar to that of *O. mykiss* when they are tested under similar conditions (Chapter III, Novales Flamarique and Hawryshyn 1996). Second, it may be that the sockeye are entraining on the feeder box more quickly. This seems to be impossible because the box containing the feeder was rotated on the days when video recordings were made to prevent landmark orientation to features inside the pen. Similarly, the net-pens were moved around the lake to avoid landmark orientation to landscape features outside the pen. Thus, this leaves us with the third possibility that the fish are orienting using another cue, such as the geomagnetic, chromatic, or brightness cues. However, because sunlight or brightness cues typically yield unimodal responses or bimodal responses with a strong bias in one direction (Taylor 1987, Levin and Belmonte 1988), this is not consistent with the observations in this particular case. In contrast, magnetic-orientation responses appear to have either a unimodal or a bimodal axial distribution in chinook (*O. tshawytscha*) and sockeye salmon (Quinn 1984, Taylor 1986,

1987). It is difficult to preclude magnetic orientation under these “natural experiments” because of the lack of a control for magnetic cues. Similarly, polarised light has been shown to calibrate the magnetic compass in passerine birds (Phillips and Moore 1992), further confounding the issue. This type of correlative data, therefore, cannot easily address why differences are observed among the species. Thus, the importance of experimental manipulations must be stressed for such experiments (e.g., Fig. 34 A-K), even when conducted in outdoor testing situations.

The results of the conflict experiments provide some interesting insights into the importance of polarised light information used by salmonids. It is unlikely fish would encounter a similar situation in the natural environment. However, the fact that rotation of the e-vector changed the orientation of test fish when provided with the choice of zenith light cues (for at least steelhead) suggests that polarised light can serve as a dominant visual cue for orientation. This conflict test corroborates previous observations based on the rotation of polarisers presented by Groot (1965), Forward *et al.* (1972), Forward and Waterman (1973), and Waterman and Forward (1972). It also demonstrates that polarised light supersedes other sensory modalities such as magnetic orientation as a cue under the conditions outlined in these experiments.

Evidence from Chapters II and III, Hawryshyn and McFarland (1987), Hawryshyn *et al.* (1990), Parkyn and Hawryshyn (1993), Coughlin and Hawryshyn (1995), and Novales Flamarique *et al.* (in press) suggests an

important role for UV light in the perception of the e-vector by fishes. The implications of this finding for the interpretation of previous studies using polarising filters are potentially profound. For example, although the Polaroid HN-32 and HN-38 filter (two commonly used polarising filters) transmit only 2% of available UV light at 380 nm, the Polaroid HNP'B filter transmits 27% of available UV light (personal observation). Furthermore, the light transmitted by the latter is 96% polarised in this region of the spectrum (personal observation). Thus, if the UV light content in the celestial hemisphere is high enough, it is conceivable that fish in some of the older studies were detecting UV-polarised light. At twilight, however, when total intensity of light is low (Novales Flamarique *et al.* 1992), this appears less likely.

As an alternative explanation for orientation of fish in the absence of UV light, it is prudent to consider the potential importance of interactions of the polariser with inherent patterns of ambient polarised light. This has been discussed in depth by Coemans *et al.* (1990) and Vos Hzn *et al.* (1995) with respect to design of experimental systems for tests of polarisation sensitivity. Such interactions would be evident as light and dark regions, depending on the angular relationship of the polariser to polarised light from the light source. To avoid this potential problem, a diffuser or depolariser must be used in tandem with the polariser. However, inherent polarisation would not be a problem when ambient levels of polarised light are low (cloudy conditions). Similarly, brightness patterns should not be as conspicuous when the polariser is aligned with the

ambient plane of maximally-polarised light. Typically, to date, diffusers have not been used in tandem with a polariser for most studies of polarisation sensitivity and orientation. Any diffuser, of course, attenuates some light and must be selected carefully for its spectral transmission properties. Additionally, attenuation of some wavelengths of light also may result from the spectral transmission properties of the polariser. This knowledge, coupled with the availability of polarising filters with a relatively high UV-light transmittance (such as the Polaroid HNP'B), should help to facilitate new investigations, less impacted by brightness artefacts in the visual field.

Some authors have theorised that salmonids perform their precise migrations through stochastic processes, with little requirement for precision in orientation (Patten 1964, Salla and Shappy 1963, Jones 1968, Leggett 1977). Indeed, more recent evidence suggests that random movements coupled with a simple compass, such as a sun-compass or polarised-light compass, may be sufficient to account for observations of movements of salmonids in structurally complex coastal areas (Pascual and Quinn 1991). However, empirical evidence also suggests that for several phases in the life history of salmonids, fish display oriented swimming responses in the wild (Johnson and Groot 1963, Groot 1965, Quinn 1990). Compass orientation may be particularly important for juvenile fish that have had no chance to form a cognitive map for navigation. Similarly, it may be important for adult fish travelling through regions where few cues for piloting are available. For example, the open ocean phase of the spawning migration

appears to be a directed and non-random movement. This is because the observed time intervals between release and recovery of fish tagged at sea appear to be too brief for the fish to travel in anything but a directed manner for thousands of kilometres across the ocean (Neave 1964, Dunn 1969, Quinn 1980, 1984, 1988, Quinn and Groot 1984b, Hartt and Dell 1986, Groot and Quinn 1987).

When polarised light is used as a cue for orientation, the typical response appears to be bimodal (Groot 1965, Taylor and Adler 1973, Hawryshyn and Bolger 1990, Hawryshyn *et al.* 1990, the present study). It should be noted that quadrimodal responses have been observed in some invertebrates (Jacobs-Jessen 1959, Jander and Waterman 1960). However, the bimodality of orientation responses should be replaced by a unimodal response in the presence of the sun, because it should serve to indicate to the fish the direction of South, West, or East. This type of unimodal response is evident in Figs. 34A, E. In addition, the underwater polarised-light field is not distributed symmetrically around a fish (McFarland 1991, Hawryshyn 1992, Novales Flamarique and Hawryshyn 1997a). Consequently, aquatic organisms monitoring polarised-light distribution outside of Snell's window might be expected to show unidirectionality in orientation responses. The relative importance of the underwater polarised-light field versus the celestial polarised-light field as viewed through Snell's window are still unknown. However, shrimp also appear to orient using the celestial pattern of polarised light from this region (Goddard and Forward 1991).

The shallow depth of the tanks in both the laboratory and the field testing situations in this study (15 cm) resulted in a short path length for light. Thus, the proportion of polarised light resulting from Rayleigh and Mie scattering in the water would have been less than would be observed with a deeper water column.

Summary

Orientation responses of juvenile rainbow trout, steelhead, and brook char to a polarised-light stimulus were examined under controlled laboratory conditions. As a group, naïve rainbow trout were found to show no directional tendency prior to training. Individual untrained fish were tracked using a digital image tracking system. Fish trained under a linearly-polarised light field learned to discriminate the orientation of a polarised light field after 3-5 training sessions, in a two-choice test. With implementation of an intermittent reinforcement schedule, the proportion and the number of correct responses increased further. In addition, trained fish of all three species oriented to the plane of polarised light. In contrast, trained fish could not orient when a diffuser was used to depolarise the light source. Rainbow trout trained to orient parallel to polarised light in the laboratory were tested at twilight and found to orient relative to the bearing of maximum e-vector. Fish in floating net-pens were provided food rewards at specific compass bearings at both dawn and dusk from one of four feeder boxes set apart by 90°. Following eight weeks of entrainment, fish were tested for their responses under various natural and artificial conditions. The two

species displayed different forms of movement behaviour within the training tank. The steelhead tended not to move much but rather maintained stations near one of the four feeder boxes on the training tank. In contrast, the sockeye salmon swam around the training tanks, almost constantly. Meteorological conditions also affected fish distribution during testing. Under testing conditions, steelhead were uniformly distributed under heavy overcast or foggy conditions, whereas sockeye appeared to assume a bimodal axial distribution. When the sun was visible, fish were unimodally distributed, fish also were unoriented when presented with only brightness and spectral cues at Zenith. In contrast, both steelhead and sockeye were oriented relative to the compass bearing of their feeder box at civil twilight if the horizon was not obscured by heavy cloud or if blue sky was visible at Zenith.

CHAPTER VI

EPILOGUE

The aim of this chapter is to summarise and synthesise the major contributions of this dissertation research and to discuss important areas for future research. First, this dissertation contains the first physiological characterisation of polarised-light sensitivity from the optic nerve of a fish (Parkyn and Hawryshyn 1993, Chapters II and III). General descriptive and quantitative models of both on- and off-responses also were provided. Prior to this, the spectral sensitivity of these salmonine fishes was largely unknown, with the exception of rainbow trout. In particular, this research establishes the presence of UV and polarisation sensitivity in the genus *Salvelinus*, as well as additional forms of *Oncorhynchus*, specifically cutthroat trout, kokanee, and steelhead. Despite differences in habitat, reproductive strategy, diet, and colouration of the salmonids examined, their spectral and polarised-light sensitivities have an overall similarity. For example, all species have four photopic photoreceptors when parr, including a UV photoreceptor. This overall similarity mirrors the observations of Bridges and Yoshikami (1969) for rod photopigments (rhodopsin and porphyropsin). Some differences were observed, however, and in particular the hyperlimnetic-dwelling kokanee appeared to differ the most from the other fishes examined.

Although orientation data collected under laboratory conditions differed from those data collected under semi-natural (field) conditions (i.e., statistical treatment and sample size), it is nonetheless a worthwhile endeavour to compare orientation error among juveniles of these salmonine species. A comparative summary of percent error in orientation responses was calculated from the data in Chapter V. The data from Chapter V were summarised to provide a comparison of orientation abilities among species under an artificial polarised-light source (HNP'B) (Fig. 35A) and a natural polarised-light (celestial) source (Fig. 35B). Rainbow trout and brook char had approximately 50% of their responses within 20° of their entrained angle (Fig. 35A). Steelhead (from laboratory and field) had 70% and 80% of their responses, respectively, within 20° of the entrained angle of orientation. This similarity in responses in steelhead using different training methodologies indicated that relative comparisons, in general, were probably valid. Ultimately, 100% of the sockeye were distributed within 20° of the angular location of their feeder box during training. Thus, under a UV-transmissive linear polariser, the more migratory forms, sockeye and steelhead, responded more accurately than the two non-migratory representatives, rainbow trout and brook char.

When tested under natural polarised light (see Chapter V for details), 60% of sockeye responded between 11° and 20° of their trained angle (Fig. 35B), a marked decrease from their performance with the polarising filter. In contrast, 60% of rainbow trout responded within 10° of the correct angle and 90% responded within 20° of their entrained angle. Similar to sockeye, steelhead

