The influence of the spectral quality of underwater light on visual sensitivity and the perception of optical signals in the threespine stickleback (*Gasterosteus aculeatus*).

by

Craig G. McDonald
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We accept this thesis as conforming
to the required standard

[redacted]

Dr. C.W. Hawryshyn, Co-supervisor (Department of Biology)

[redacted]

Dr. T.E. Reimchen, Co-supervisor (Department of Biology)

[redacted]

Dr. M. Edgell, Outside member (Department of Geography)

[redacted]

Dr. J.D. McPhail, External Examiner (Department of Zoology,
University of British Columbia)

University of Victoria

[redacted]

Supervisors: Dr. C.W. Hawryshyn
Dr. T.E. Reimchen

ABSTRACT

This work addresses the influence of female sensory perception on epigamic signal design in male threespine sticklebacks. Specifically, I investigated the hypothesis that red nuptial colour loss in red-shifted (stained) habitats is the result of reduced signal efficacy. Stickleback populations from different photic regimes expressing both divergent (black) and ancestral (red) nuptial colour were examined. It was found that stickleback populations from different photic regimes possessed different spectral sensitivities, with sensitivity in each population spectrally-tuned to the prevailing ambient spectra. Despite considerable differences in spectral sensitivity of fish from clear and stained aquatic habitats, it is probable that the efficacy of red nuptial colour would be similar in both habitats. Constraints on the perception of red nuptial colour based on colour discrimination would likely be readily compensated for by luminance discrimination in stained habitats. Thus, differences in sensory perception can not readily account for the loss of red nuptial colour in populations from stained habitats. However, nuptial colour preference experiments showed that female preference for red nuptial colour dropped significantly when nuptial colour contrast against the visual background was reduced. This suggests that preference for an epigamic optical signal is likely a function of its efficacy in generating visual contrast, rather than its intrinsic spectral characteristics.

Examiners:

[redacted]

Dr. C.W. Hawryshyn, Co-supervisor (Department of Biology)

[redacted]

Dr. T.E. Reimchen, Co-supervisor (Department of Biology)

[redacted]

Dr. M. Edgell, Outside Member (Department of Geography)

[redacted]

Dr. J.D. McPhail, External Examiner (Department of Zoology, University of British Columbia)

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GENERAL INTRODUCTION

The epigamic (mate attracting) optical signals of the males of many species have long been the subject of scientific investigation and debate (Darwin 1871, Houde 1993). Consequently, several hypotheses regarding their evolution have been put forth. These hypotheses are not mutually exclusive and are difficult to distinguish. Generally, it is suggested that an epigamic signal and preference for it continuously co-evolve by mutual reinforcement ("Fisherian runaway" hypothesis). In addition, it is hypothesized that epigamic signals serve as indicators of genetic quality ("Good genes" hypothesis), as well as facilitate conspecific mate recognition. All of these hypotheses require the assumption that signal expression (by the male) and preference (of the female) are genetically correlated (Houde 1993).

Recent evidence has led to the formulation of a new hypothesis, sensory drive, which suggests that epigamic signals exploit sensory biases of the signal receiver (Endler 1992, Ryan and Keddy-Hector 1992). These sensory biases are presumed to be the result of a selection pressure unrelated to mate preference (e.g. prey detection). Hence, the assumption of a genetic correlation between the trait and preference for it is not required. The sensory drive hypothesis deals primarily with ascertaining how the sensory perception of the signal receiver influences signal design. Although the sensory drive hypothesis is not independent of other hypotheses, its predictions can be independently tested. That is to say that based on knowledge of the parameters which contribute to the detectability of an optical signal, predictions about the evolution of its design can be tested. It is the intent of this work to examine the role of sensory bias in the evolution of divergent nuptial colouration in the threespine stickleback.

If we are to understand the evolution of signal design we must first understand how sensory and environmental factors interact to influence the efficacy of optical signal detection. Although considerable research has been devoted to the visually guided behaviours of the threespine stickleback (*Gasterosteus aculeatus*), very little is known of the visual system of this species. The only published work to date is that of Cronly-Dillon and Sharma (1968), who, using an optomotor response technique, found evidence for spectral mechanisms sensitive to medium (green) and long (red) wavelength light. However, the optomotor response technique fails to detect input from spectral mechanisms sensitive to short (blue) wavelength light, owing to the poor spatial resolution of these mechanisms. (Muntz 1974). Thus, it is evident that a more complete analysis of vision in sticklebacks is required if questions related to the perception of epigamic (mate attracting) optical signals are to be appropriately addressed.

The task of determining visual sensitivity in the threespine stickleback is complicated by the fact that it inhabits a variety of photic regimes. Since it has been demonstrated that the photic environment plays an integral role in shaping the visual sensitivity of different species (Loew and Lythgoe 1978, Levine and MacNichol 1983, Crescitelli et al. 1985), it can be expected that the visual system of a single species with populations resident to different spectral habitats would also exhibit variation in visual sensitivity. It is evident that optical signal perception would be affected by differences in visual sensitivity. Indeed, divergent nuptial colour expression in certain stickleback populations from the Pacific Northwest may relate to population specific sensory bias.

The males of several stickleback populations in the Pacific Northwest lack the red nuptial colouration typical of this species (McPhail 1969, Moodie 1972a, Hagen et al. 1980, Reimchen 1989). Instead, reproductive males are highly melanic. Melanism in these populations is strongly correlated with the spectral quality of underwater light, with melanic populations often being found in aquatic habitats which are substantially red-shifted (dsytrophic systems). It has been hypothesized that red nuptial colour is masked by the red-shifted ambient spectra, thus resulting in its loss in these populations (Reimchen 1989). It is also hypothesized that the melanic appearance of breeding males in these populations provides a highly conspicuous optical signal suited to dystrophic habitats. It is the aim of this work to provide insight into the possible role of sensory and environmental influences on the development of divergent nuptial colour in the threespine stickleback. The specific objectives of this study are as follows:

1. To characterize the diversity of spectral habitats which a single species may encounter.

2. To determine spectral sensitivity of stickleback from different spectral habitats.

3. To test whether sensory drive can play a role in the divergence of male nuptial colouration.

CHAPTER 1

THE PHOTIC ENVIRONMENT OF DYSTROPHIC AND CLEAR WATER AQUATIC HABITATS IN THE QUEEN CHARLOTTE ARCHIPELAGO

Abstract

The spectral characteristics of a number of aquatic habitats in the Queen Charlotte archipelago were examined using an underwater spectroradiometer. Several of the habitats experienced substantial attenuation of short wavelength light. Short wavelength attenuation resulted in considerable narrowing of the spectral band width and preferential transmission of long wavelength light. The spectral characteristics of these systems exhibited little variation, with the wavelength of maximum transmission ranging from 705 to 710 nm. One clear water system was also characterized. This system exhibited spectral characteristics similar to that of a typical mesotrophic "green water" lake. The marked diversity of aquatic spectral habitats in the Queen Charlotte archipelago can be expected to influence sensory perception of resident fish populations. It follows that differential perception of biologically important visual cues may result in alteration of behavioural responses to these cues.

Introduction

Although it is generally well accepted that visual sensitivity in fishes is correlated with the spectral quality of underwater light, virtually all work to date has examined fishes from short (blue) or medium (green) wavelength dominated photic regimes (Loew and Lythgoe 1978, Crescitelli et al. 1985). Most freshwater systems are dominated by medium wavelength light that is red-shifted in comparison to oceanic waters (Lythgoe 1979). Accordingly, fish in these systems tend to exhibit a red-shift in visual sensitivity (Levine and MacNichol 1979). Interestingly, the ambient spectra of some freshwater habitats exhibit a more extensive red shift. These habitats are typically dominated by long wavelengths as a result of the absorption of short wavelength light by humic acids (Bowling et al. 1986). Such habitats are generally referred to as dystrophic systems. In addition to being dominated by long wavelength light, dystrophic (or stained) habitats are subject to substantially reduced levels of ambient illumination. The extreme spectral characteristics of dystrophic habitats provide an unparalleled and unexplored opportunity to examine visual system optimization.

Much of the research addressing the functional association between visual sensitivity and the spectral characteristics of underwater light is comprised of analyses of the visual pigments of deep sea fishes. These analyses have provided the strongest evidence for what has come to be known as the sensitivity hypothesis (Muntz 1968, Crescitelli et al. 1985). This hypothesis predicts that the visual sensitivity of fishes should match the ambient spectra in order to maximize photon catch. Indeed, fishes from deep, blue-shifted waters tend to possess blue-shifted visual pigments (Crescitelli et al.

1985). However, deep sea fishes have occupied their particular photic niches for considerable periods of evolutionary time. As a result, a direct test of the sensitivity hypothesis cannot be made. By contrast, there are several non-deep sea species that dwell in distinctly different photic regimes. For example, threespine stickleback are resident to a variety of photic regimes, ranging from deeply stained dystrophic habitats to comparatively clear oceanic waters (see Table 1 for further examples). Comparison of intraspecific variation of visual sensitivity in fish with populations resident to different spectral habitats would permit rigorous examination of hypotheses regarding visual system evolution.

The purpose of this chapter is to provide a general overview of the spectral characteristics of dystrophic and clear water habitats in the Queen Charlottte archipelago, with special reference to the implications for the study of underwater vision. It is also the intent of this analysis to illustrate the value of employing within species comparisons of visual sensitivity when examining the role of the photic environment in determining visual sensitivity.

Methods and Materials

To evaluate the spectral characteristics of underwater light in a number of Queen Charlotte water systems (Table 2), measurements were made with an underwater LiCor Spectroradiometer at various depths. Downwelling, sidewelling and upwelling irradiance measures were made for each depth sampled in most systems. Although spectral irradiance curves provide considerable information and are simple to interpret, a means of data reduction was required (over 300 hundred measurements were made). To characterize light intensity in the different systems, the area under the spectral irradiance curves was summed between 360 and 720 nm. Calculations were made to determine quantal capture for downwelling light at a depth of 1 meter (except for Gold Creek and the Heillen River). The λP_{50} was also calculated and used as an index to compare the various spectra. The λP_{50} is the wavelength which halves the total number of photons between 360 and 720 nm (the visible spectrum). This index is useful for visual studies, as it denotes the wavelength about which quanta are most likely to be abundant (McFarland and Munz 1975). The λP_{50} does not, however, indicate how broad or narrow the spectral band width may be. Thus, spectral irradiance curves representative of dystrophic and clear water systems at different depths and lines of sight were also generated.

Results

All but two of the systems (Yakoun Lake and Mosquito Lake) sampled in the Queen Charlotte archipelago can be classified as dystrophic. The spectral characteristics of these dystrophic systems were found to be markedly similar, as evidenced by the λP_{50} and λ_{max} values (Table 2). Short wavelength attenuation is extensive and occurs over extremely short distances (Figure 1). Substantial attenuation of short wavelength light is evident at depths as shallow as 0.02m (Figure 1). Hence, even at depths less than 1 meter the photic environment is dominated by long wavelength light for all lines of sight (Figure 2) Total spectral irradiance is approximately two orders of magnitude lower than a typical "green" water mesotrophic lake (Table 2). Depths beyond 3.5 meters marked the lower limit of sensitivity of the Licor spectroradiometer in Drizzle (Figure 3) and Mayer Lake (data not shown). Upwelling irradiance is accordingly extremely low and irradiance measures could not be obtained at depths greater than 1 meter (Figure 2).

It should be noted that these measurements were all taken during summer months (July 28 - August 2, 1991), a period when staining from organic matter is extensive. During winter periods ambient light levels are elevated and short wavelength attenuation is diminished. This is a consequence of high precipitation and runoff in the autumn which saturates the peat bogs such that the residence time of water in the organic matrix is greatly reduced (Reimchen pers. comm.). It is therefore evident that these measurements represent the most "extreme" photic conditions encountered in dystrophic systems.

Extensive data was collected for only one clear water system. Yakoun Lake has spectral characteristics similar to that of a typical "green" water mesotrophic lake (Novales-Flamarique *et al.* 1992, Table 2). In comparison to the dystrophic systems the spectral band width (for all lines of sight) is broader and the ambient light levels are substantially higher (Figures 2 and 3, Table 2). The λP_{50} is also substantially blue-shifted. (Table 2). In addition, the λP_{50} undergoes a blue-shift with respect to depth, reaching a value of 589 nm at 6 m. A similar blue-shift was also observed for Cowichan Lake (Novales-F. unpublished data). By contrast, the λP_{50} of dytrophic systems undergoes a slight-red shift with respect to depth. For example, the λP_{50} for Drizzle Lake shifts from 683 nm at 1 m to 700 nm at a depth of 3 m.

Table 1. Queen Charlotte fish species with populations that are either resident to different spectral habitats or anadromous.

Species	Habitat type	Anadromous or Resident
Threespine stickleback	dystrophic, clear fw & sw	anadromous resident to dystrophic and clear fw
Prickly sculpin	dystrophic & clear fw	resident
Coho salmon	dystrophic, clear fw & sw	anadromous
Sockeye salmon	dystrophic, clear fw & sw	anadromous
Cutthroat trout	dystrophic, clear fw & sw	anadromous resident to dystrophic and clear fw
Dolly Varden	dystrophic, clear fw & sw	anadromous resident to dystrophic and clear fw

Table 2. Spectral irradiance, spectral distribution (λP_{50}) and wavelength of maximum transmission (λ_{max}) for downwelling light in a number of Queen Charlotte systems at various depths. Percent transmission (calibrated to distilled water) at 400 nm (T_{400}) is provided (from Reimchen 1989) to allow for comparison of this index of water colour with measures of underwater spectral irradiance (Figures 1,2 and 3). Data for Cowichan Lake (Vancouver Island) is also provided as an example of a typical "green water" system (Novales-Flamarique. unpublished data).

System	Depth (m)	Irradiance (photons/m^2/s)	λP_{50} (nm)	λ_{max} (nm)	T_{400}
Cowichan Lake	1	1.030e+21	560	560	NA
Yakoun Lake	1	2.204e+21	600	560	NA
Drizzle Lake	1	2.953e+19	683	705	67.0
Mayer Lake	1	7.709e+18	681	710	57.1
Heillen River	0.5	7.487e+16	690	710	NA
Gold Creek	0.3	1.467e+19	678	710	NA

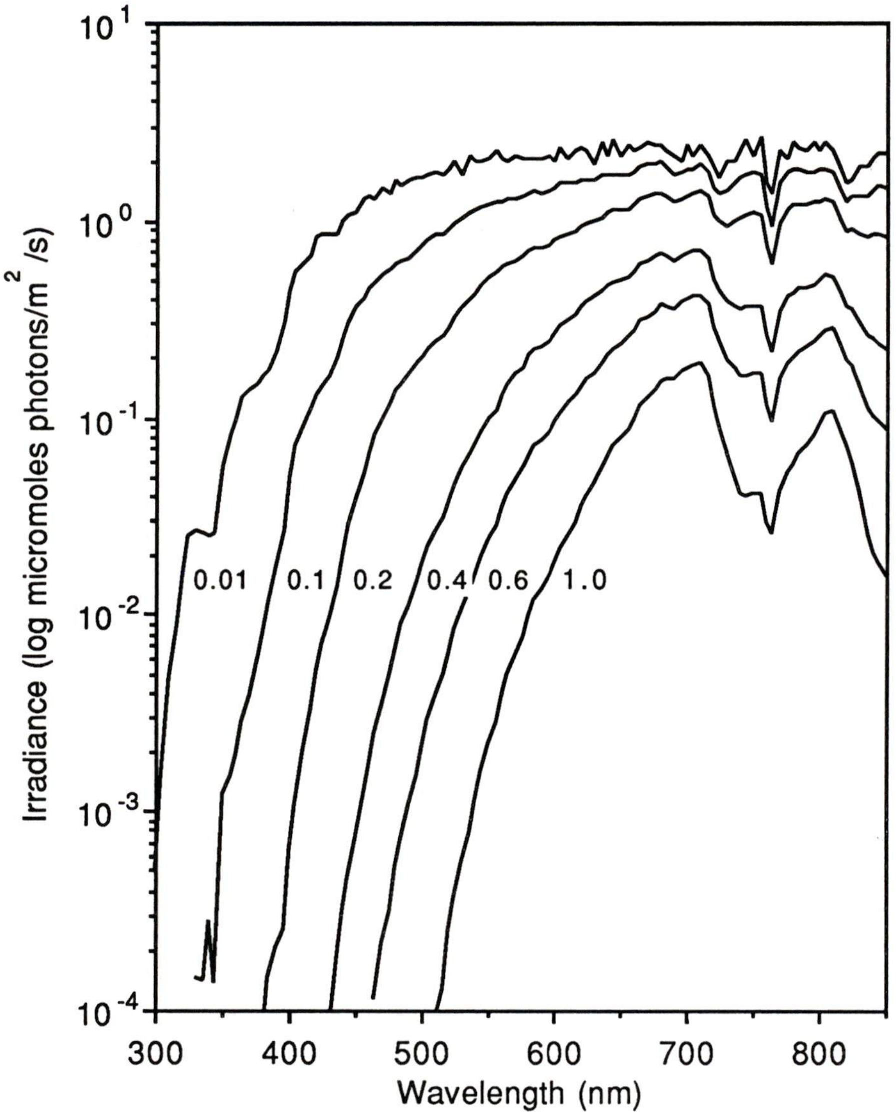


Figure 1. Downwelling spectral irradiance for Mayer Lake at various depths, ranging from 0.01 to 1.0 m. Note the rapid attenuation of short wavelength light over a modest increase in depth.

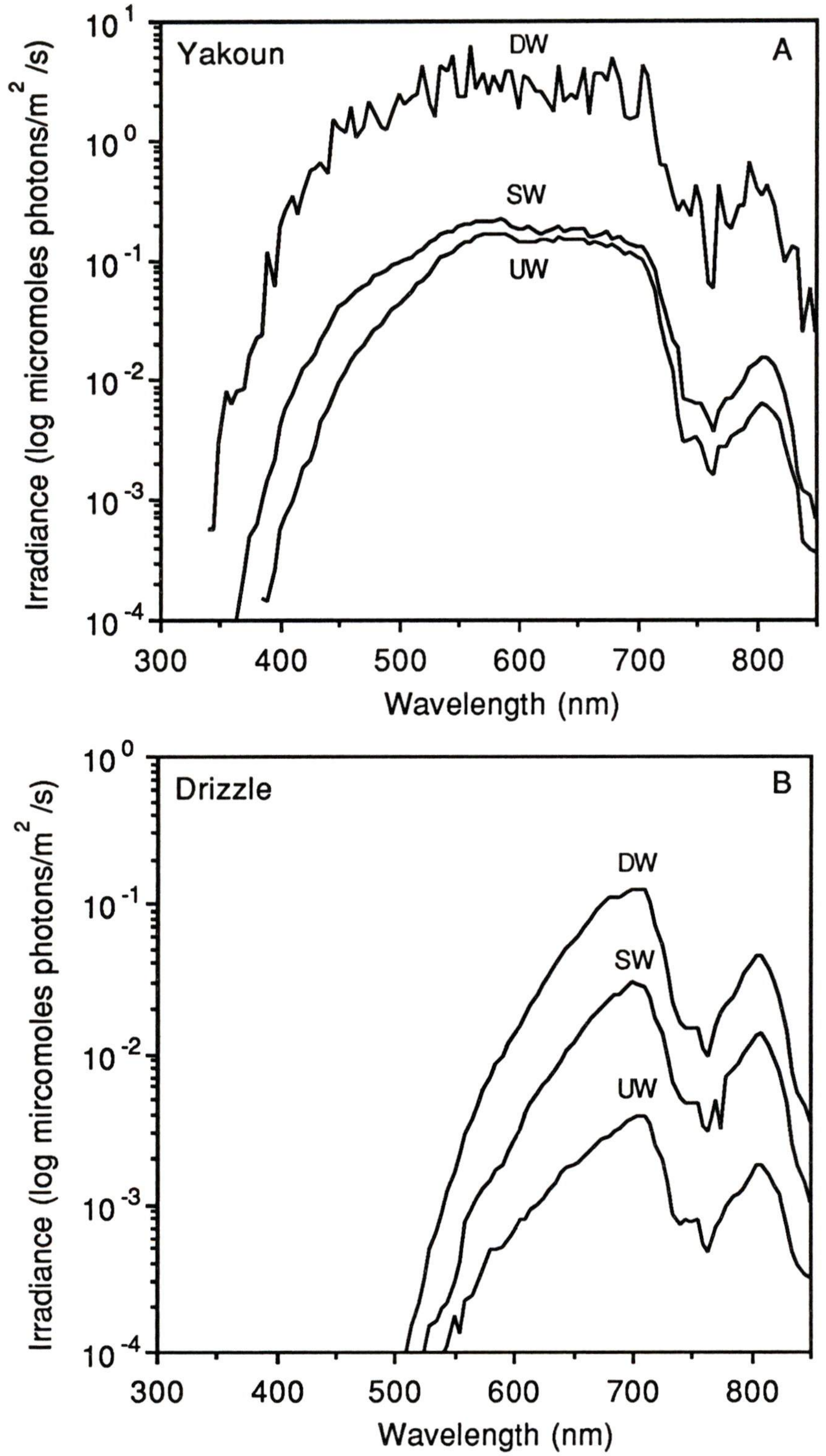


Figure 2. Downwelling (DW), sidewelling (SW) and upwelling (UW) spectral irradiance for Yakoun (a) and Drizzle Lake (b) at a depth of 1.0 m.