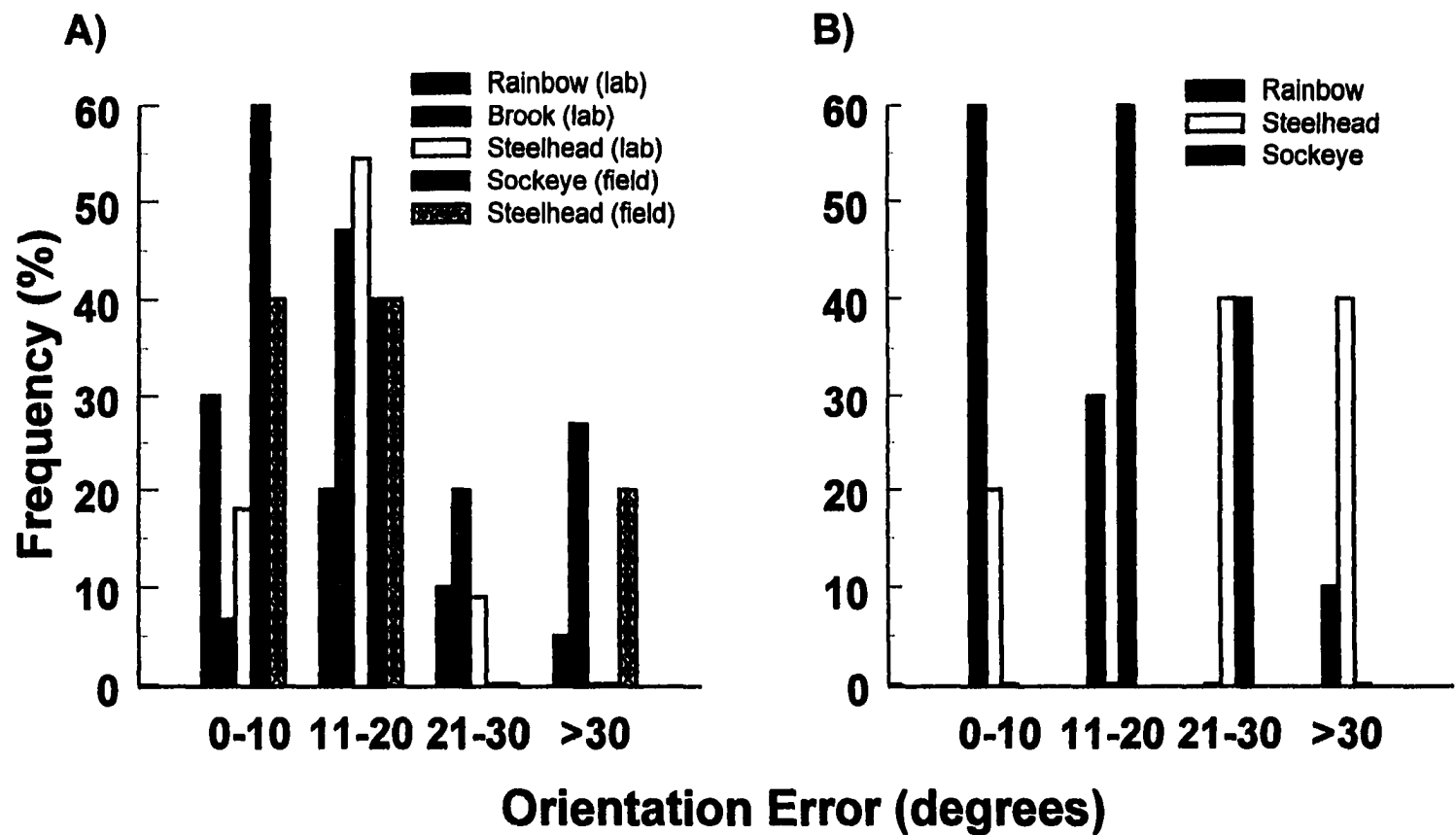


Figure 35. Percent frequency of responses as a function of orientation error in degrees: A) Responses obtained using an HNP'B polariser as the source of polarised light in lab and controlled field conditions B) Responses to natural polarised light fields under clear, sunny conditions.

showed a behavioural deficit relative to their performance under a polariser, and only 20% responded within 10° of the angle to which they were entrained. Given that the fish compared under laboratory conditions using a polariser were subject to roughly the same polarised-light conditions (~100% polarised light) (Fig. 35A), it is tempting to conclude that the differences in orientation under natural polarised light reflect differences in per cent polarised light on the specific days of testing. Indeed, when rainbow trout were tested, the % polarisation was between 59% and 63% (Fig. 32). However, the celestial-polarised light was 51% for sockeye exposed to only the zenith of the sky (a more restrictive scenario than the testing for either the rainbow or the steelhead) (Fig. 34F) and only 45% for steelhead exposed to all cues (Fig. 34A). Alternatively, water clarity in the testing ponds may have affected the relative comparability of the results. Rainbow trout (Fig. 32) were tested in dechlorinated municipal water, whereas the steelhead and sockeye were tested in lake water. This, however, should not have been a great factor because the steelhead and sockeye oriented more accurately under the polariser than the rainbow trout. Therefore, it is more probable that the lower levels of polarised light contributed to the decreased orientation ability in the steelhead, and to a lesser extent the sockeye, under semi-natural testing conditions. Hence, at 45% polarised light, we may have been at or approaching the limit for orientation of salmonids to polarised light.

The biological roles of polarisation sensitivity only recently have begun to be explored. Certainly, fish can be trained to orient to the plane of polarised light and to perform orientation-responses under natural polarised-light fields.

However, is polarised light used by wild fish orienting in nature? This is a challenging question to address. To date, Groot (1965), Forward *et al.* (1972), Waterman and Forward (1972), and Forward and Waterman (1973) provide the most extensive studies examining orientation responses of wild fish to polarised light. Although, it is known that parr trout in the laboratory can orient to the plane of polarised light, few studies have examined larger fish. Pseudo-smoltified rainbow trout do not orient to plane-polarised light (Hawryshyn *et al.* 1990), but it is not known if reproductive fish can orient using this cue. Because adult salmonids often undergo extensive migrations, the utility of polarised light as a compass mechanism has great appeal. This is an area that requires extensive investigation to understand if a role exists for polarised-light sensitivity in migrating adult salmonids.

Polarised-light and Orientation of Adult Salmonids

Following some initial work that indicated the presence of UV and polarised-light sensitivity in large juvenile and adult salmonids (Parkyn, unpublished data), a small-scale study was initiated to train adult salmonids to orient using linearly-polarised light. The same methodology was used for these fish as detailed for the laboratory studies in Chapter V. Concomitantly, an histological examination of several species of mature salmonids identified relic populations of UV receptors in the retina (Beaudet *et al.* 1997).

Twenty-two reproductive rainbow trout were trained and tested. All fish were obtained from Mountain Trout Sales, Sooke, B.C. Reproductive status was

confirmed by the presence of milt in males and eggs in females. Twelve of the fish were late fall/winter-spawning rainbow trout and were tested in late November 1994. An additional ten fish were spring-spawning rainbow trout and were tested in April 1995. Typically, adult fish swam directly to the edge of the testing-tank (Fig. 36A-D). However, 9 of 22 fish swam in one direction and then doubled back in the opposite direction, and thus had bi-directional movements (Fig. 36E,F). This behaviour was not the result of the fish coming into close contact with the wall of the tank because the fish did not approach the tank wall any closer than 40 cm. The mean behavioural responses of the trout were significantly oriented (V-test: $P < 0.005$) (Fig. 37). Thus, it is evident that mature rainbow trout do have the ability to orient to linearly polarised light in a laboratory situation. However, the significance of the role of polarised light in migration of salmonids remains to be assessed. The native, ocean habitat of salmonids in the North Pacific is subject to much rain and overcast conditions. This does not seem to be ideal for such a compass. However, this compass may be important only at certain times of the year. For example, summers and early fall in the North Pacific tend to be less rainy than winters. Logically, studies should be conducted on migrating fish to assess under which conditions polarised-light sensitivity could serve migrating adult salmonids. Because such studies are difficult to conduct with free-ranging fish, initial work should be conducted on a larger scale under semi-natural conditions, for example in large sea pens. In addition, many mature salmonids cease to feed in the nearshore phase of their

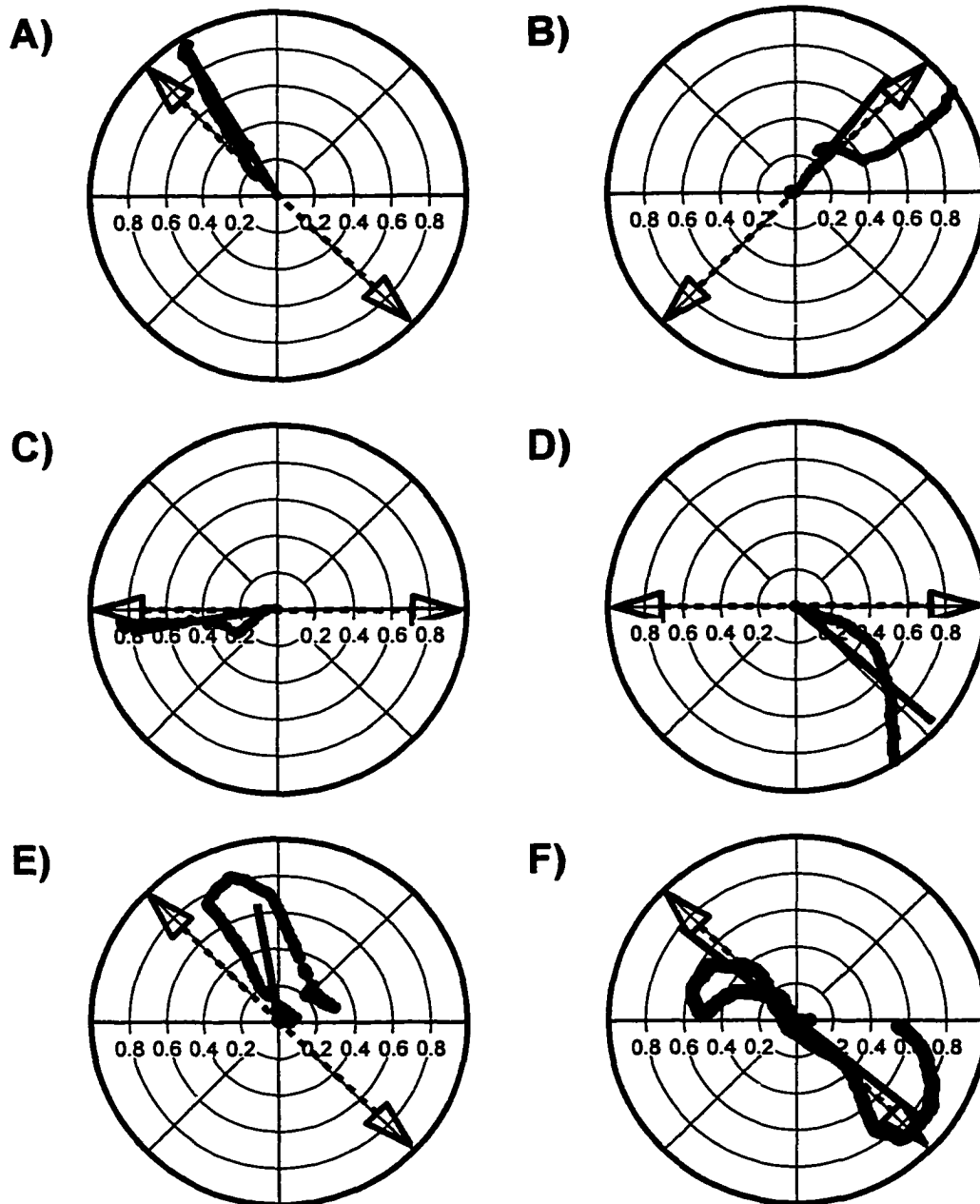


Fig. 36. A-F. Orientation responses of individual adult rainbow trout trained to orient parallel to a linearly-polarised light source, as tracked using the F-chase algorithm. Heavy line = $\vec{\phi}_s$. Radius of heavy line = r_v .

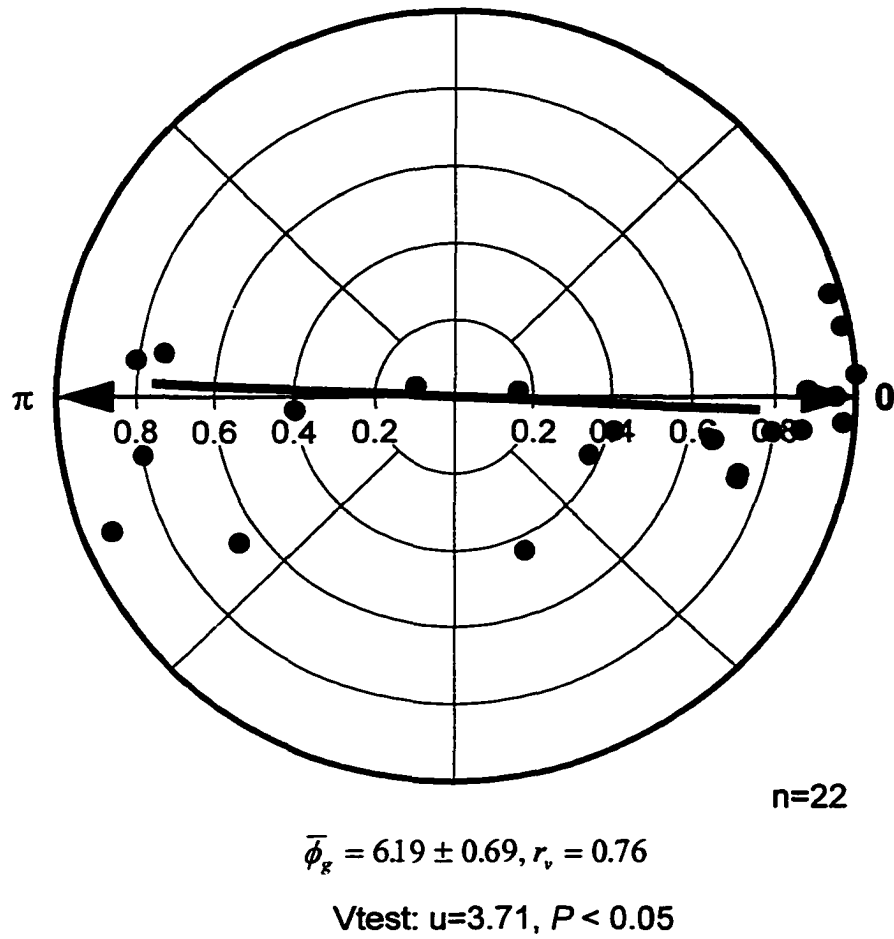


Fig. 37. Orientation responses of sexually-reproductive adult rainbow trout. Symbols equal individual $\bar{\phi}_s$ of fish. Length of vector = \bar{r}_v . Heavy line represents $\bar{\phi}_g$. Double headed arrow indicates responses were standardised to the bearing $0/\pi$ ($0/180^\circ$).

spawning migrations, it would be necessary to use fish that are still feeding voraciously in the open-ocean phase of their migration. Alternatively, a methodological technique that does not require the fish to eat would be necessary.

Other Roles for Polarised Light Sensitivity

Are there other roles for polarised-light sensitivity? Certainly, polarising structures such as corneal iridophores (Chapter I) can be understood in terms of an analogy to sunglasses. As well, polarised-light sensitivity has been modelled with video-cameras and can enhance visibility of objects in turbid media by 2 to 3 times (Bains 1996, Tyo *et al.* 1996). These researchers speculated that this system mimics the visual system of sunfish (Cameron and Pugh 1991, Rowe *et al.* 1994), a species inhabiting eutrophic and often turbid water (however, see Novales and Hawryshyn 1997b for a contrasting view of polarisation sensitivity in this fish). Accordingly, if their model is correct, this polarisation subtraction would serve as a mechanism for enhancing contrast, because the percentage and relative orientation of the e-vectors from a target can differ from the background veiling illumination.

Recently, *Octopus vulgaris* has been shown to discriminate objects on the basis of differences in the patterns of polarisation or polarisation contrast (Shashar and Cronin 1996). It is conceivable that salmonids also may be capable of similar discriminations, because analysis of patterns of polarised light in water would require extraction of information relating the orientation and

relative polarisation of light across the visual field. Patterns of polarised light also differ on the bodies of salmonids (Parkyn, unpublished data). These patterns undoubtedly result from the differences in the deposition of guanine and hypoxanthine crystals in the skin on the sides of the fish.

Several explanations have been offered for the biophysical mechanisms mediating polarised-light sensitivity (Underwood 1968, Snyder 1973, Young and Martin 1984, Gerharz 1982, Hawryshyn and McFarland 1987, Rowe *et al.* 1994, Novales *et al.* 1998). Although the latter model appears encouraging, several issues still remain to be addressed, including an experimental demonstration of the mechanism of differential e-vector absorption at the level of the photoreceptor. In addition, it is not known if polarised light information is processed at the level of the retina, or to what extent polarisation sensitivity is distributed across the retina. Excellent single-unit measurements have been made, however, that demonstrate polarisation opponency at the level of the *torus semicircularis* (Coughlin and Hawryshyn 1995).

One future area of research I will address is the potential effect of the nasal groove at the anterior portion of the eye on polarisation sensitivity. Although this region undoubtedly confers binocular vision in salmonids, under some states of accommodation of the lens, the space between the lens and the edge of the pupil is quite large. In an adult rainbow trout, this aphakic space can, at times, exceed 1 mm (personal observation). When the light path does not include the lens, the light would not be useful for image formation. However, this region could potentially subserve to transmit information on the state of

polarisation of the ambient light field, because it should illuminate photoreceptors axially. This is because living photoreceptors are believed to be oriented toward the lens and the aperture of the pupil (Webb 1977). Unfortunately, to date, no *in vivo* measurements of the effects of this form of axial illumination on polarisation sensitivity of fishes have been undertaken. Alternatively, such light could act as noise for a polarisation sensitive system that is mediated by information that strikes the retina via the lens.

An additional area of research that warrants further investigation is the adaptive properties of polarisation sensitivity. Kreithen and Keeton (1974) noted that e-vector sensitivity rapidly adapts. Thus, they chose a rotating polarised-light source for their studies, considering it more similar to what the animal would encounter in nature. A similar observation was made by de Groot (1979) and de Groot and de Pender (1979), who noted an increase in perceived brightness when a polarised-light stimulus was rotated following a period of adaptation to a non-moving polarised-light background. De Groot termed this perceived brightness a "transient threshold increase", because it manifested itself as a flicker-like phenomenon. He also believed that this effect arose as a result of the Stiles-Crawford Effect (see Marriott 1962 for further details). A similar phenomenon may be present in fishes, although the effect appears to more stable and pronounced (personal observation). Such an adaptation effect would be useful for enhancement of contrast. If this effect manifests itself through opponent interactions of horizontal and vertical polarised-light sensitive mechanisms, then it would operate in a manner similar to chromatic adaptation.

Therefore, the dominant e-vector in the light field would adapt the retina, increasing the relative sensitivity of the less dominant orthogonal e-vector. This perceived change in intensity could serve as a feature detector. Alternatively, it could operate to reduce glare or in a contrast enhancement function, as has been suggested by Bains (1996), Tyo *et al.* (1996), and Rowe *et al.* (1994).

Thus, it is important for researchers to be cognisant that major differences can exist in architecture and function of sensory systems among humans (as the researchers) and other animals. Because these differences greatly influence what they, and we, can perceive in our respective environments, it is important that sensory studies be consistent with what the animal potentially perceives, rather than our own particularly human perspective.

LITERATURE CITED

- Able, K.P. 1982. Skylight polarization patterns at dusk influence the migratory orientation of birds. *Nature* 299: 550-551.
- Able, K.P. 1989. Skylight polarization patterns and the orientation of migratory birds. *Journal of Experimental Biology* 141: 241-256.
- Adler, K., and D.H. Taylor. 1973. Extraocular perception of polarized light by orienting salamanders. *Journal of Comparative Physiology* 87: 203-212.
- Adron, J.W., P.T. Grant, and C.B. Cowey. 1973. A system for the quantitative study of the learning capacity of rainbow trout and its application to the study of food preferences and behaviour. *Journal of Fish Biology* 5: 625-636.
- Ahlbert, J.B. 1976. Organization of the cone cells in the retinae of salmon (*Salmo salar*) and trout (*Salmo trutta trutta*) in relation to their feeding habits. *Acta Zoologica (Stockholm)* 57: 13-35.
- Ali, M.A. 1975. Retinomotor responses. *Pages 313-355 in Vision in fishes: New approaches in research. Edited by M.A. Ali. Plenum Press, New York, NY. 836 pp.*
- Ali, M.A., and M. Anctil. 1976. *Retinas of fishes: An atlas. Springer-Verlag, Berlin. 284 pp.*
- Allen, D.M., and F.W. Munz. 1983. Visual pigment mixtures and scotopic spectral sensitivity in rainbow trout. *Environmental Biology of Fishes* 8: 185-190.
- Allen, D.M., W.N. McFarland, F.W. Munz, and H.A. Poston. 1973. Changes in visual pigments of trout. *Canadian Journal of Zoology* 51: 901-914.
- Altmann, J. 1974. Observational study of behavior: Sampling methods. *Behaviour* 49: 227-265.
- Arnold, G.P., and P.H. Cook. 1984. Fish migration by selective tidal stream transport: First results with a computer simulation model for the European continental shelf. *Pages 227-261 in Mechanisms of migration in fishes. Edited by J.D. McKeave, G.P. Arnold, J.J. Dodson, and W.H. Neill. Plenum Press, New York. 574 pp.*

- Arnold, K., and C. Neumeyer. 1987. Wavelength discrimination in the turtle *Pseudemys scripta elegans*. *Vision Research* 27: 1501-1511.
- Aronson, L.R. 1951. Orientation and jumping behavior in the gobiid fish (*Bathygobius soporator*). *American Museum Novitates* 1486:1-22.
- Avery, A.V., and J.K. Bowmaker. 1982. Visual pigments in the four-eyed fish *Anableps anableps*. *Nature (London)* 298: 62-63.
- Avery, J.A., J.K. Bowmaker, M.B.A. Djamgoz, and J.L.G. Downing. 1983. Ultra-violet sensitive receptors in a freshwater fish. *Journal of Physiology (London)* 334: 23-24.
- Baburina, E.A., P.B. Bogatyrev, and V.R. Protasov. 1968. A study of age variation of acuity of sight of some fish. *Zoologicheskii Zhurnal* 47: 1364-1369.
- Bagarinao, T., and J.R. Hunter. 1983. The visual feeding threshold and action spectra of northern anchovy (*Engraulis mordax*) larvae. *California Cooperative Oceanic Fisheries Investigative Report* 14: 245-54.
- Bains, S. 1996. Machine vision - sunfish shows the way through the fog. *Science*: 272: 653-653.
- Bartsch, K. 1995. Polarization-sensitive photoreceptors of different spectral types in the the compound eye of waterstriders. *Naturwissenschaften* 82: 292-293.
- Bassi, C.J., and M.K. Powers. 1987. Circadian rhythm in goldfish visual sensitivity. *Investigative Ophthalmology and Vision Science* 28: 1811-15.
- Batschelet, E. 1979. Second-order statistical analysis of directions. *Pages 3-26 in Animal migration, navigation, and homing. Edited by K. Schmidt-Koenig and W.T. Keeton. Springer-Verlag, New York. 462 pp.*
- Batschelet, E. 1981. *Circular Statistics in Biology*. Academic Press, New York, NY. 371 pp.
- Bayliss, L.E., R.J. Lythgoe, and K. Tansley. 1936. Some new forms of visual purple found in sea fishes, with a note on the visual cells of origin. *Proceedings of the Royal Society, Series B*, 816: 95-113.
- Beatty, D.D. 1966. A study of the succession of visual pigments in Pacific salmon (*Oncorhynchus*). *Canadian Journal of Zoology* 44: 429-455.

- Beatty, D.D. 1984. Visual pigments and the labile scopic visual system of fish. *Vision Research* 1984: 1563-1573.
- Beaudet, L., H.I. Browman, and C.W. Hawryshyn. 1993. Optic nerve response and retinal structure in rainbow trout of different sizes. *Vision Research* 33: 1739-1746.
- Beaudet, L., I. Novales Flamarique, and C.W. Hawryshyn. 1997. Cone photoreceptor topography in the retina of sexually mature Pacific salmonid fishes. *Journal of Comparative Neurology* 383: 49-59.
- Beauchamp, R.D., and J.S. Rowe. 1977. Goldfish spectral sensitivity: A conditioned heart rate measure in restrained or curarized fish. *Vision Research* 17: 617-624.
- Beauchamp, R.D., J.S. Rowe, and L.A. O'Reilly. 1979. Goldfish spectral sensitivity: Identification of the three cone mechanisms in heart-rate conditioned fish using colored adapting backgrounds. *Vision Research* 19: 1295-1302.
- Behnke, R.J. 1992. Native trout of western North America. American Fisheries Society, Monograph 6. Bethesda, Maryland. 273 pp.
- Bennett, Andrew T.D., Innes C. Cutthill, Julian C. Partridge, and Erhard J. Maier. 1996. Ultraviolet vision and mate choice in zebra finches. *Nature*. 380: 433-435.
- Bernard, G.D. 1987. Spectral characteristics of butterfly L-receptors using extended Dartnall/MacNichol template functions. Abstracts of Annual Meeting of the Optical Society of America. *Journal of the Optical Society of America, Series A*, 4: 123.
- Bernard, G.D., and R. Wehner. 1977. Functional similarities between polarization vision and color vision. *Vision Research* 17: 1019-1028.
- Bilotta, J., R.J. DeMarco, Jr., and M.K. Powers. 1995. The contributions of ON- and OFF- pathways to contrast sensitivity and spatial resolution in goldfish. *Vision Research* 35: 103-108.
- Boehm, G. 1940. Über ein neues entoptisches Phänomen im polarisierten Licht. 'Periphere' Polarsationsbüschel. *Acta Ophthalmologica* 18: 143-169.
- Boehlert, G.W. 1978. Intraspecific evidence for the function of single and double cones in the teleost retina. *Science* 202: 309-311.