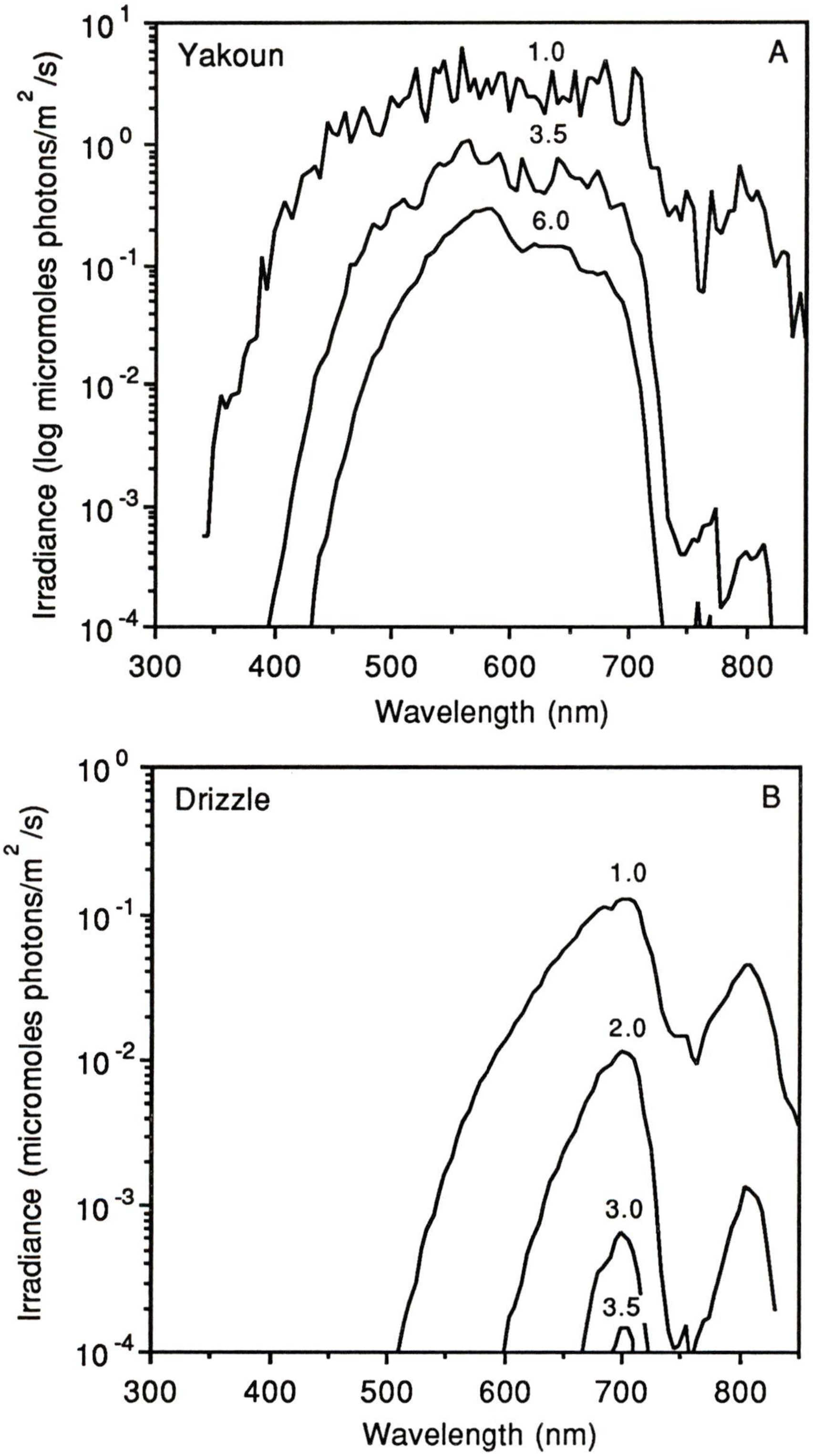


Figure 3. Downwelling spectral irradiance for Yakoun (a) and Drizzle Lake (b) at various depths (meters).

Discussion

There are several species in the Queen Charlotte archipelago with populations resident to both clear and dystrophic systems (Table 1). An intraspecific comparative analysis of the visual capabilities of resident populations from different photic regimes would most certainly provide considerable insight into visual system function and evolution. An intraspecific analysis would permit elimination of a number of confounding variables associated with interspecific comparisons. For example, phylogenetic constraints or interspecific differences in visually guided behaviour may make investigation of visual system - photic environment interactions difficult. Although the vast majority of work addressing visual ecology has examined interspecific differences in visual systems (Loew and Lythgoe 1978, Levine and MacNichol 1979, Crescitelli et al. 1985), examining intraspecific variation may be more appropriate, especially when exploring the role of the photic environment in determining visual sensitivity. An analysis of Queen Charlotte fishes provides an optimal opportunity for this type of approach.

Examination of dystrophic populations would provide considerably more insight into the evolutionary process of spectral tuning. Although deep sea fishes undoubtedly possess spectrally-matched visual sensitivity (Crescitelli 1985 et al.), it is difficult to obtain evidence indicating the mechanisms which facilitate spectral tuning. Study of populations from various photic regimes selected to provide a spectral gradient can be expected to provide a glimpse of visual system evolution. Furthermore, if populations from dystrophic habitats were found to undergo a substantial red-shift in sensitivity, with a concomitant loss of short wavelength sensitivity, the finding would provide direct evidence that the visual systems of fishes with populations resident to spectrally-narrow photic regimes undergo tuning to maximize photon capture. Here, it should be noted that increased absolute sensitivity does not necessarily imply greater contrast sensitivity. Reflective targets (targets that radiate more photons than the background), for example, would be difficult to detect with a spectrally-matched visual pigment, since the backwelling spectra would degrade target contrast (McFarland and Munz 1975). Should the backwelling irradiance be extremely low, however, a spectrally-matched pigment would provide strong luminance contrast for a reflective target.

The marked spectral diversity of aquatic habitats in the Queen Charlotte archipelago also provides an excellent opportunity for studying how modification of the visual system influences behaviour. Undoubtedly, the perception of many important visual cues, such as epigamic optical signals, would be altered in different spectral habitats. It follows that changes in visual perception may alter the behavioural responses to biologically significant visual cues (Levine and MacNichol 1979).

CHAPTER 2

INTRASPECIFIC VARIATION OF SPECTRAL SENSITIVITY IN THREESPINE STICKLEBACK (*GASTEROSTEUS ACULEATUS*) FROM DIFFERENT PHOTIC REGIMES.

Abstract

To examine the influence of the spectral characteristics of underwater light on spectral sensitivity of the ON and OFF visual pathways, compound action potential recordings were made from retinal ganglion cells of threespine stickleback from different photic regimes. In fish from a red-shifted photic regime (λP_{50} 680 nm for downwelling light at 1 m), peak sensitivity of both the ON and OFF pathways was limited to long wavelength light (λ_{max} 600 - 620). In contrast, the ON pathway of fish from a comparatively blue-shifted (λP_{50} 566 nm) photic regime exhibited sensitivity to medium (λ_{max} 540 - 560) and long (λ_{max} 600 nm) wavelengths, while the OFF pathway exhibited peak sensitivity to only medium (λ_{max} 540 nm) wavelength light. In a third population, where the the ambient light is moderately red-shifted (λP_{50} 629 nm), the ON pathway once again exhibited only a long wavelength sensitivity peak at 620 nm, while the OFF pathway exhibited sensitivity to both medium (λ_{max} 560 nm) and long (λ_{max} 600 - 620 nm) wavelength light. These findings suggest that the photic environment plays an integral role in shaping spectral sensitivity of the ON and OFF pathways.

Introduction

Although considerable research has been devoted to the visually mediated behaviours of the threespine stickleback (*Gasterosteus aculeatus*), only limited data exist for the visual capabilities of this species. Cronly-Dillon and Sharma (1968) employed an optomotor response technique to provide a measure of spectral sensitivity in the threespine stickleback, and found sensitivity to medium and long wavelength light. Interestingly, they also found that long wavelength sensitivity in females increased during the breeding season, when males develop red nuptial colouration. Cronly-Dillon and Sharmas' findings reveal the value of detailed examination of intraspecific variation in visual sensitivity; however, despite the intriguing implications of their work, no subsequent research on the visual ecology of the threespine stickleback has been undertaken.

Photic regimes vary considerably in different aquatic habitats (McFarland and Munz 1975, Lythgoe 1979). Moreover, it has been demonstrated that spectral sensitivity of many fishes relates to the spectral characteristics of the ambient spectra. Since species which live in different habitats exhibit differences in visual sensitivity (Loew and Lythgoe 1978, Crescitelli et al. 1985), it seems plausible that populations of the same species dwelling in different habitats would also exhibit variation in visual sensitivity. It was therefore the intention of this work to document not sex, but rather population related variation in the visual capabilities of the threespine stickleback. To this end, compound action potential (CAP) recordings were made from retinal ganglion fibers within the optic nerve of stickleback from different photic regimes.

CAP recordings from retinal ganglion fibers of rainbow trout and goldfish confirm the presence of ON and OFF visual pathways in teleosts. These pathways encode increments (ON pathway) and decrements (OFF pathway) of light as independent, excitatory events. In addition, the ON and OFF pathways exhibit different spectral sensitivities, and may therefore encode colour opponency (Wheeler 1979, DeMarco and Powers 1991, Beaudet et al. 1993 and Coughlin and Hawryshyn 1994). These differences in spectral sensitivity can be attributed to differential input from cones with different λ maxima.

Adult rainbow trout (Hawryshyn and Harosi 1994) and goldfish (Harosi 1976, Bowmaker et al. 1991) possess three classes of cone maximally sensitive to short, medium and long wavelength light. However, processing of chromatic information provided by these cones differs greatly between the two species. In goldfish, the OFF response receives input primarily from long (L) wavelength sensitive cones, whereas the ON response receives input primarily from short wavelength sensitive (S) cones. In contrast, the OFF response of rainbow trout receives input primarily from medium wavelength sensitive (M) cones, while the ON response receives considerable input from S and L cones (Beaudet et al. 1993, Coughlin and Hawryshyn 1994). The functional significance of the interspecific differences in spectral sensitivity of the ON and OFF pathways remains largely unaddressed.

It is probable that spectral sensitivity of the ON and OFF pathways relates to functional demands imposed by the spectral composition of the photic regime. Beaudet et al. (1993) noted that spectral sensitivity of the OFF response in rainbow trout may relate to the nature of the photic environment. In particular, they suggested that the OFF response is likely tuned to the prevailing ambient light, and therefore optimally suited to subserve a shadow detection function. Coughlin and Hawryshyn (1994) also argue that colour coding of ganglion cells relates to the spectral characteristics of underwater light.

The stickleback populations examined in the present study are from markedly different photic regimes and thus provided an ideal system for studying the influence of aquatic spectra on visual sensitivity. The specific aims of this study were (1) to examine how different photic regimes influence spectral sensitivity at the ganglion cell level and (2) to determine if the OFF pathway exhibits spectral tuning to the prevailing ambient spectra.

Methods and Materials

Underwater Light Measurements

Spectral irradiance measurements were made in two dystrophic lakes (Drizzle and Swan Lake) and a eutrophic lake (Quamichan Lake) to determine whether visual sensitivity of threespine sticklebacks from these lakes is optimally adapted to the photic environment. Measurements were made in Drizzle Lake in August 1991. In Swan and Quamichan Lake, measurements were made in April and May 1993, respectively. Downwelling, sidewelling and upwelling spectral irradiance measures were taken at 0 m (just beneath the surface), 20 cm and at 50 cm intervals thereafter to a depth of 1.5 m using an underwater LiCor spectroradiometer (LI-1800 UW model). For a detailed discussion of the use of the LiCor spectroradiometer for underwater spectral measurements see Novales et al. (1992). Spectral measurements were taken within one hour of solar noon. The total number of incident photons between 360 and 720 nm was calculated by measuring the area under the spectral irradiance curve. The λP_{50} was also calculated for downwelling, sidewelling and upwelling spectral irradiance at a depth of 1 m. The spectral index, λP_{50}, is the wavelength that halves the total number of photons between 360 and 720 nm and is a suitable measure for visual studies as it denotes the spectral position of the prevailing ambient quanta (McFarland and Munz 1975).

Electrophysiology

Animals. Threespine sticklebacks were collected from two dystrophic lakes (Swan Lake, Drizzle Lake, British Columbia) and a eutrophic lake (Quamichan Lake, British Columbia) from February to July, 1993. Fish were fed euphausids and live tubifex worms daily. Fish were held at the ambient temperature of their source lake and maintained under natural photoperiod. The photic conditions of the holding facility were broad spectrum (fluorescent lights), with a spectral distribution of 360 to 700 nm. The majority of experiments were conducted less than seven days after capture to obviate variability resulting from holding conditions. Experiments were conducted between 1000 h and 2000 h.

Preparation. Fish were immersed in a 100mg/l solution of tricaine methanosulphate (ms222) to Stage 4 anaesthesia and then administered intramuscular injections of an immobilizing agent (Pavulon, 0.0008 mg/g body weight) at several sites. Fish were restrained and respired with aerated water with a 20 mg/l solution of tricaine at 300 ml/min at the ambient temperature of their source lake. After 10 min. the right optic tectum was exposed to provide access to the optic nerve. A local anaesthetic salve (Pontocaine) was then applied to the periphery of the surgical incision. Following completion of each experiment, the fish was killed by a brain and spinal pith. All procedures and care of the animals were in accordance with the guidelines of the Canadian Council for Animal Care.

Apparatus and experimental procedure. Test fish were placed in a Faraday cage and respired with aerated water. UV-transmissive light guides projected light from a three-channel optical system to the left eye (see Parkyn and Hawryshyn 1993 for a detailed description of the optical system). The light guides were positioned 2.5 cm from the eye at a 20° angle above the horizontal midline of the pupil to project stimulus and background channels onto the ventral surface of the retina.

Ganglion fiber CAP recordings made without chromatic adaption do not permit determination of the cone mechanisms responsible for sensitivity to specific regions of the spectrum. It is therefore necessary to isolate the cone mechanism(s) of interest by depressing sensitivity of (chromatically adapting) the other cone mechanisms. The use of coloured backgrounds permitted isolation of the putative cone mechanisms. Specifically, 500 nm longwave pass filters were used to isolate the UV and S cone mechanisms, while 450 nm shortwave pass filters were used to isolate the M and L cone mechanisms. A 60 min. chromatic adaptation period allowed for isolation of the cone mechanism(s) sampled. Inconnel-coated neutral density filters were used to control background intensity. Background illumination was provided by 250 W Tungsten-Halogen lamps.

A 750 ms light stimulus, controlled by an electronic shutter (Uniblitz) was projected onto the eye via a third light guide from a 400 W Xenon lamp (Oriel). Sampling of multi-unit ganglion cell action potentials was at 20 s intervals and was controlled by the experimenter through a 386 microcomputer A/D interface. The wavelength and intensity of the light stimulus were manipulated by a monochromator (SA Instruments) and a neutral density wedge (Optikon). Both the monochromator and the neutral density wedge were controlled by a stepper motor interface.

Electrophysiological recording from ganglion fibers was carried out using nickel-plated chromium electrodes. A reference electrode was placed in the olfactory epithelium of the right nares. The recording electrode was inserted into the lumen of the optic nerve near its insertion point into the optic tectum. Voltage potentials (Figure 4a) were amplified through a Grass Instrument P5 pre-amplifier and exported to the computer via an A/D port.

Sensitivity to a particular wavelength was tested by presenting the fish with a light stimulus which increased in intensity by increments of 0.2 log units. Typically, 14 test wavelengths ranging over the visible spectrum (360 nm to 720 nm) were presented in a randomized order, which ensured that none of the cone mechanisms became selectively adapted. At each wavelength, the amplitude of the ON and OFF response was plotted against the incremental increase in photon irradiance (Figure 4b). A third order polynomial provided a best fit for the ON and OFF amplitude versus intensity plots (Beaudet el al. 1993). Threshold intensities were determined by calculating the irradiance required to evoke a criterion response of 30 μV (Figure 4b).

Results

Underwater light

Both the λ p50 values and the spectral irradiance curves indicate that Quamichan Lake is medium wavelength dominated, while Swan and Drizzle Lake are long wavelength dominated (Figure 5, Table 3). Swan and Drizzle Lake are dystrophic systems, which are typically dominated by long wavelength light as result of absorption of shorter wavelengths by humic acids (Bowling et al. 1986). Short wavelength attenuation in Swan Lake is moderate in comparison to that of Drizzle Lake, where there is virtually no transmission of sidewelling light below 500 nm at a depth of 1 m (Figure 5). Although the spectral band width varies considerably with line of sight in each lake (Figure 5) , variation in the λ p50 is low, the largest difference being 18 nm between downwelling and sidewelling irradiance for Quamichan Lake (Table 3).

Spectral sensitivity

The ON response.

Differences in spectral sensitivity of the ON response were found among the three populations (Figure 6). In the Swan and Drizzle Lake populations, only a long wavelength ON sensitivity peak was observed, regardless of the isolating background (Figures 6a and 6c, Table 4). Long wavelength sensitivity in these populations was, however, approximately 0.5 log units higher under the M - L isolating background. In the Quamichan Lake population, a medium wavelength ON sensitivity peak was observed under a UV - S isolating background (Figure 6a); a long wavelength ON sensitivity peak was present only under a M - L isolating background (Figure 6c). Sensitivity of the long wavelength arm of the long wavelength sensitive ON response of the Swan and Drizzle Lake fish was found to be significantly greater than that of the Quamichan Lake fish, attesting to a correlation between spectral sensitivity and the extent of red-shift in the ambient spectra (Figure 6c). Although secondary sensitivity peaks in the UV - S region of the spectrum were observed in the long wavelength sensitive action spectra of the Swan (ON response) and Drizzle Lake (ON and OFF responses) populations (Figure 6), their amplitude relative to the α peaks was found to be similar under both UV - S and M - L isolating backgrounds, suggesting that they represent β band absorption of the long wavelength sensitive visual pigment.

The OFF response.

Spectral sensitivity of the OFF response varied substantially among the three populations (Figures 6b and 6d, Table 4). The greatest difference in spectral sensitivity was found to be between the fish from the most disparate photic regimes (Quamichan and Drizzle Lake). The OFF response λ_{max} of the Drizzle Lake fish was at wavelengths 60 - 80 nm (Figures 6b and 6d, Table 4) longer than that of the Quamichan Lake fish. The Swan Lake photic regime has spectral characteristics intermediate to those of Quamichan and Drizzle Lake (Figure 5, Table 3), and the OFF sensitivity peaks of the Swan Lake fish appear to be correlated with this intermediate photic condition (Figures 6b and 6d, Table 4). The action spectra of the OFF response in all populations were markedly similar under both UV - S and M - L isolating backgrounds (Figure 6). However, under the M - L isolating background, sensitivity was approximately 0.5 log units higher in the Swan and Drizzle Lake fish.

Table 3. $\lambda\ P_{50}$ values for downwelling, sidewelling and upwelling light at a depth of 1 m.

Direction	Quamichan Lake	Swan Lake	Drizzle Lake
Downwelling	566 nm	629 nm	680 nm
Sidewelling	584 nm	628 nm	684 nm
Upwelling	578 nm	642 nm	695 nm

Table 4. Summary of ON and OFF response λ_{max} values for stickleback from three different spectral regimes. The type of isolating background is given in parentheses. Sample sizes are provided in Figure 3.

Pathway	Quamichan Lake	Swan Lake	Drizzle Lake
		λ_{max} (nm)	
OFF (UV - S)	540	560, 600	600 - 620
OFF (M - L)	540	560, 620	620
ON (UV - S)	540 - 560	620	600 - 620
ON (M - L)	600	620	620

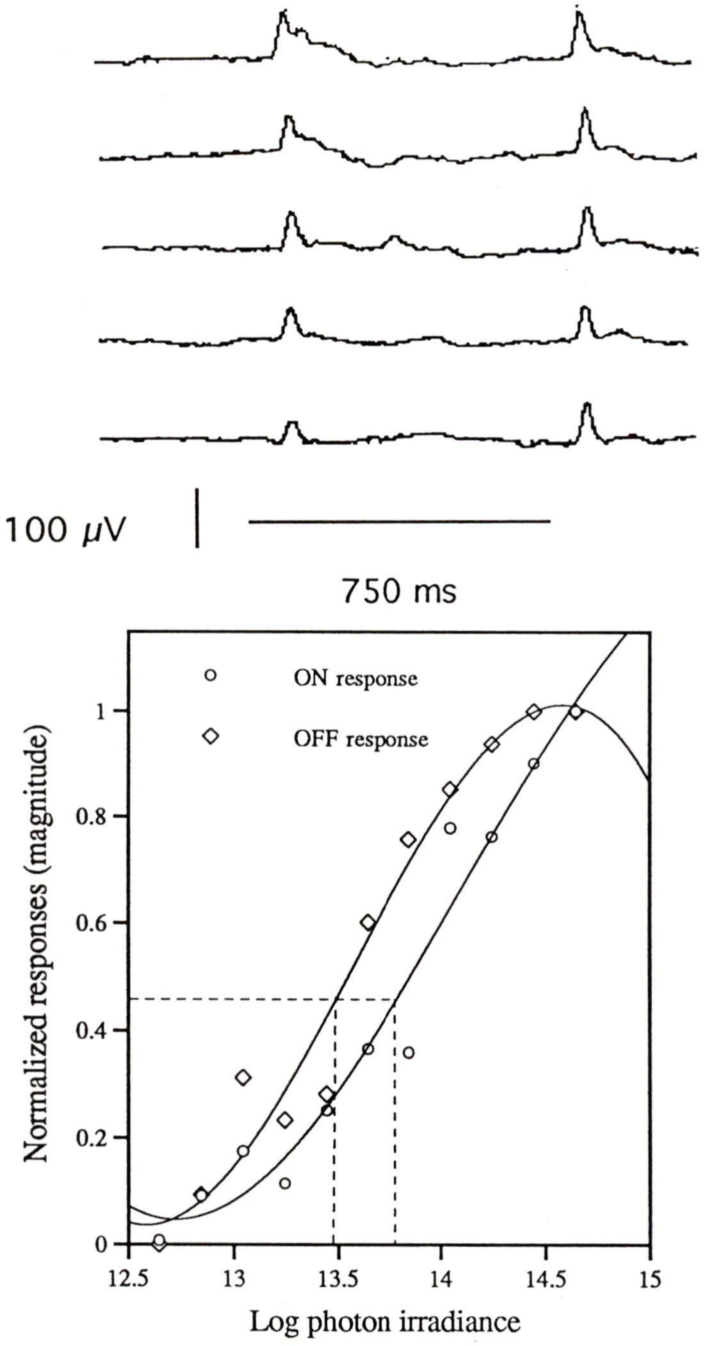


Figure 4. Compound action potential recordings from retinal ganglion fibers of a Swan Lake fish presented with a 560 nm stimulus (a). Each trace (moving from bottom to top) represents a response to an increase in photon irradiance of 0.2 log units starting from a neutral density of 0.8. Note that the responses presented here are suprathreshold. (b) A plot of response versus intensity for the same fish using a 560 nm stimulus. Intensity of light at threshold (30μV) is indicated by the intersection of the slope and the dashed lines.