- Bowmaker, J.K. 1990. Visual pigments of fish. *Pages 81-107 in Vision in fishes. Edited by R.H. Douglas and M.B.A. Djamgoz. Chapman and Hall, London. 526 pp.*
- Bowmaker, J.K., and Y.W. Kunz. 1987. Ultraviolet receptors, tetrachromatic colour vision and retinal mosaics in the brown trout (*Salmo trutta*): Age-dependent changes. *Vision Research 27: 2101-2108.*
- Bradbury, J.W. and S.L. Verencamp. 1998. Principles of animal communication. Sinauer Associates, Sunderland New Jersey. 882 pp.
- Braemer, W.A. 1960. A critical review of the sun-azimuth hypothesis. *Cold Spring Harbor Symposium on Quantitative Biology 25: 413-428.*
- Bridges, C.D.B., and S. Yoshikami. 1970. Distribution and evolution of visual pigments in salmonid fishes. *Vision Research 10: 609-626.*
- Browman, H.I., and C.W. Hawryshyn. 1992. Thyroxine induces a precocial loss of ultraviolet photosensitivity in rainbow trout (*Oncorhynchus mykiss*, Teleosti). *Vision Research 32: 2303-2312.*
- Browman, H.I., and C.W. Hawryshyn. 1994a. The developmental trajectory of ultraviolet photosensitivity in rainbow trout is altered by thyroxine. *Vision Research 34: 1397-1406.*
- Browman, H.I., and C.W. Hawryshyn. 1994b. Retinoic acid modulates retinal development in the juveniles of a teleost fish. *Journal of Experimental Biology 193: 191-207.*
- Browman, H.I., I. Novales Flamarique, and C.W. Hawryshyn. 1994. Ultraviolet photoreception contributes to prey search behaviour in two species of zooplanktivorous fishes. *Journal of Experimental Biology 186: 187-198.*
- Brown, P.K., and G. Wald. 1963. Visual pigments in human and monkey retinas. *Nature 200: 37-43.*
- Buckland, F. 1880. Natural history of British fishes. Unwin, London. 420 pp.
- Cameron, N.E. 1982. The photopic spectral sensitivity of a dichromatic teleost fish (*Perca fluviatilis*). *Vision Research 22: 1341-1348.*
- Cameron, D.A., and E.N. Pugh. 1991. Double cones as a basis for a new type of polarization sensitivity in vertebrates. *Nature (London) 353: 161-164.*

- Campton, D.E., and F.E. Utter. 1985. Natural hybridization between steelhead trout (*Salmo gairdneri*) and coastal cutthroat trout (*Salmo clarki clarki*) in two Puget Sound streams. *Canadian Journal of Fisheries and Aquatic Sciences* 42: 110-119.
- Cavanaugh, G.M. 1956. Formulae and methods of the marine biological laboratory chemical room. Marine Biological Laboratory, Woods Hole, MA.
- Chen, D.M., J.S. Collins, and T.H. Goldsmith. 1984. The ultraviolet receptors of bird retinas. *Science* 225: 337-340.
- Chew, G.L., and G.E. Brown. 1989. Orientation of rainbow trout (*Salmo gairdneri*) in normal and null magnetic fields. *Canadian Journal of Zoology* 67: 641-643.
- Chittka, L. 1996. Does bee color vision predate the evolution of flower color? *Naturwissenschaften* 83: 136-138.
- Churmasov, A.V., and A.S. Stepanov. 1977. Sun orientation and guideposts of humpback salmon. *Soviet Journal of Marine Biology* 3 (5): 55-63.
- Clarke, G.L. 1936. On the depth at which fish see. *Ecology* 17: 452-456.
- Coemans, M., J. Vos Hzn, and J. Nuboer. 1990. No evidence for polarization sensitivity in the pigeon. *Naturwissenschaften* 77: 138-142.
- Conover, W.J. 1980. *Practical nonparametric statistics*, 2nd ed. John Wiley & Sons, Toronto. 493 pp.
- Corion Corporation. 1990. *Optical filters and coatings*. Holliston, MA. 110 pp.
- Coughlin, D.J., and C.W. Hawryshyn. 1994a. The contribution of ultraviolet and short-wavelength sensitive cone mechanisms to color vision in rainbow trout. *Brain, Behaviour and Evolution* 43: 219-232.
- Coughlin, D.J., and C.W. Hawryshyn. 1994b. Ultraviolet sensitivity in the *torus semicircularis* of juvenile rainbow trout (*Oncorhynchus mykiss*). *Vision Research* 34: 1407-1413.
- Coughlin, D.J., and C.W. Hawryshyn. 1995. A cellular basis for polarized-light vision in rainbow trout. *Journal of Comparative Physiology A* 176: 261-272.
- Craigie, E.H. 1926. A preliminary experiment upon the relation of the olfactory sense to the migration of the sockeye salmon (*Oncorhynchus nerka*, Walbaum). *Transactions of the Royal Society of Canada* 20: 215-224.

- Crescitelli, F. 1991. The natural history of visual pigments: 1990. *Pages 1-32 in Progress in retinal research, volume II. Edited by N.N. Osborne and G.J. Chader. Pergamon Press, Oxford. 391 pp.*
- Crescitelli, F., M. McFall-Ngai, and J. Horwitz. 1985. The visual pigment sensitivity hypothesis: Further evidence from fishes of varying habitats. *Journal of Comparative Physiology A 157: 323-333.*
- Cronin, T.W., N.J. Marshall, and R.L. Caldwell. 1993. Photoreceptor spectral diversity in the retinas of squilloid and lysiosquilloid stomatopod crustaceans. *Journal of Comparative Physiology A 172: 339-350.*
- Cronin, T.W., N.J. Marshall, R.L. Caldwell, and N. Shashar. 1994. Specialization of retinal function in the compound eyes of mantis shrimps. *Vision Research 34: 2639-2656.*
- Cserháti, P., A. Szél, and P. Röhlich. 1989. Four cone types characterized by anti-visual pigment antibodies in the pigeon retina. *Investigative Ophthalmology and Visual Science 30: 74-81.*
- Dartnall, H.J.A. 1975. Visual pigments and photic environments. *Pages 543-563 in Vision in fishes. Edited by M.A. Ali. Plenum Press, New York, NY. 836 pp.*
- Dartnall, H.J.A., M.R. Lander, and F.W. Munz. 1961. Periodic changes in the visual pigments of a fish. *Pages 203-213 in Progress in Photobiology. Edited by B.C. Christensen and B. Buchmann. Elsevier, Amsterdam.*
- Davitz, M.A., and K.R. McKaye. 1978. Discrimination between horizontally and vertically polarised light by the cichlid fish *Pseudotropheus macropthalmus*. *Copeia 2: 333-334.*
- Daw, 1968. Colour-coded ganglion cells in the goldfish retina: Extension of their receptive fields by means of new stimuli. *Journal of Physiology 197: 567-592.*
- Deegan, J.F., III., and G.H. Jacobs. 1993. On the identity of the cone types of the rat retina. *Experimental Eye Research 56: 375-377.*
- DeMarco, P.J., Jr., and M.K. Powers. 1991. Spectral sensitivity of ON and OFF responses from the optic nerve of goldfish. *Visual Neuroscience 6: 207-217.*
- DeMarco, P.J., Jr., and M.K. Powers. 1994. APB alters photopic spectral sensitivity of the goldfish retina. *Vision Research 34: 1-9.*

- Denton, E.J. 1954. A method of easily observing the dichroism of the visual rods. *Journal of Physiology* 124: 16-17.
- Deutschlander, M. E. and J.B. Phillips. 1995. Ultraviolet sensitivity in the Axotyl, *Ambystoma mexicana*. *Neuroscience Letters*. 197: 93-96.
- Dietz, M. 1972. Erdkröten können UV-Licht sehen. *Naturwissenschaften* 59: 316.
- Dill, P.A. 1965. Polarized light perception and orientation by yearling sockeye salmon (*Oncorhynchus nerka*). M. Sc. thesis, University of British Columbia, Vancouver, B.C.
- Dill, P.A. 1971. Perception of polarized light by yearling sockeye salmon (*Oncorhynchus nerka*). *Journal of the Fisheries Research Board of Canada* 28: 1319-1322.
- Douglas, R.H. 1982. The function of photomechanical movements in the retina of rainbow trout (*Salmo gairdner*). *Journal of Experimental Biology* 96: 389-403.
- Douglas, R.H. 1983. Spectral sensitivity of rainbow trout (*Salmo gairdner*). *Revue Canadienne de Biologie* 42: 117-122.
- Douglas, R.H. 1986. Photopic spectral sensitivity of a teleost fish, the roach (*Rutilus rutilus*), with special reference to its ultraviolet sensitivity. *Journal of Comparative Physiology A* 159: 415-421.
- Douglas, R.H. 1989. The spectral transmission of the lens and cornea of the brown trout (*Salmo trutta*) and goldfish (*Carssius auratus*): Effect of age and implications for ultraviolet vision. *Vision Research* 29: 861-869.
- Douglas, R.H., and H.J. Wagner. 1982. Endogenous patterns of photomechanical movements and their relationship to activity rhythms in teleosts. *Cell Tissue Research* 226: 133-144.
- Douglas, R.H., and C.M. McGuigan. 1989. The spectral transmission of freshwater teleost ocular media- and interspecific comparison and guide to potential ultraviolet sensitivity. *Vision Research* 29: 871-879.
- Douglas, R.H., and C.W. Hawrsyhyn. 1990. Behavioural studies of fish vision: An analysis of visual capabilities. *Pages 373-417 in The visual system of fish. Edited by R.H. Douglas and M.B.A. Djamgoz. Chapman and Hall, London. 526 pp.*

- Douglas, R.H., J.K. Bowmaker, and Y.W. Kunz-Ramsay. 1989. Ultraviolet vision in fish. *Pages 601-616 in Seeing Contour and Colour. Edited by J.J. Kulikowski, C.M. Dickinson, and I.J. Murray. Pergamon Press, Oxford.*
- Dowling, J.E. 1987. *The retina: An approachable part of the brain. Belknap Press of Harvard University, Cambridge, Massachusetts. 282 pp.*
- Dunn, J. R. 1969. Direction of movement of salmon in the North Pacific Ocean, Bering Sea, and Gulf of Alaska as indicated by surface gillnet catches. *International North Pacific Fisheries Commission Bulletin 26: 27-55.*
- Durand, D., and G.W. Greenwood. 1958. Modification of the Rayleigh test for uniformity in analysis of two-dimensional orientation data. *Journal of Geology 66: 229-238.*
- Eakin, R.M. 1968. Evolution of photoreceptors. *American Scientist 2: 194-242.*
- Easter, S.S. 1975. Retinal specialisations for aquatic vision: Theory and facts. *Pages 609-617 in Vision in Fishes. Edited by M.A. Ali. Plenum Press, New York. 836 pp.*
- Ekman, S. 1932. Prinzipielles über die Wanderungen und die tiergeographische Stellung des europäischen Aales, *Anguilla anguilla* L. *Zeogeographica 1: 85-106.*
- Emlen, S.T. 1969. Bird migration: Influence of physiological state upon celestial orientation. *Science 165: 716-718.*
- Fein, A., and E.Z. Szuts. 1982. Photoreceptors and their role in vision. *IUPAB Biophysics Series. Cambridge University Press, Cambridge. 212 pp.*
- Fernald, R.D. 1988. Aquatic adaptations in fish eyes. *Pages 435-466 in Sensory biology of aquatic animals. Edited by J. Atema, R.R. Fay, A.N. Popper, and W.N. Tavolga. Springer-Verlag, New York. 936 pp.*
- Fernald, R.D. 1990. The optical system of fishes. *Pages 45-80 in The visual system of fish. Edited by R.H. Douglas and M.B.A. Djamgoz. Chapman and Hall, London. 526 pp.*
- Fineran, B.A., and J.A.C. Nichol. 1978. Studies on the photoreceptors of *Anchoa mitchelli* and *Anchoa hepsetus* (Engraulidae) with particular reference to the cones. *Philosophical Transactions of the Royal Society of London, Series B, 283: 25-60.*