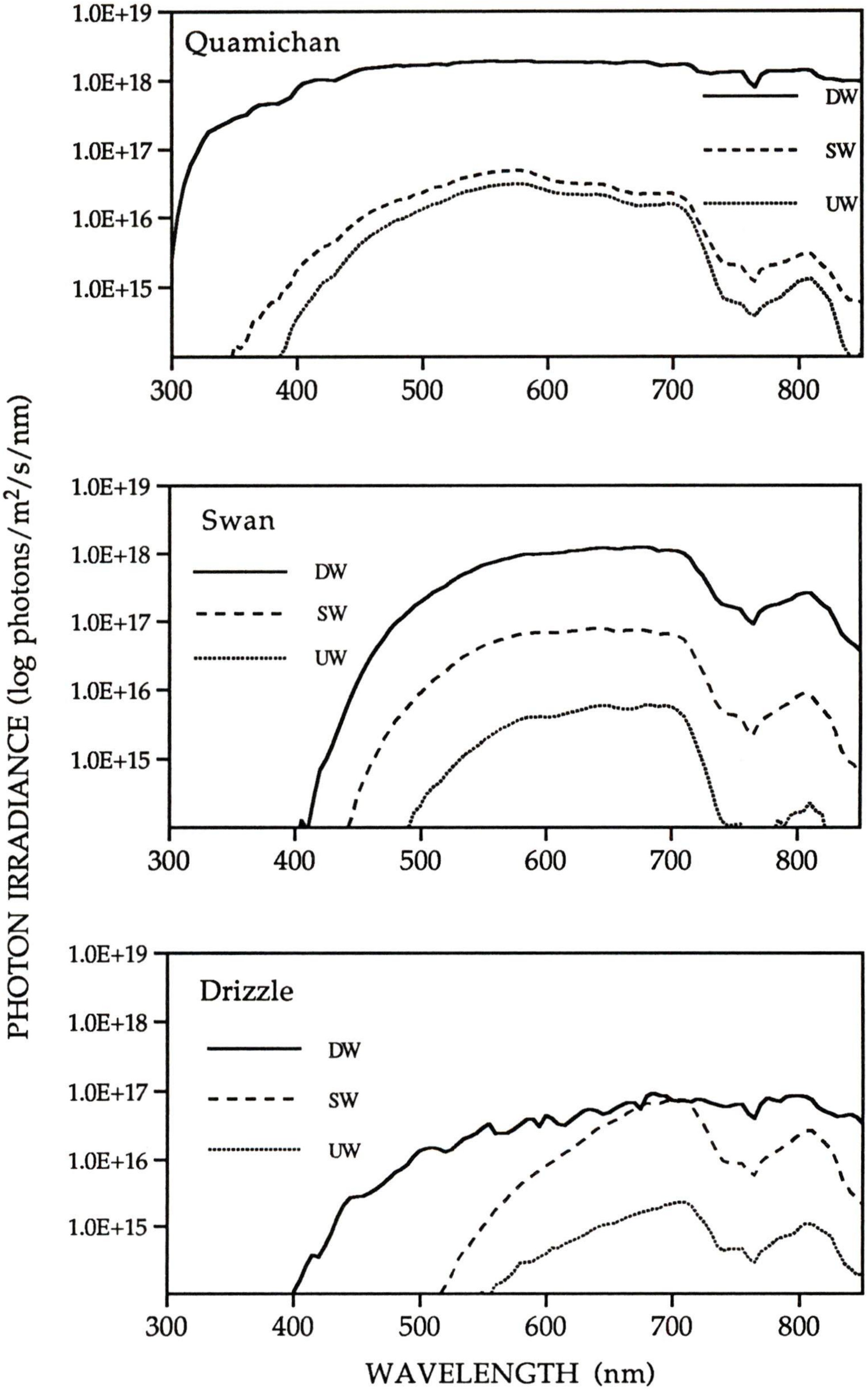


Figure 5. Downwelling (DW), sidewelling (SW) and upwelling (UW) spectral irradiance at a depth of 1 m.

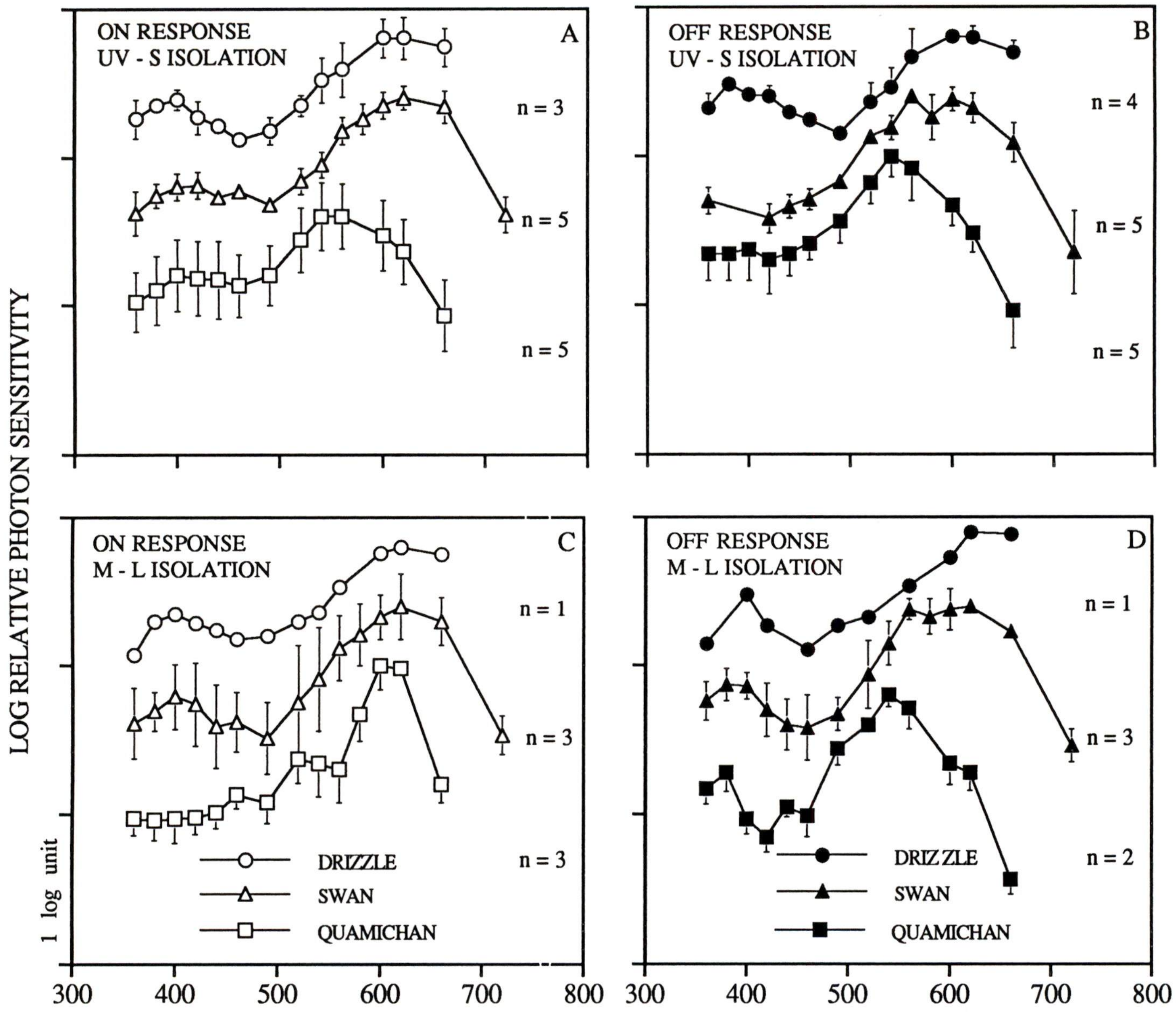


Figure 6. Mean spectral sensitivity of ON (a,c) and OFF (b,d) optic nerve responses under a UV-S (a,b) and a M-L isolating background (c,d). Spectral sensitivity curves have been displaced on the ordinate to enable comparison of the three populations. Error bars respresent 1 standard error of the mean, except where positive or negative error is given.

Discussion

This study provides evidence for substantial intraspecific variation of spectral sensitivity in threespine sticklebacks (*Gasterosteus aculeatus*), and further suggests that this variation relates to the nature of the photic environment. Remarkably, in the Drizzle lake population, peak sensitivity of both the ON and OFF pathways was limited to long wavelengths. The absence of sensitivity peaks at shorter wavelengths might be expected in this population, as attenuation of short wavelength ambient light is extensive. Indeed, in the remaining populations, where attenuation of short wavelength light is not as extensive, distinct medium wavelength sensitivity peaks were found. Medium wavelength sensitivity was expressed by both the ON and OFF pathways in the Quamichan Lake population, while in the Swan Lake population, where attenuation of short wavelength light is moderate, peak medium wavelength sensitivity was expressed by only the OFF pathway and red-shifted. These findings strongly suggest that the photic regime has played a role in determining spectral sensitivity in these populations.

Possible spectral mechanisms underlying variation in spectral sensitivity.

The presence of medium and long wavelength sensitivity peaks in the Quamichan and Swan Lake fish provide evidence for medium and long spectral mechanisms in these populations. Although the medium wavelength OFF sensitivity peak observed for the Swan Lake fish is not as distinct as that observed for the Quamichan Lake fish, its presence does suggest the existence of a medium spectral mechanism in the Swan Lake population. My data for these populations is in good agreement with microspectrophotometric data for an estuarine stickleback population, in which M and L cones numerically dominate the retina (Baube and Loew pers. comm.).