- Fisher, N.I. 1993. *Statistical analysis of circular data*. Cambridge University Press, Cambridge. 277 pp.
- Fleishman, L., and E.R. Loew. 1993. Ultraviolet vision in lizards. *Nature* 30: 397.
- Fleishman, L.J., Bowman, M. Saunders, D, Miller, W.E., Rury, M.J., and E.R. Loew. 1997. The visual ecology of Puerto Rican anoline lizards: habitat, light and spectral sensitivity. *Journal of Comparative Physiology*. 181: 446-460.
- Fontaine, M., and R. Vibert. 1952. Migration fluviale anadrome du saumon (*Salmo salar* L.) et gradient de salinité. *Annales de la Station Central Hydrobiologique* 4: 339-346.
- Foote, C.J., C.C. Wood, and R.E. Withler. 1989. Biochemical genetic comparison of sockeye salmon and kokanee, the anadromous and nonanadromous forms of *Oncorhynchus nerka*. *Canadian Journal of Fisheries and Aquatic Sciences* 46: 149-158.
- Foote, C.J., C.C. Wood, W.C. Clarke, and J. Blackburn. 1992. Circannual cycle of seawater adaptability in *Oncorhynchus nerka*: Genetic differences between sympatric sockeye and kokanee. *Canadian Journal of Fisheries and Aquatic Sciences* 49: 99-109.
- Forward, R.B., Jr., and T.H. Waterman. 1973. Evidence for e-vector and light intensity pattern discrimination by the teleost *Dermogenys* sp. *Journal of Comparative Physiology A* 87: 189-202.
- Forward, R.B., Jr., K.W. Horch, and T.H. Waterman. 1972. Visual orientation at the water surface by the teleost *Zenarchopterus*. *Biological Bulletin* 143: 112-126.
- Foster, D.H., and P.A. Ward. 1991. Asymmetries in oriented-line detection indicate two orthogonal filters in early vision. *Proceedings of the Royal Society of London B* 243: 75-81.
- Frisch, K., von. 1949. Die Polarisation des Himmelslichtes als orientierender Faktor bei den tanzender Bienen. *Experientia* 4: 142-148.
- Gerharz, R. 1982. Peripheral response of the retina to polarized light. *Ophthalmologica* 185: 26-31.

- Goddard, S.M., and R.B. Forward, Jr. 1991. The role of the underwater polarized light pattern, in sun compass navigation of the grass shrimp, *Palaemonetes vulgaris*. *Journal of Comparative Physiology A* 169: 479-491.
- Goldsmith, T.H. 1980. Hummingbirds see U.V. *Science* 207: 786-788.
- Goldsmith, T.H. 1994. Ultraviolet receptors and color vision: Evolutionary implications and a dissonance of paradigms. *Vision Research* 34: 1479-1487.
- Goodyear, C.P. 1970. Terrestrial and aquatic orientation in the starhead topminnow, *Fundulus notti*. *Science* 168: 603-605.
- Govardovskii, V.I., and L.V. Zueva. 1974. Spectral sensitivity of the frog eye in the ultraviolet and visible region. *Vision Research* 32: 1317-1321.
- Groot, C. 1965. On the orientation of young sockeye salmon (*Oncorhynchus nerka*) during their seaward migration out of lakes. *Behaviour* [Supplemental] 14: 198 pp.
- Groot, C., and T.P. Quinn. 1987. Homing migration of sockeye salmon, *Oncorhynchus nerka*, to the Fraser River. *Fishery Bulletin* 85: 455-469.
- Groot, C., and L. Margolis. 1991. Pacific salmon life histories. UBC Press, Vancouver. 564 pp.
- de Groot, P.J. 1979. Transient threshold increase due to combined changes in direction of propagation and plane of polarization. *Vision Research* 19: 1253-1259.
- de Groot, P.J., and E. de Pender. 1979. A transient effect contingent on the direction of polarization of light incident on the cornea. *Vision Research* 19: 1065-1066.
- Hädinger, W. 1844. Über das direkte Erkennen des polarisierten Lichtes und der Lage der Polarisationssebene. *Annalen der Physik und Chemie* 63: 29-39.
- Hailman, J.P. 1977. *Optical signals: Animal communication and light*. Indiana University Press, Bloomington, Indiana. 362 pp.
- Hairston, N.G., Jr., K.T. Li, and S.S. Easter, Jr. 1982. Fish Vision and the detection of planktonic prey. *Science* 218: 1240-1242.

- Harden-Jones, F.R. 1968. Fish migration. Arnold Publishing, London. 325 pp.
- Hárosi, F.I. 1975. Linear dichroism of rods and cones. *Pages 55-65 in Vision in fishes. Edited by M.A. Ali. North American Treaty Organisation (NATO) Advanced Study Institute Series, Volume 1. Plenum Press, New York. 836 pp.*
- Hárosi, F.I. 1994. An analysis of two spectral properties of vertebrate visual pigments. *Vision Research 34: 1359-1368.*
- Hárosi, F.I., and Y. Hashimoto. 1983. Ultraviolet visual pigment in a vertebrate: A tetrachromatic cone system in the dace. *Science 222: 1021-1023.*
- Hart, J.L. 1973. Pacific fishes of Canada. Fisheries Research Board of Canada. *Bulletin 180: 740 pp.*
- Hartline, H.K. 1940. Receptive fields of optic nerve fibres. *American Journal of Physiology 130: 690-699.*
- Hartt, A.C., and M.B. Dell. 1986. Early oceanic migrations and growth of juvenile Pacific salmon and steelhead trout. *International North Pacific Fisheries Commission Bulletin 46: 105 pp.*
- Hasler, A.D. 1971. Orientation and fish migration. *Pages 429-510 in Fish physiology, volume 6. Edited by W.S. Hoar and D.J. Randall. Academic Press, London.*
- Hasler, A.D., and W.J. Wisby. 1951. Discrimination of stream odors by fishes and relation to parent stream behaviour. *American Naturalist 85: 223-238.*
- Hasler, A.D., and A.T. Scholz. 1983. Olfactory imprinting and homing in salmon. Springer-Verlag, Berlin. 134 pp.
- Hasler, A.D., R.M. Horrall, W.J. Wisby, and W. Braemer. 1958. Sun-orientation and homing in fishes. *Limnology and Oceanography 3: 353-61.*
- Hawryshyn, C.W. 1984. Ultraviolet photoreception in fish. Ph.D. Dissertation, University of Waterloo, Ontario. 294 pp.
- Hawryshyn, C.W. 1991a. Light-adaptation properties of the ultraviolet-sensitive cone mechanism in comparison to the receptor mechanisms in goldfish. *Visual Neuroscience 6: 293-301.*

- Hawryshyn, C.W. 1991b. Spatial summation of cone mechanisms in goldfish. *Vision Research* 31: 563-566.
- Hawryshyn, C.W. 1992. Polarization vision in fishes. *American Scientist* 80: 164-175.
- Hawryshyn, C.W., and R. Beauchamp. 1985. Ultraviolet photosensitivity in goldfish: An independent U.V. retinal mechanism. *Vision Research* 25: 11-20.
- Hawryshyn, C.W., and W.N. M^cFarland. 1987. Cone photoreceptor mechanisms and detection of polarised light in fish. *Journal of Comparative Physiology A* 160: 459-465.
- Hawryshyn, C.W., and A.E. Bolger. 1990. Spatial orientation of trout to partially polarised light. *Journal of Comparative Physiology A* 167: 691-697.
- Hawryshyn, C.W., and F.I. Hárosi. 1994. Spectral characteristics of visual pigments in rainbow trout (*Oncorhynchus mykiss*). *Vision Research* 34: 1385-1392.
- Hawryshyn, C.W., M.G. Arnold, D.J. Chaisson, and P.C. Martin. 1989. The ontogeny of ultraviolet photosensitivity in rainbow trout (*Salmo gairdner*). *Visual Neuroscience* 2: 247-254.
- Hawryshyn, C.W., M.G. Arnold, E. Bowering, and R.L. Cole. 1990. Spatial orientation of rainbow trout to plane-polarised light: The ontogeny of E-vector discrimination and spectral sensitivity characteristics. *Journal of Comparative Physiology A* 166: 565-574.
- Heggenes, J., T.G. Northcote, and A. Peter. 1991. Spatial stability of cutthroat trout (*Oncorhynchus clarki*) in a small, coastal stream. *Canadian Journal of Fisheries Research Board of Canada* 48: 757-762.
- Heiligenberg, W. 1974. Electrolocation and jamming avoidance in a *Hypopygus* (Rhamphthyichthyidae, Gymnotoidei), and electric fish with pulse-type discharges. *Journal of Comparative Physiology* 91: 233-240.
- Heiligenberg, W. 1977. Principles of electrolocation and jamming avoidance in electric fish: A neuroethological approach. *Studies of Brain Function, Volume I*. Springer-Verlag, Berlin. 85 pp.
- Hinton, D.E. 1990. Histological techniques. Pages 191-221 in *Methods for fish biology*. Edited by C.B. Schreck and P.B. Moyle. American Fisheries Society, Bethesda, Md. 684 pp.

- Hisatomi, O., T. Satoh, L.K. Barthelemy, D.L. Stenkamp, P.A. Raymond, and F. Tokunaga. 1996. Molecular cloning and characterization of the putative ultraviolet-sensitive visual pigment of goldfish. *Vision Research* 36: 933-939.
- Hoar, W.S. 1953. Control and timing of fish migration. *Biological Reviews* 28: 437-452.
- Hoar, W.S. 1954. The behavior of juvenile Pacific salmon with particular reference to the sockeye (*Oncorhynchus nerka*). *Journal of the Fisheries Research Board of Canada* 11: 69-97.
- Hoar, W.S. 1989. The physiology of smolting salmonids. Pages 275-332 in *Fish physiology. XI. The physiology of developing fish. Part B. Viviparity and posthatching juveniles. Edited by W.S. Hoar and D.J. Randall.* Academic Press, Toronto. 436 pp.
- Horváth, G., and D. Varjú. 1995. Underwater refraction-polarization patterns of skylight perceived by aquatic animals through Snell's window of the flat water surface. *Vision Research* 35: 1651-1666.
- Hubel, D.H., and T.N. Weisel. 1960. Receptive fields of optic nerve fibers in the spider monkey. *Journal of Physiology* 154: 572-580.
- Huth, H.H., and D. Burkhardt. 1972. Der spektrale Sehbereich eines Violettohr Kolibris. *Naturwissenschaften* 59: 650.
- Huynh, H., and L.S. Feldt. 1976. Estimation of the box correction for degrees of freedom from sample data in randomized block and split-plot designs. *Journal of Educational Statistics* 1: 69-82.
- Huynh, H., and G.K. Mandeville. 1979. Validity conditions in repeated measures designs. *Psychological Bulletin* 86: 964-973.
- Iman, R.L., and J.M. Davenport. 1980. Approximations of the critical region of the Friedman statistic. *Communications in Statistics*, A9: 571-595.
- Jacobs, G.H. 1992. Ultraviolet vision in vertebrates. *American Zoologist* 32: 544-554.
- Jacobs, G.H., and J. Neitz. 1987. Inheritance of color vision in a New World monkey (*Saimiri sciureus*). *Proceedings of the National Academy of Sciences USA* 84: 2545-2549.

- Jacobs, G.H., and J.F. Deegan, III. 1993. Photopigments underlying color vision in ringtail lemurs (*Lemur catta*) and brown lemurs (*Eulemur fulvus*). *American Journal of Primatology* 30: 243-256.
- Jacobs, G.H., J. Neitz, and J.F. Deegan, III. 1991. Retinal receptors in rodents maximally sensitive to ultraviolet light. *Nature* 353: 655-656.
- Jacobs-Jessen, U.F. 1959. Zur Orientierung der Hummeln und einiger anderer Hymenopteren. *Zeitschrift für vergleichende Physiologie* 41: 597-641.
- Jagger, W.S. 1992. The optics of the spherical fish lens. *Vision Research* 32: 1271-1284.
- Jagger, W.S. 1996. Image formation by the crystalline lens and eye of the rainbow trout. *Vision Research* 36: 2641-2655.
- Jagger, W.S. 1997. Chromatic and monochromatic optical resolution in the rainbow trout. *Vision Research* 37: 1249-1254.
- Jander, R., and T.H. Waterman. 1960. Sensory discrimination between polarised light and light intensity patterns by arthropods. *Journal of Cellular and Comparative Physiology* 56: 136-160.
- Jander, R., K. Daumer, and T.H. Waterman. 1963. Polarized light orientation by two Hawaiian decapod cephalopods. *Zeitschrift für vergleichende Physiologie* 46: 383-394.
- Jane, S.D., and J.K. Bowmaker. 1988. Tetrachromatic colour vision in the duck (*Anas platyrhynchos* L.): Microspectrophotometry of visual pigments and oil droplets. *Journal of Comparative Physiology* 162: 225-235.
- Jerlov, N.G. 1976. *Marine optics*. Elsevier, Amsterdam. 231 pp.
- Johnson, J.H., and N.H. Ringler. 1980. Diets of juvenile coho salmon (*Oncorhynchus kisutch*) and steelhead trout (*Salmo gairdneri*) relative to prey availability. *Canadian Journal of Zoology* 58: 553-558.
- Johnson, P.B., and A.D. Hasler. 1980. The use of chemical cues in the upstream migration of coho salmon, *Oncorhynchus kisutch* Walbaum. *Journal of Fish Biology* 17: 67-73.
- Johnson, W.E., and C. Groot. 1963. Observations on the migration of young sockeye salmon (*Oncorhynchus nerka*) through a large, complex lake system. *Journal of the Fisheries Research Board of Canada* 20: 919-938.

- Jolly, D.W., L.E. Mawdesley-Thomas, and D. Bucke. 1972. Anaesthesia of fish. *Veterinary Record* 91: 424-426.
- Jones, F.R.H. 1968. Fish migration. St. Martins, New York. 325 pp.
- Kawamura, G., A. Shigata, and T. Yonemori. 1981. Response of teleosts to the plane of polarized light as determined by heart rate. *Bulletin of the Japanese Society of Scientific Fisheries* 47: 727-729.
- Kleerekoper, H. 1969. Olfaction in fishes. Indiana University Press, Bloomington. 222 pp.
- Kleerekoper, H., and E.C. Chagnon. 1954. Hearing in fish with special reference to *Semotilus atromaculatus* (Mitchell). *Journal of Fisheries Research Canada* 11:130-152.
- Kleerekoper, H., J.H. Matis, A.M. Timms, and P. Gensler. 1973. Locomotor response of goldfish to polarised light and its e-vector. *Journal of Comparative Physiology* 141: 19-30.
- Klimley, A.P. 1993. Highly directional swimming by scalloped hammerhead sharks, *Sphyrna lewini*, and subsurface irradiance, temperature, bathymetry, and geomagnetic field. *Marine Biology* 117: 1-22.
- Knowles, A. and H.J.A. Dartnall. 1975. The photobiology of vision. In H. Davson (editor) *The eye, Volume 2*. London/New York: Academic Press.
- Koelz, W. 1929. Coregonid fishes of the Great Lakes. *Bulletin of the United States Bureau of Fisheries* 43: 297- 643.
- Kohler, K., E. Zrenner, and R. Weiler. 1992. Ethambutol induced alterations of spinule expression in the fish retina. *Investigative Ophthalmology and Vision Science Supplement* 33: 939.
- Kong, K.-L., Y.M. Fung, and G.S. Wasserman. 1980. Filter-mediated color vision with one visual pigment. *Science* 207: 783-786.
- Können, G.P. 1985. Polarized light in nature. Translated by G.A. Beerling. Cambridge University Press, Cambridge. 172 pp.
- Kramer, G. 1950. Orientierte Zugaktivität gekäfigter Singvogel. *Naturwissenschaften* 37: 188.

- Kreithen, M.L., and W.T. Keeton. 1974. Detection of polarized light by the homing pigeon, *Columba livia*. *Journal of Comparative Physiology* 89: 83-92.
- Kreithen, M.L., and T.H. Eisner. 1978. Ultraviolet light detection by the homing pigeon. *Nature (London)* 272: 247-348.
- Kunz, Y.W. 1987. Tracts of putative ultraviolet receptors in the retina of the two-year old brown trout (*Salmo trutta*) and the Atlantic salmon (*Salmo salar*). *Experientia* 43: 1202-1204.
- Kunz, Y.W., and E. Callaghan. 1989. Embryonic fissures in teleost eyes and their possible role in detection of polarized light. *Transactions of the American Fisheries Society* 118: 195-202.
- Kunz, Y.W., G. Wildenburg, L. Goodrich, and E. Callaghan. 1994. The fate of ultraviolet receptors in the retina of the Atlantic salmon (*Salmo salar*). *Vision Research* 34: 1375-1383.
- Kusmic, C., L. Barsanti, V. Passarelli, and P. Gualtieri. 1993. Photoreceptor morphology and visual pigment content in the pineal organ and in the retina of juvenile and adult trout, *Salmo irideus*. *Micron* 24: 279-286.
- Lamb, T.D. 1995. Photoreceptor spectral sensitivities: Common shape in the the long-wavelength region. *Vision Research* 35: 3083-3091.
- LaVail, M.M. 1976. Rod outer segment disc shedding in relation to cyclic lighting. *Experimental Eye Research* 23: 277-280.
- Leggett, W.C. 1977. The ecology of fish migrations. *Annual Review of Ecology and Systematics* 8: 285-308.
- Lehner, P.N. 1979. *Handbook of ethological methods*. Garland STPM Press, New York. 403 pp.
- Lehninger, A.H. 1982. *Principles of biochemistry*. Worth Publishers, New York. 1011 pp.
- Levin, L.E., and P. Belmonte. 1988. Oriented swimming at an angle to light in a schooling characid, *Moenkhausia dichroua* Kner. *Journal of Fish Biology* 32: 169-177.
- Levine, J.S., and E.F. MacNichol. 1979. Visual pigments in teleost fishes: Effects of habitat, microhabitat, and behavior on visual system evolution. *Sensory Processes* 3: 95-131.

- Loew E.R. and Dartnall. 1976. Vitamin A1/A2-based visual pigment mixtures in cones of the rudd. *Vision Research* 16:891-896.
- Loew, E.R., and J.N. Lythgoe. 1978. The ecology of cone pigments in teleost fishes. *Vision Research* 18: 715-722.
- Loew, E.R., and W.N. McFarland. 1990. The underwater visual environment. *Pages 1-43 in The visual system of fish. Edited by R.H. Douglas and M.B.A. Djamgoz. Chapman and Hall, London. 526 pp.*
- Loew, E.R., and W.N. McFarland. 1994. Ultraviolet visual pigments in marine fishes of the Family Pomacentridae. *Vision Research* 8: 997-1012.
- Loew, E.R., W.N. McFarland, E.L. Mills, and D. Hunter. 1993. A chromatic action spectrum for plankton predation by juvenile yellow perch (*Perca flavescens*). *Vision Research* 31: 353-360.
- Lorenz, K.Z. 1981. The foundations of ethology. Springer-Verlag, New York, NY. 380 pp.
- Lubbock, J. 1883. Ants, bees and wasps: A record of observations on the habits of social hymenoptera (New edition, based on the 17th, 1929 edition). D. Appleton and Company, New York. 448 pp.
- Lyall, A.H. 1957. The growth of the trout retina. *Quarterly Journal of Microscopical Science* 98: 101-110.
- Lythgoe, J.N. 1966. Visual pigments and underwater vision. *Pages 375-391 in Light as an ecological factor. A symposium of the British Ecological Society; 30th March-1st April, 1965. Edited by R. Bainbridge, G.C. Evans, and O. Rackham. Blackwell Scientific, Oxford. 425 pp.*
- Lythgoe, J.N. 1968. Visual pigments and visual range underwater. *Vision Research* 8: 997-1011.
- Lythgoe, J.N. 1971. Iridescent comeas in fishes. *Nature, London* 23: 205-207.
- Lythgoe, J.N. 1975a. The iridescent comeas of the sand goby. *Pages 263-278 in Vision in fishes. Edited by M.A. Ali. North American Treaty Organisation Advanced Study Institutes Series, Volume 1. Plenum Press, New York. 836 pp.*

- Lythgoe, J.N. 1975b. Problems of seeing colours under water. Pages 619-634 in *Vision in Fishes*. Edited by M.A. Ali. Plenum Press, New York. 836 pp.
- Lythgoe, J.N. 1975c. The structure and phylogeny of iridescent corneas in fishes. Pages 253-262 in *Vision in fishes*. Edited by M.A. Ali. North American Treaty Organisation (NATO) Advanced Study Institutes Series, Volume 1. Plenum Press, New York. 836 pp.
- Lythgoe, J.N. 1979. *The ecology of vision*. Clarendon Press, Oxford. 244 pp.
- Lythgoe, J.N., and J.C. Partridge. 1989. Visual pigments and the acquisition of visual information. *Journal of Experimental Biology* 146: 1-20.
- Lythgoe, J.N., and D.P.M. Northmore. 1973. Colours underwater. Pages 77-98 in *Colour '73*. Adam Hilger, London.
- Lythgoe, J.N., W.R.A. Muntz, J.C. Partridge, J. Shand, and D.McB. Williams. 1994. The ecology of the visual pigments of snappers (Lutjanidae) on the Great Barrier reef. *Journal of Comparative Physiology A* 174: 461-467.
- Maier, E.J., and J.K. Bowmaker. 1993. Colour vision in the passeriform bird, *Leiothrix lutea*: Correlation of visual pigment absorbance and oil droplet transmission with spectral sensitivity. *Journal of Comparative Physiology A* 172: 295-301.
- Marc, R.E., and D.M.K. Lam. 1981. Glycinergic pathways in the goldfish retina. *Journal of Neuroscience* 1: 152-165.
- Mardia, K.V. 1972. *Statistics of directional data*. Academic Press, London. 220 pp.
- Mardia, K.V. 1976. Linear-circular correlation coefficients and rhythmometry. *Biometrika* 63: 403-405.
- Marriott, F.H.C. 1962. Colour vision: The two-colour threshold technique of Stiles. Pages 251-273 in *The eye, Volume II*. Edited by H. Davson. Academic Press, New York.
- Marquart, D.W. 1963. An algorithm for least squares estimation of nonlinear parameters. *Journal of the Society for Industrial and Applied Mathematics* 2: 431-441.
- Marshall, N.J. 1988. A unique colour and polarisation vision system in mantis shrimp. *Nature* 333: 557-560.

- Marshall, N.J., M.F. Land, C.A. King, and T.W. Cronin. 1991. The compound eyes of mantis shrimps (Crustacea, Hoplocarida, Stomatopoda). I. Compound eye structure: The detection of polarized light. *Philosophical Transactions of the Royal Society of London B* 334: 33-56.
- Martin, G.R. 1991. The question of polarization. *Nature* 350: 194.
- Mazur, J.E. 1986. *Learning and behavior*. Prentice Hall, Englewood Cliffs, New Jersey. 387 pp.
- McCandless, R.L., J.R. Hoffert, and P.O. Fromm. 1969. Light transmission by corneas, aqueous humor and crystalline lenses of fishes. *Vision Research* 9: 223-232.
- McDonald, C.G., and C.W. Hawryshyn. 1995. Intraspecific variation of spectral sensitivity in threespine stickleback (*Gasterosteus aculeatus*) from different photic regimes. *Journal of Comparative Physiology A* 176: 255-260.
- McFarland, W.N. 1986. Light in the sea-correlations with behaviors of fishes and invertebrates. *American Zoologist* 26: 389-401.
- McFarland, W.N. 1991. Light in the sea: The optical world of elasmobranchs. *Journal of Experimental Zoology Supplement* 5: 3-12.
- McFarland, W., and F.W. Munz. 1975. Part III: The evolution of photopic visual pigments in fishes. *Vision Research* 15: 1071-1080.
- McFarland, W.N., F.H. Pough, T.H. Cade, and J.B. Heiser. 1979. *Vertebrate life*. McMillan Publishing Co., New York. 875 pp.
- McInerney, J.E. 1964. Salinity preference: An orientation mechanism in salmon migration. *Journal of the Fisheries Research Board of Canada* 21: 995-1018.
- Merker, E. 1934. Die Sichtbarkeit ultravioletten Lichtes. *Biological Reviews (Cambridge Philosophical Society)* 9: 49-78.
- Metzner, W., and S. Viete. 1996. The neuronal basis of communication and orientation in the weakly electric fish, *Eigenmannia*. *Naturwissenschaften* 83: 71-77.
- Mie, Gustav. 1908. Beiträge zur Optik trüber Medien, speziell Kolloidaler Metallösungen. *Annalen der Physik*, 25: 377-445.