Spectral shifts in visual pigment absorption may well underlie the intraspecific differences in spectral sensitivity reported here. Without a microspectrophotometric analysis, it is difficult to determine the exact mechanism(s) underlying the variation in spectral sensitivity reported here. My finding of a 540 nm OFF sensitivity peak in the Quamichan Lake population does, nonetheless, compare well to the absorption spectra of the M cones (λ_{max} 530 nm) in the estuarine population (Baube and Loew unpublished data). Baube and Loew also report the presence of a small population of red-shifted M cones. The similarity in λ_{max} (566 nm) of these cones with the 560 nm OFF sensitivity peak observed in the Swan Lake population suggests that red-shifted M cones may underlie this sensitivity. Long wavelength sensitivity peaks were found in all populations, and it is probable that they reflect input primarily from L cones. Although Baube and Loew report the presence of UV and S cones, they appear to be considerably less abundant than M and L cones (Loew pers. comm.). It is possible that my recording technique was not sensitive enough to detect input from UV and S cones, if they are indeed present in the populations examined in the present study. Since several red environment dwelling fishes do not possess S cones (Fukurotani and Harosi 1985, Sillman et al. 1993, Loew and Lythgoe unpublished data), it seems plausible that UV and S cones may be absent in the stickleback from red-shfted photic regimes.

Spectral sensitivity in an extreme photic regime

It is significant to note that long wavelength dominated spectral sensitivity was observed in the Drizzle Lake population. The action spectra of the ON and

OFF pathways were found to be virtually identical, and both exhibited peak sensitivity in the range of 600 - 620 nm. In contrast to the other populations and findings for goldfish (Wheeler 1979, DeMarco and Powers 1991) and rainbow trout (Beaudet et al. 1993, Coughlin and Hawryshyn 1994), it appears that spectral sensitivity of both the ON and OFF pathways in the Drizzle Lake population is a function of equal input from the same class of cone(s). The ON and OFF pathways may receive input primarily from L cones, which possibly dominate the retina of this population. My experimental conditions did not, however, permit determination of whether input solely from L cones underlies this sensitivity. If input from M cones contributes to the long wavelength sensitivity peak, it may do so by acting additively with L cone input to form a single sensitivity peak. Given that a small population of red-shifted M cones (λ_{max} 566 nm) are present in the estuarine population (Baube and Loew unpublished data), it might be expected that selection would favour red-shifted M cones in the Drizzle Lake population. Regardless of the mechanism underlying this phenomenon, it is important to note that the retinal organization of this population likely maximizes sensitivity to the substantially red-shifted ambient spectra (Crescitelli et al. 1985).

Functional implications of spectral tuning of the OFF pathway

Because the OFF pathway encodes decrements of light, it is reasonable to postulate that it facilitates detection of targets that appear darker than the visual background. According to the contrast sensitivity hypothesis (Lythgoe 1968), optimal contrast for a dark target would be generated with an OFF response maximally sensitive to the prevailing ambient light. The finding by Northmore (1973) that the escape behaviour of the rudd is driven primarily by a red OFF stimulus provides neuroethological evidence that the OFF pathway functions as a shadow detector (Wheeler 1979). Moreover, as spectral absorption of the visual pigments in the ventral retina of the rudd is red-shifted in comparison to that of the dorsal retina (Muntz and Northmore 1971), it is possible that the ventral retina exhibits tuning to the prevailing downwelling light. Beaudet et al. (1993) noted that the OFF response of rainbow trout probably exhibits peak sensitivity to the prevalent aquatic spectra and, like Wheeler, postulated that the OFF response subserves a shadow detection function. Significantly, the OFF response λ maxima of the stickleback populations we examined are shifted towards the prevalent wavelengths of ambient light, and thus appear to exhibit spectral tuning. The progression towards longer wavelengths dominating spectral sensitivity of the OFF response relates directly to the extent of attenuation of short wavelength ambient light. These findings provide further evidence for a spectrally-matched shadow detector.

Implications for study of visual ecology

My results reveal the importance of considering intraspecific variation when adopting a comparative approach in examining ecological or phylogenetic influences on the visual systems of different species. From these findings it becomes apparent that ecological constraints, such as those imposed by an extreme photic regime, can potentially mask phylogenetic relationships. Conversely, phylogenetic constraints or interspecific differences in visually guided behaviour may make investigation of visual system - photic environment interactions equally difficult. The vast majority of work addressing visual ecology has examined interspecific differences in visual systems (Loew and

Lythgoe 1978, Levine and MacNichol 1979, Crescitelli et al. 1985). However, when exploring the role of the photic environment in determining visual sensitivity, it may be advantageous to examine intraspecific variation. It is also evident that behavioural studies addressing issues such as sexual selection (e.g. female preference for nuptial colouration) can be properly evaluated only if the visual capabilities of the population(s) in question have been established.

CHAPTER 3

PREFERENCE FOR THROAT COLOUR IN MALE THREESPINE STICKLEBACKS (*GASTEROSTEUS ACULEATUS*) AS A FUNCTION OF BACKGROUND COLOURATION.

Abstract

I used a video imaging technique in an attempt to determine if the spectral composition of the visual background influences female preference for nuptial colouration in male threespine sticklebacks. Throat colour preference tests were carried out in which females chose between video images of red and black-throated males superimposed on either a red or a blue background. Female responses were generally characterized by direct orientation towards one of the two video images while moving in sync with it. My results demonstrate that preference for throat colouration can be altered by manipulating throat colour contrast against the visual background. Despite belonging to a red-throated population, females were found to respond preferentially to black-throated males viewed against a red background, indicating that preference was not related to throat patch hue. This suggests that preference for an epigamic, optical signal is likely a function of its efficacy in generating visual contrast rather than its intrinsic spectral characteristics.

Introduction

Although variation in nuptial colour expression in the threespine stickleback (*Gasterosteus aculeatus*) has been extensively documented (McPhail 1969, Semler 1971, Moodie 1972, Hagen and Moody 1979, Reimchen 1989), a satisfactory explanation addressing the functional significance of this phenomenon remains elusive. A number of hypotheses have been proposed to explain the melanic phenotype which is common in the Pacific Northwest. Until recently, the most widely accepted hypothesis held that melanic colouration facilitated crypsis in populations where salmonid predation levels are high (Moodie 1972*a,b*, Semler 1971). A second hypothesis (Hagen et al. 1980) suggests that melanic nuptial colour functions as an interspecific threat display in areas where stickleback are sympatric with the black Olympic mudminnow (*Novumbra hubbsi*). However, the validity of these hypotheses is called into question by the fact that there are several melanic populations in the Pacific Northwest where salmonid predators and/or*Novumbra* are not present (Reimchen 1989, pers. comm.)

The only consistent correlative element associated with melanic colouration has been found to be the spectral quality of the underwater photic regime (Reimchen 1989). Reimchen found that in deeply red stained (dystrophic) lakes, breeding males tend to be highly melanic and lack red nuptial colour. This observation led Reimchen (1989) to hypothesize that red nuptial colour may be masked by the ambient spectra and furthermore, to propose that melanic nuptial colour would provide a highly conspicuous optical signal suited to stained habitats. Reimchen's hypothesis, signal masking, is consistent with the predictions of the sensory drive hypothesis, which suggests that signal design is largely determined by the sensory perception of the signal receiver (Endler 1992, Ryan and Keddy-Hector 1992).

Several studies indicate that sensory bias of the signal receiver plays a role in determining optical signal design in a number of species (Endler 1991, Fleishman 1988 and Endler 1983). However, few studies have attempted to manipulate preference for epigamic signals by imposing a sensory constraint on the signal receiver (but see Long and Houde 1989 and Sigmund 1983). In the present study, a video imaging technique was used to constrain the ability of female sticklebacks to discriminate between red throat colour and the visual background. By experimentally altering the visual background to produce a sensory bias, it was possible to investigate whether signal masking can result in a loss of female preference for red nuptial colour in a population that is monomorphic for this trait. The experimental design allowed for females to choose between spectrally modified mirror images of a single male, thus making it possible to isolate signal contrast as the sole variable related to nuptial colour preference.

Materials and Methods

Origin of experimental fish

Threespine stickleback were collected from Quamichan Lake, British Columbia for a period extending from May to July, 1993. Males from the Quamichan Lake population develop the carotenoid based throat pigmentation characteristic of this species. Males were housed solitarily in 20 L home tanks to elicit establishment of breeding territories and nest building behaviour. Only gravid females and reproductive males were used for throat colour preference experiments. Females were collected on a weekly basis and housed communally in 80 L aquaria. Females were tested as soon as possible after capture as they often exuded their egg masses shortly after capture.

Video Image Processing

The Video Imaging System

A unique feature of the imaging system was that it allowed spectral alteration of a video image in real time. This permitted image processing while a trial was in progress. Video images were first exported from a VCR to a video imaging board (PIP 512). Since the video imaging board did not have colour capability, colouring of the video image was accomplished with a 486 microcomputer and costumized software. Pseudocolouration software (developed by R. Racca of Racca Scientific Consulting) converted assigned intensities of the video image to a hue of choice (within the capabilities of the monitor). Spectrally altered images were exported from the video board to an RGB colour computer monitor (Sony Trinitron).

Choice of spectral composition of the video images

At least two differentially sensitive spectral mechanisms are required for colour discrimination. Electrophysiological recordings from retinal ganglion cells of the Quamichan Lake fish indicate that medium (green) and long (red) spectral mechanisms are present in this population. Microspectrophotometric data for an estuarine population (Baube and Loew unpublished data) suggest the possible existence of UV (ultraviolet) and S (blue) mechanisms as well. Thus, it is probable that the Quamichan Lake fish are capable of colour discrimination, although behavioural colour discrimination tests would be required to confirm this. The visual backgrounds used for the throat colour preference experiments were chosen to provide different levels of colour contrast for red throat colouration. The spectral composition of the red background and red throat patch were similar (Table 5), and thus likely provided a constraint on the ability to disriminate on the basis of wavelength (colour). Black (the absence of incident photons) can be expected to provide luminance contrast against both the red and blue backgrounds (Table 5). The spectral output of the blue, green and red phosphorus of the monitor are provided in Figure 7.

The Filming Chamber

To facilitate relative ease of spectral alteration of the video images, three walls of the filming chamber were painted flat black, while the floor (which consisted of clear aquarium glass) was rendered highly reflective by placing the chamber on glossy white cardboard. This permitted high contrast between the fish, which was illuminated from above by a 22 watt florescent light, and the background.

The ambient illumination was manipulated such that the ventral portion of the male was the brightest component of the visual scene. This was necessary because spectral alteration of video taped males involved colouring the ventral portion of the male by converting the highest range of intensities in the visual scene to a hue of choice. The background, which exhibited the lowest range of intensities in the visual scene, was spectrally altered in the same fashion. The dorsal and lateral surfaces of the male remained unaffected by the spectral alteration process and appeared as a black and white image.

Filming

Males that exhibited nest building behaviour were placed in a filming chamber and filmed with a high sensitivity video camera (Panasonic). The camera possessed a mirror imaging function, which permitted generation of mirror images of a single male. To film courtship interactions, nest building males were placed in the filming chamber and provided visual access to a gravid female. A 10 to 15 min. acclimation period was provided for both the male and the female. The use of a one way mirror permitted the male to view a gravid female placed in a 20 L aquarium positioned orthogonally to the filming chamber. The one way mirror also prevented view of the experimenter, while permitting direct sight of the courting male for filming (Figure 8). Although ritualized courtship behaviours were not observed, the male was often observed to vigorously swim up and down against the front wall of the chamber in unison with the female. This behaviour provided the criterion for operational courtship used in the present study. A six minute segment of vigorous vertical swimming motion of a single male was chosen for use in the colour preference trials.