- Montgomery, K.C., and E.G. Heinemann. 1952. Concerning the ability of homing pigeons to discriminate patterns of polarized light. *Science* 116: 454-456.
- Moore, F., and J.B. Phillips. 1988. Sunset, skylight polarization and the migratory orientation of yellow-rumped warblers, *Dendroica coronata*. *Animal Behaviour* 36: 1770-1778.
- Muntz, W.R.A., and D.P.M. Northmore. 1973. Scotopic sensitivity in a teleost fish (*Scardinius erythrophthalmus*) adapted to different daylengths. *Vision Research* 13: 245-252.
- Munz, F.W. 1958. Photosensitive pigments from the retinæ of certain deep sea fishes. *Journal of Physiology London* 140: 220-235.
- Munz, F.W., and W.N. McFarland. 1977. Evolutionary adaptations of fishes to the photic environment. *Pages 193-274 in Handbook of sensory Physiology, VII/5. Edited by F. Crescitelli. Springer-Verlag, Berlin. 813 pp.*
- Naka, K.I., and W.A.H. Rushton. 1966a. An attempt to analyse colour reception by electrophysiology. *Journal of Physiology* 185: 556-586.
- Naka, K.I., and W.A.H. Rushton. 1966b. S-potentials from colour units in the retina of fish (Cyprinidae). *Journal of Physiology* 185: 536-555.
- Naka, K.I., and W.A.H. Rushton. 1966c. S-potentials from luminosity units in the retina of fish (Cyprinidae). *Journal of Physiology* 185: 587-599.
- Neave, F. 1964. Ocean migrations of Pacific salmon. *Journal of the Fisheries Research Board of Canada* 21: 1227-1235.
- Needham, P. 1940. Trout streams. Comstock Publishing, Ithaca, N.Y.
- Nelson, J.S. 1994. *Fishes of the world*, 3rd edition. J. Wiley, New York. 600 pp.
- Neumeyer, C. 1985. An ultraviolet receptor as a fourth receptor type in goldfish color vision. *Naturwissenschaften* 72: 162-163.
- Nevitt, G.A., A.H. Dittman, T.P. Quinn, and W.J. Moody. 1994. Evidence for a peripheral olfactory memory in imprinted salmon. *Proceedings of the National Academy of Sciences* 91: 4288-4292.

- Norušis, M.J, and SPSS Inc. 1993. SPSS® for Windows™. Advanced Statistics User's Guide, Release 6. SPSS Inc., Chicago, Illinois. 578 pp.
- Novales Flamarique, I. 1997. Polarisation sensitivity in vertebrates. Ph.D. Dissertation, Univeristy of Victoria, Victoria, B.C.
- Novales Flamarique, I., and C.W. Hawryshyn. 1993. Spectral characteristics of salmonid migratory routes from southern Vanvouver Island (British Columbia). *Canadian Journal of Fisheries and Aquatic Sciences* 50: 1706-1716.
- Novales Flamarique, I., and C.W. Hawryshyn. 1996. Retinal development and visual sensitivity of young Pacific sockeye salmon (*Oncorhynchus nerka*). *Journal of Experimental Biology* 199: 869-882.
- Novales Flamarique, I., and C.W. Hawryshyn. 1997a. Is the use of underwater polarized light by fish restricted to crepuscular time periods? *Vision Research* 37: 975-989.
- Novales Flamarique, I., and C.W. Hawryshyn. 1997b. No evidence of polarization sensitivy in freshwater sunfish from multi-unit optic nerve recordings. *Vision Research* 37: 967-973.
- Novales Flamarique, I., A.P. Hendry, and C.W. Hawryshyn. 1992. The photic environment of a salmonid nursery lake. *Journal of Experimental Biology* 169: 121-141.
- Novales Flamarique, Iñigo, Craig W. Hawryshyn, and Ferenc I. Hárosi. 1998. Double-cone internal reflection as a basis for polarisation detection in fish. *Journal of the Optical Society of America*. 15: 1-10.
- O'Benar, J.D. 1976. Electrophysiology of neural units in goldfish optic tectum. *Brain Research Bulletin* 1: 529-541.
- van Oosten, J. 1929. The age, growth, sexual maturity, and sex ratio of the Lake Superior longjaw (*Leucichthys zenithicus*) (Jordan and Evermann). *Papers of the Michigan Academy of Sciences and Arts Letters* 22 (1936): 691-711.
- van Oosten, J. 1939. The age, growth, sexual maturity, and sex ratio of the common whitefish, *Coregonus clupeaformis* (Mitchill), of Lake Huron. *Papers of the Michigan Academy of Science Arts Letters* 24: 195-221.

- Parkyn, D.C., and C.W. Hawryshyn. 1993. Polarized-light sensitivity in rainbow trout (*Oncorhynchus mykiss*): Characterization from multi-unit responses in the optic nerve. *Journal of Comparative Physiology A* 172: 493-500.
- Partridge, J.C., S.N. Archer, and J.N. Lythgoe. 1988. Visual pigments in individual rods of deep-sea fishes. *Journal of Comparative Physiology A* 162: 543-550.
- Partridge, J.C., S.N. Archer, and J. van Oostrum. 1992. Single and multiple visual pigments in deep-sea fishes. *Journal of Marine Biological Association U.K.* 72: 113-130.
- Pascual, M.A., and T.P. Quinn. 1991. Evaluation of alternative models of the coastal migration of adult Fraser River sockeye salmon (*Oncorhynchus nerka*). *Canadian Journal of Fisheries and Aquatic Sciences* 48: 799-810.
- Patten, B.C. 1964. The rational decision process in salmon migration. *Journal du Conseil International pour l'Exploration de la Mer* 28: 410-417.
- Pau, H., and M. Wahl. 1972. Myambutol-Schädigung des Auges. *Berichte der Deutschen Ophthalmologischen Gesellschaft* 72: 176-181.
- Peitsch, D., A. Fietz, H. Hertel, J. de Souza, D. Ventura, and R. Menzel. 1992. The spectral input systems of hymenoptera insects and their receptor-based colour vision. *Journal of Comparative Physiology A* 170: 23-40.
- Phillips, J.P., and F.R. Moore. 1992. Calibration of the sun compass by sunset polarized light patterns in a migratory bird. *Behavioural Ecology and Sociobiology* 31: 189-193.
- Phillips, J.P., and J.A. Waldvogel. 1988. Celestial polarized light patterns as a calibration reference for sun compass of homing pigeons. *Journal of Theoretical Biology* 131: 55-67.
- Pitcher, T. 1986. Functions of schooling behaviour in teleosts. *Pages 294-338 in The behaviour of teleost fishes. Edited by T. Pitcher. John Hopkins University Press, Baltimore. 553 pp.*
- Quinn, T.P. 1980. Evidence for celestial and magnetic compass orientation in lake migrating sockeye salmon fry. *Journal of Comparative Physiology* 137: 243-248.

- Quinn, T.P. 1984. An experimental approach to fish compass and map orientation. Pages 113-123 in *Mechanisms of migration of fishes*. Edited by J.D. McCleave, G.P. Arnold, J.J. Dodson, and W.H. Neill. Plenum Press, New York.
- Quinn, T.P. 1988. Estimated swimming speeds of migrating adult sockeye salmon (*Oncorhynchus nerka*). *Canadian Journal of Zoology* 66: 2160-2163.
- Quinn, T.P. 1990. Current controversies in the study of salmon homing. *Ethology, Ecology and Evolution* 2: 49-63.
- Quinn, T.P., and E.L. Brannon. 1982. The use of celestial and magnetic cues by orienting sockeye salmon smolts. *Journal of Comparative Physiology* 147: 547-552.
- Quinn, T.P., and C. Groot. 1984a. The effect of water flow rate on bimodal orientation of juvenile chum salmon, *Oncorhynchus keta*. *Animal Behaviour* 32: 628-629.
- Quinn, T.P., and C. Groot. 1984b. Pacific salmon (*Oncorhynchus*) migrations: Orientation versus random movement. *Canadian Journal Fisheries and Aquatic Sciences* 41: 1319-1324.
- Quinn, T.P., and C. Groot. 1983. Orientation of chum salmon (*Oncorhynchus keta*) after internal and external magnetic field alternation. *Canadian Journal of Fisheries and Aquatic Sciences* 40: 1598-1606.
- Robins, C.R., R.M. Bailey, C.E. Bond, J.R. Brooker, E.A. Lachner, R.N. Lea, and W. B. Scott. 1991. *Common and scientific names of fishes from the United States and Canada*, 5th ed. American Fisheries Society, Bethesda, Md. 183 pp.
- Robinson, S.R. 1994. Early vertebrate colour vision. *Nature* 367: 121.
- Rossel, S. 1989. Polarization sensitivity in compound eyes. Pages 298-316 in *Facets of vision*. Edited by D.G. Stavenga and R.C. Hardie. Springer, New York, New York.
- Rowe, M.P., N. Engheta, S.S. Easter, Jr., and E.N. Pugh. 1994. Graded-index model of a fish double cone exhibits differential polarization sensitivity. *Journal of the Optical Society of America* 11: 55-70.

- Rowell, C.H.F., and M.J. Wells. 1961. Retinal orientation and the discrimination of polarized light by octopuses. *Journal of Experimental Biology* 38: 827-831.
- Ruggerone, G.T., T.P. Quinn, I.A. McGregor, and T.D. Wilkinson. 1990. Horizontal and vertical movements of adult steelhead trout, *Oncorhynchus mykiss*, in the Dean and Fisher Channel, British Columbia. *Canadian Journal of Fisheries and Aquatic Sciences* 47: 1963-1969.
- Russell, D.F. 1990. Nervous system. *Pages 417-450 in Methods for fish biology. Edited by C.B. Schreck and P.B. Moyle. American Fisheries Society, Bethesda, Md. 684 pp.*
- Saidel, W.M., J.Y. Lettvin, and E.F. McNicol. 1983. Processing of polarized light by squid photoreceptors. *Nature* 304: 534-536.
- Saila, S.B., and R.R. Shappy. 1963. Random movement and orientation in salmon migration. *Journal du Conseil international pour l'Exploration de la Mer* 28: 153-166.
- Santschi, F. 1923. L'orientation sidérale des fourmis, et quelques considérations sur leurs différentes possibilités d'orientation. *Mémoires de la Société Vaud Science Naturel* 4: 137-175.
- Schiemenz, F. 1924. Über den Farbensinn der Fische. *Zeitschrift für vergleichende Physiologie* 1: 175-200.
- Schmidt, W.J. 1938. Polarisations-optische Analyse eines EiweissLipoid-systems, erläutert am Aussenglied der Sehzellen. *Kolloid-Zeitschrift* 85: 137-148.
- Scott, W.B., and E.J. Crossman. 1973. *Freshwater fishes of Canada. Fisheries Research Board of Canada Bulletin* 184: 966 pp.
- Shand, J. 1993. Changes in the spectral absorption of cone visual pigments during the settlement of the goatfish *Upeneus tragula*: The loss of red sensitivity as a benthic existence begins. *Journal of Comparative Physiology A* 173: 115-121.
- Shand, J., J.C. Partridge, and J.N. Lythgoe. 1990. Catacholamine-induced colour changes in the corneal iridophores of the sand goby, *Pomatoschistus minutus*. *Comparative Biochemistry and Physiology* 94: 351-355.