Experimental chamber design

Female preference for throat colour was determined by placing a female in a chamber which permitted view of a 14" colour computer monitor. The floor and the two lateral walls of the chamber (36 x 30 cm) were painted flat black to prevent confounding effects from extraneous light. The two remaining walls of the chamber (25.4 x 30 cm) consisted of clear plexiglass and permitted direct view of the monitor by both the female stickleback and the experimenter. The chamber was filled to a water depth of 25.4 cm. The ambient light levels were low in the experimental room, with the only ambient illumination, aside from the monitor itself, being a 60 watt incandescent bulb.

Experimental Design

Females were provided a choice between red and black-throated mirror images of a single male superimposed on either a red or a blue background. The choice regime allowed the female to view adjacent mirror images of the male on the computer monitor (Figure 9). Females were introduced to the test chamber and provided an adaptation period of 10 min. Before presentation of the video image, the experimenter allowed the female to reach the central region of the tank. This ensured that the female's response was not biased by her initial position. The position of red and black throat colouration was switched randomly among trials to further control for side bias. On the few occasions when females remained responsive after the initial 6 min. trial, throat colours were again switched and the trial repeated. Female responses were recorded with a high sensitivity video camera (Panasonic).

Successful trials were those in which females tracked male video images. Tracking behaviour was said to occur when a female oriented directly toward one of the two video images and moved, more or less (females tended to lag marginally in responding to the movements of the image), in unison with it. Females that exhibited tracking behaviour repeatedly altered their orientation such that they swam in the same direction as the image was moving. In some cases, females remained stationary, but rotated their body about the vertical axis such that they maintained direct alignment with the video image.

Table 5. Spectral composition of the throat patches and visual backgrounds. The spectral composition of each spectrally altered element in the visual scene is expressed as a percentage of the total red, green and blue output of the RGB computer monitor.

	Percent (%) RGB output		
Spectral element	Red	Green	Blue
Red throat patch	70.59	7.84	0.00
Black throat patch	0.00	0.00	0.00
Red background	78.43	0.00	0.00
Blue background	0.00	0.00	78.43

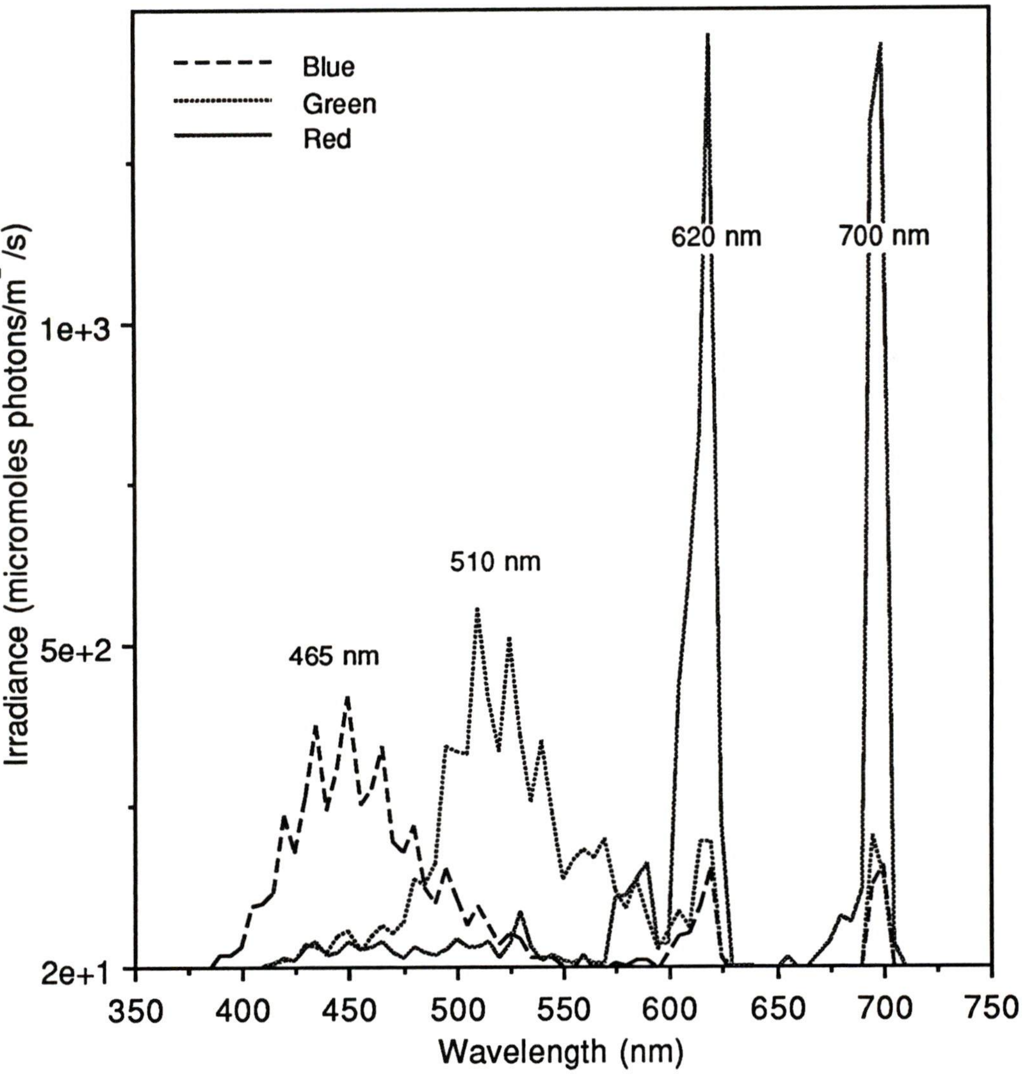


Figure 7. Spectral output of the blue, green and red phosphorus of the Sony Trinitron computer monitor. The wavelength(s) of peak emission for each phosphorus is also provided.

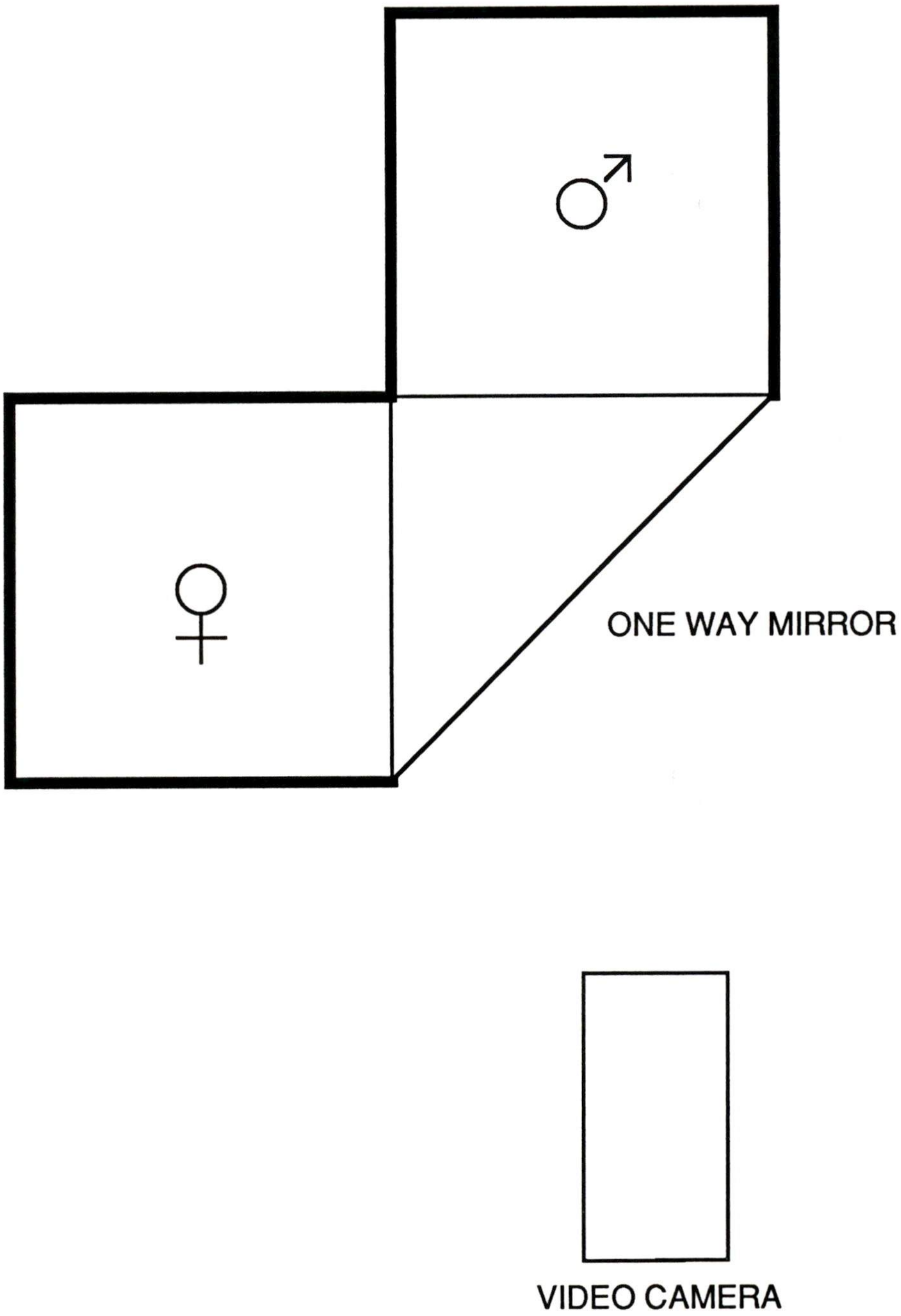


Figure 8. Set-up for filming males. The one way mirror permitted the male to observe the female without detecting the experimenter. All chamber walls were painted flat black (▬), with the exception of the orthogonally situated walls which permitted the male and female to view one and other.

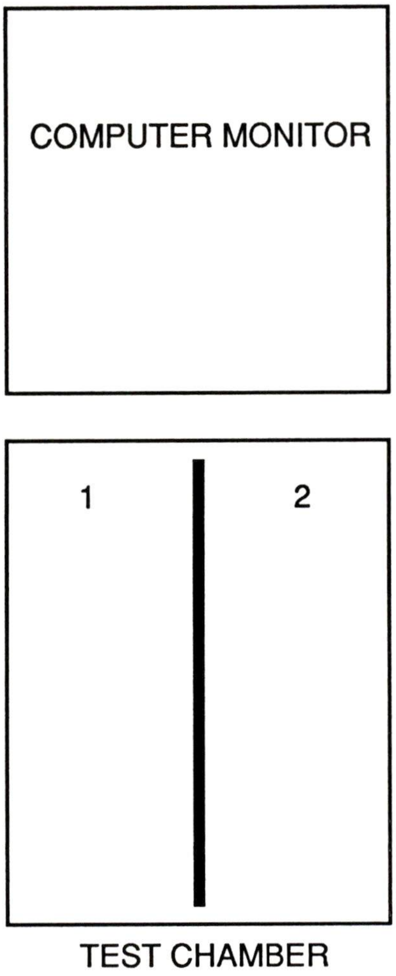


Figure 9. Throat colour preference experimental set-up. The test chamber was positioned 5 cm away from the video screen. A line bisected the chamber into two equal halves. For the time/side analysis, females were considered to be in front of the left video image if they were in the region designated with a 1 and in front of the right video image if they were in the region designated with a 2.