- Shashar, N., and T.W. Cronin. 1996. Polarization contrast vision in *Octopus*. *The Journal of Experimental Biology* 199: 999-1004.
- Sillman, A.J., V.I. Goverdovski, P.I. Rölich, J.A. Southard, and E.R. Loew. 1997. The photoreceptors and visual pigments of the garter snake (*Thamnophis sirtalis*): a microspectrophotometric, scanning electron microscopic and immunocytochemical study. *Journal of Comparative Physiology A*. 181: 89-101.
- Sivak, J.G., and T. Mandelman. 1982. Chromatic dispersion of the ocular media. *Vision Research* 22: 997-1003.
- Smit, S.A., and G.C. Anker. 1997. Photopic sensitivity to red and blue light related to retinal differences in two zooplanktivorous Haplochromine species (Teleosti, Cichlidae). *Netherlands Journal of Zoology* 47: 9-20.
- Smith, K.C., and E.R. Macagno. 1990. UV photoreceptors in the compound eye of *Daphnia magna* (Crustacea, Branchiopoda). A fourth spectral class in single ommatidia. *Journal of Comparative Physiology* 166: 597-606.
- Snyder, A.W. 1973. How fish detect polarized light. *Investigative Ophthalmology* 12: 78-79.
- Spekreijse, H., H.G. Wagner, and M.L. Wolbarsht. 1972. Spectral and spatial coding of ganglion cell responses in goldfish retina. *Journal of Neurophysiology* 35: 73-86.
- Spekreijse, H., J.J. Wietsma, and C. Neumeyer. 1991. Induced color blindness in goldfish: A behavioral and electrophysiological study. *Vision Research* 31: 551-562.
- Sperling, H.G., and R.S. Harwerth. 1971. Red-green cone interactions in the increment-threshold spectral sensitivity of primates. *Science* 172: 180-184.
- Stearley, R.F., and G.R. Smith. 1993. Phylogeny of the Pacific trouts and salmon (*Oncorhynchus*) and genera of the family Salmonidae. *Transactions of the American Fisheries Society* 122: 1-33.
- Stewart, K.W. Observation on the morphology and optical properties of the adipose eyelid of fishes. 19: 1161-1162.

- Stys, P.K., B.R. Ransom, and S.G. Waxman. 1991. Compound action potential of nerve recorded by suction electrode: A theoretical and experimental analysis. *Brain Research* 546: 18-32.
- Szél, A., and P. Rölich. 1992. Two cone types in rat retina detected by anti-visual pigment antibodies. *Experimental Eye Research* 55: 47-52.
- Tamura, T., and W.J. Wisby. 1963. The visual sense of pelagic fishes, especially the visual axis and accommodation. *Bulletin of Marine Science of the Gulf and Caribbean* 13: 433-448.
- Tasaki, K., and K. Karita. 1966a. Discrimination of horizontal and vertical planes of polarized light by the cephalopod retina. *Japan Journal of Physiology* 16: 205-216.
- Tasaki, K., and K. Karita. 1966b. Intraretinal discrimination of horizontal and vertical planes of polarized light by Octopus. *Nature* 209: 934-935.
- Taylor, D.H., and K. Adler. 1973. Spatial orientation by salamanders using plane-polarized light. *Science (Washington, D.C.)* 181: 285-287.
- Taylor, E.B., and C.J. Foote. 1991. Critical swimming velocities of juvenile sockeye salmon and kokanee, the anadromous and nonanadromous forms of *Oncorhynchus nerka* (Walbaum). *Journal of Fish Biology* 38: 407-419.
- Taylor, P.B. 1986. Experimental evidence for geomagnetic orientation in juvenile salmon, *Oncorhynchus tshawytscha*, Walbaum. *Journal of Fish Biology* 28: 607-623.
- Taylor, P.B. 1987. Experimental evidence for juvenile chinook salmon, *Oncorhynchus tshawytscha* Walbaum, orientation at night and in sunlight after a 7° change in latitude. *Journal of Fish Biology* 31: 89-111.
- Thorpe, J., and R. Douglas. 1993. Spectral transmission and shortwave absorbing pigments in the fish lens. II. Effects of age. *Vision Research* 33: 301-307.
- Tierney, J.A. 1975. *Calculus and analytical geometry*, 3rd ed. Allyn and Bacon, Toronto, Ontario. 720 pp.
- Tovée, Martin J. 1995. Ultraviolet vision in the animal kingdom. *Trends in Ecology and Evolution* 10: 455-460.
- Trusciewicz, D. 1975. Farnsworth 100-Hue test in diagnosis of ethambutol-induced damage to optic nerve. *Ophthalmologica* 171: 425-431.

- Tsin, A.T.C., and D.D. Beatty. 1977. Visual pigment changes in rainbow trout in response to temperature. *Science* 195: 1358-1359.
- Tyler, J.E., and R.C. Smith. 1970. Measurement of spectral irradiance underwater. Gordon and Breach, New York.
- Tyto, J.S., M.P. Rowe, E.N. Pugh, and N. Engheta. 1996. Target detection in optically scattering media by polarization-difference imaging. *Applied Optics* 35: 1855-1870.
- Underwood, G. 1968. Some suggestions concerning vertebrate visual cells. *Vision Research* 8: 483-488.
- Underwood, G. 1970. The eye. *Pages 1-97 in Biology of the Reptilia. Morphology B. Edited by C. Gans and T.S. Parsons. Volume 2. Academic Press, New York.*
- Van Dijk, B.W., and H. Spekreijse. 1983. Ethambutol changes the color coding of carp retinal ganglion cell reversibly. *Investigative Ophthalmology and Visual Science* 24: 128-133.
- Vigh-Teichmann, I., M.A. Ali, A. Szél, and B. Vigh. 1991. Ultrastructure and opsin immunocytochemistry of the pineal complex of the larval Arctic charr *Salvelinus alpinus*: A comparison with the retina. *Journal of Pineal Research* 10: 196-209.
- Vos Hzn, J.J., M.A.J.M. Coemans, and J.F.W. Nuboer. 1995. No evidence for polarization sensitivity in the pigeon electroretinogram. *The Journal of Experimental Biology* 198: 325-335.
- Wald, G. 1936-37. Pigments of the retina. II. Sea robin, sea bass, and scup. *Journal of General Physiology* 22: 45-56.
- Wald, G. 1938-39. On the distribution of vitamins A₁ and A₂. *Journal of General Physiology* 22: 391-415.
- Wald, G. 1941. The visual systems of euryhaline fishes. *Journal of General Physiology* 25: 235-245.
- Wald, G. 1957. The metamorphosis of visual systems in the sea lamprey. *Journal of General Physiology* 40: 901-914.
- Wald, G., P.K. Brown, and P.S. Brown. 1957. Visual pigments and depth of habitat of marine fishes. *Nature, London*, 180: 969-971.

- Walls, G.L. 1942. The vertebrate eye and its adaptive radiation. Hafner, New York, NY. 785 pp.
- Waterman, T.H. 1954. Polarization patterns in submarine illumination. *Science* 120: 927-932.
- Waterman, T.H. 1955. Polarization of scattered sunlight in deep water. *Deep Sea Research (Supplement)* 3: 426-434.
- Waterman, T.H. 1959. The problem of polarized light sensitivity. *Proceedings of the International Congress of Zoology* 15: 537-539.
- Waterman, T.H. 1966. Systems analysis and the visual orientation of animals. *American Scientist* 54: 15-45.
- Waterman, T.H. 1972. Visual direction finding by fishes. *Pages 437-456 in Animal orientation and navigation: A symposium. Edited by S.R. Galler, K. Schmidt-Koenig, G.J. Jacobs, and R.E. Belleville. National Aeronautics and Space Administration, Washington, D.C.*
- Waterman, T.H. 1981. Polarization sensitivity. *Pages 281-469 in The handbook of sensory physiology, Volume VII/6B. Edited by H. Autrum. Springer-Verlag, New York, NY. 729 pp.*
- Waterman, T.H. 1989. Animal migration. W.H. Freeman and Company, New York, New York. 243 pp.
- Waterman, T.H., and W.E. Westell. 1956. Quantitative effect of the sun's position on submarine light polarization. *Journal of Marine Research* 15: 149-169.
- Waterman, T.H., and K. Horch. 1966. Mechanisms of polarized light perception. *Science* 154: 467-475.
- Waterman, T.H., and R.B. Forward, Jr. 1972. Field demonstration of polarotaxis in the fish *Zenarchopterus*. *Journal of Experimental Biology* 180: 33-54.
- Waterman, T.H., and K. Aoki. 1974. E-vector sensitivity patterns in the goldfish optic tectum. *Journal of Comparative Physiology* 95: 13-27.
- Waterman, T.H., and H. Hashimoto. 1974. E-vector discrimination by the goldfish optic tectum. *Journal of Comparative Physiology A* 95: 1-12.
- Webb, N.G. 1977. Orientation of retinal rod photoreceptor membranes in the intact eye using X-ray diffraction. *Vision Research* 17: 625-631.

- Wehner, R. 1976. Polarized-light navigation by insects. *Scientific American* 238: 106-115.
- Wehner, R. 1983. The perception of polarized light. *Pages 331-369 in The biology of photoreception. Edited by D.J. Cosens and D. Vince-Price. Society for Experimental Biology. Cambridge University Press, New York. 610 pp.*
- Wehner, R. 1987. 'Matched filters' - neural models of the external world. *Journal of Comparative Physiology A* 161: 511-531.
- Wehner, R. 1989. Neurobiology of polarization vision. *Trends in Neuroscience* 12: 353-359.
- Wehner, R. 1994. The polarization-vision project: Championing organismic biology. *Pages 103-143 in Neural basis of behavioural adaptation. Edited by K. Schildberg and N. Elsner. G. Fischer, New York.*
- Wehner, R. 1997. Sensory systems and behaviour. *Pages 19-41 in Behavioural ecology: An evolutionary approach. 4th Edition. Edited by J.R. Krebs and N.B. Davies. Blackwell Science, Malden. 456 pp.*
- Wehner, R., and G.D. Bernard. 1993. Photoreceptor twist: A solution to the false-color problem. *Proceedings of the National Academy of Sciences U.S.A.* 90: 4132-4135.
- Wheeler, T.G. 1979a. Retinal ON and OFF responses convey different chromatic information to the CNS. *Brain Research* 160: 145-149.
- Wheeler, T.G. 1979b. Retinal red sensitivity under dark-adapted conditions. *Brain Research* 175: 140-145.
- Wheeler, T.G. 1982. Color vision and retinal chromatic information processing in teleost: A review. *Brain Research Reviews* 4: 177-235.
- Whitmore, A.V., and J.K. Bowmaker. 1989. Seasonal variation in cone sensitivity and short-wave absorbing visual pigments in the rudd *Scardinius erythrophthalmus*. *Journal of Comparative Physiology A* 166: 103-115.
- Wietsma, J.J., M. Kamermans, and H. Spekreijse. 1995. Horizontal cells function normally in ethambutol-treated goldfish. *Vision Research* 35: 1667-1674.

- Willers, W.B. 1991. Trout biology, 2nd edition. Lyons and Burford, New York. 273 pp.
- Winn, H.E., M. Salmon, and N. Roberts. 1964. Sun-compass orientation by parrot fishes. *Zeitschrift für Tierpsychologie* 21: 798-812.
- Wisby, W.J., and A.D. Hasler. 1954. Effect of olfactory occlusion on migrating silver salmon (*O. kisutch*). *Journal of the Fisheries Research Board of Canada* 11: 472-478.
- Wootton, R.J. 1990. Ecology of teleost fishes. Chapman Hall, London. 404 pp.
- Yager, D. 1969. Behavioral measures of the spectral sensitivity in the goldfish following chromatic adaptation. *Vision Research* 9: 179-186.
- Young, S.R., and G.R. Martin. 1984. Optics of retinal oil droplets: A model of light collection and polarization detection in the avian retina. *Vision Research* 24: 129-137.
- Zar, J.H. 1984. Biostatistical analysis, 2nd edition. Prentice-Hall, Englewood Cliffs, New Jersey. 718 pp.
- Zar, J.H. 1996. Biostatistical analysis, 3rd edition. Prentice-Hall, Upper Saddle River, New Jersey. 662 pp.
- Zrenner, E., and C.J. Krüger. 1981. Ethambutol mainly affects the function of red-green opponent neurons. *Documenta Ophthalmologica Proceedings Series* 27: 13-25.