Results

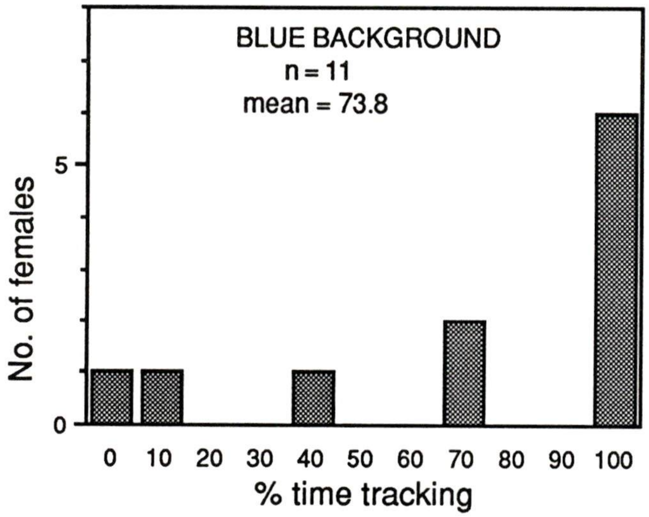
Out of a total of 120 trials, sixteen females displayed tracking behaviour. Females were scored as responding preferentially to a particular throat colour if they tracked the male expressing it for a greater period of time than the male expressing the alternate colour. This is a robust measure of preference as in all but one trial (n = 16) females tracked a focal male for at least 76.1% of the total time allocated to tracking (Figure 10). Female preference for red or black nuptial colour was shown to be influenced by background colour, as evidenced by a significant difference between red and blue background treatments (Table 6). The proportion of female responses to red-throated males dropped significantly under the red background treatment (Table 6, Figure 10).

In one trial for each of the experimental treatments, females remained responsive until the end of the initial 6 min. test period. Throat colour position was switched and these females retested. Under the red background treatment, the female tracked the black-throated male for a greater period of time, both before (100% of response duration) and after (91%) throat colours were switched. Under the blue background treatment, the female preferentially tracked the red-throated male before (76.9%) and after (100%) throat colours were switched. These findings provide evidence that side bias was not a confounding factor. As females chose between mirror images of the same male, it highly probable that preference was associated solely with throat colour contrast.

In an attempt to determine whether females which did not display tracking behaviour responded preferentially to a particular throat colour, a time per side analysis was carried out. Only females which exhibited swimming behaviour directed towards the video screen (but did not track the images) were used for this analysis. The amount of time each female spent on each side of the test chamber swimming at the video screen was recorded (Figure 9). The proportion of the total response duration that females spent swimming in front of the red-throated male image was then calculated (for both red and blue background treatments). The resulting data was arcsin transformed and a single sample t-test carried out (two-tailed). Under both the red (d.f.=15, mean=0.512, p=0.380) and blue (d.f.=13, mean=0.481, p=0.875) background treatments, time spent per side did not differ significantly. A two sample t-test was carried out to compare the two treatments. There was no significant difference between means (d.f.=28, p=0.557).

Table 6. Female responses to red and black-throated male video images as a function of background colouration. The difference between treatments is significant (Fisher's exact P=0.0128, one-tailed).

	Treatment	
Variable	Red background	Blue background
Red throat	0	8
Black throat	5	3



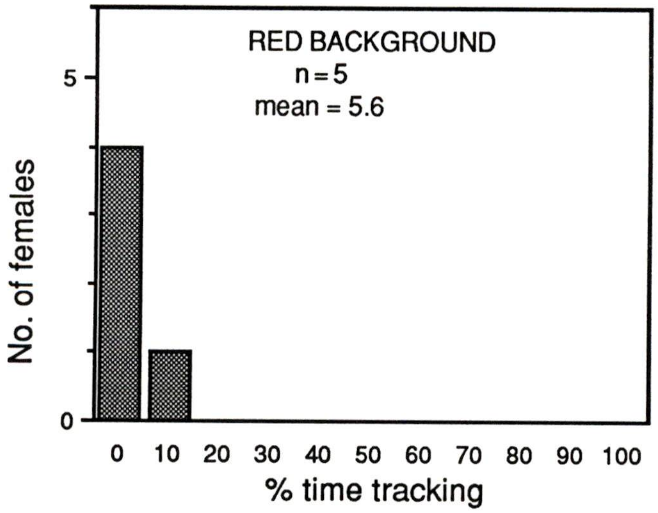


Figure 10. Percentage of the total response duration each female spent tracking a red-throated male.

Discussion

These results demonstrate that female preference for throat colour in the threespine stickleback (*Gasterosteus aculeatus*) can be altered by manipulating throat colour contrast against the visual background. Despite belonging to a red-throated population, females were found to respond preferentially to black-throated males viewed against a red background, indicating that preference was not related to throat patch hue. This suggests that preference for an epigamic, optical signal is likely a function of its efficacy in generating visual contrast rather than its intrinsic spectral characteristics.

It should be noted that the present study did not permit determination of whether females would proceed to mate with males expressing black nuptial colouration. Thus, the observed responses can not be interpreted as mate choice preference *per se*. However, at the very least these results strongly suggest that females can be expected to exhibit an initial preference for males with more conspicuous nuptial colouration, regardless of its spectral character. Indeed, Blouw and Hagen (1990) found that during the initial stages of courtship, female threespine sticklebacks responded preferentially to males of the newly described white stickleback species (*Gasterosteus*). The white stickleback is a close relative of the threespine stickleback and reproductive males of this species are highly conspicuous by virtue of their iridescent, white dorsal colouration, which threespine stickleback males do not possess. Blouw and Hagen (1990) found that there were no backgrounds that consistently matched the iridescent white colour at the nesting sites they examined, suggesting that white stickleback males are conspicuous in their natural habitat. Female threespine sticklebacks stopped responding to white stickleback males only when they were introduced to the nest which, unlike that of the threespine stickleback, is situated above the substrate and constructed of fine filamentous algae. It appears then that differences in the position and composition of the nest, rather than nuptial colouration, were factors related to the ultimate rejection of white stickleback males as potential mates. In another study by Baube (pers. comm.), it was found that female threespine sticklebacks preferred yellow-throated dummies to red-throated dummies. Using a colour vision model, Baube calculated that yellow throat colouration provided greater contrast against both the dorsal colouration of the dummies and the visual background used in his experiment. These findings (Blouw and Hagen 1990, Baube pers. comm., present study) all indicate that the primary determinants of nuptial colour hue are a function of the necessity to increase the detectability of courting males.

A number of studies on species other than the threespine stickleback also suggest that epigamic signals function primarily to improve the probability that a male will be detected by a female. Evidence for different sensory modalities also exists. For example, a study by Ryan *et al.* (1990) indicates that the mating calls of two populations of cricket frog (*Acris crepitans*) are optimally suited to transmit in structurally different habitats. Ryan *et al.* found that the efficacy of call transmission of tree frogs from an arboreal habitat was greater than that of a population from an open habitat. Open habitats likely confer fewer functional constraints on call dynamics and hence negate the need for improved call transmission in the open habitat population.

In a study by Sigmund (1983) on visual display in an *Anolis* lizard (*Anolis carolinensis*), which adopted a similar approach to that taken here, it was found that contrast of a male's dewlap with background colouration can have a significant influence on female mate choice. Females were shown to exhibit no preference between males with red or green dewlaps, provided that dewlap colour contrasted with the background. Since only red dewlaps occur in the population examined by Sigmund, one may conclude that female choice is based upon the conspicuousness of the nuptial signal and not a result of ritualized preference for a particular hue. As females rarely chose a male with a green dewlap if it did not contrast with the background, it is unlikely that green dewlaps were chosen simply because of their novelty. Sigmund suggested that these results provide evidence that red dewlap colouration evolved to enhance contrast against the green foliage in front of which *Anolis* lizards typically display.

Similarly, Endler (1983) demonstrated that female guppies (*Poecilia reticulata*) tend to favour males which contrast with background colour over those which resemble it. He was also able to show, using a multigenerational study, that selection operates to increase the frequency of males which contrast with the background. Thus, it seems likely that nuptial colouration in the guppy has evolved to enhance the detectability of males, and furthermore that a given photic regime can influence optical signal design.

Although there is evidence to suggest that preference for red nuptial colouration in threespine sticklebacks is genetically based (Bakker 1993), it is apparent that the efficacy of optical signal transmission and detection must play an important role in determining optical signal design. Perhaps, under certain photic conditions, visual contrast over-rides any genetic preference that may exist. Reimchen's (1989) finding of red nuptial colour loss in threespine stickleback populations resident to red wavelength dominated habitats suggests that this may be the case. Indeed, the findings presented here indicate that female preference for red nuptial colouration can be eliminated by its superimposition on a low contrast visual background. However, it remains to be determined whether red nuptial colour is spectrally masked in natural habitats.

It is important to note that although red nuptial colour would provide minimal hue contrast in red-shifted habitats (Reimchen 1989), it would conversely provide high luminance contrast if expressed laterally (rather than ventrally). This is because reflective surfaces that are spectrally similar to the ambient spectra reflect relatively more light than surfaces that are spectrally offset (Lythgoe 1979). Moreover, since the ambient irradiance in dystrophic systems is very low (Chapter 1), laterally expressed red would appear considerably brighter than the background (at least at proximal distances). However, Reimchen found that in red environment populations where red nuptial colour was present, expression was limited to the mid-ventral region of the throat. Upwelling irradiance in dystrophic systems is generally extremely low (Chapter 1), and thus would probably not render red ventral colouration conspicuous.

Significantly, Moodie (1972) found that in one red environment population (Mayer Lake), males lacked carotenoid based nuptial colour, but possessed dull plum colouration on the lateral surface of their opercula. This colouration is due to the fact that during the breeding season the opercula become transparent, revealing the gill arches. This phenomenon has been observed in the Swan and Drizzle Lake populations as well (pers. obs., Reimchen pers. comm.). Thus, in some populations, an alternate strategy for red nuptial expression may have evolved, possibly in response to low carotenoid availability. Indeed, Reimchen (1989) reports that there are several limnological parameters (e.g. low light levels as well as low calcium and sulfur concentrations) in many of the Queen Charlotte lakes that would likely limit carotenoid availability. The plum colouration may provide an effective optical signal since it is located laterally and furthermore, may reflect a greater proportion of the ambient light than carotenoid based pigmentation.

Reimchen argues that the melanic appearance of reproductive males from red environment systems may provide a high contrast optical signal. Specifically, he suggests that melanic nuptial colour would result in reversed countershading (Hailman 1977). However, it should again be noted that ambient light levels in dystrophic systems are extremely low. Hence, melanic nuptial expression may provide poor contrast against the low backwelling irradiance. There are, however, some scenarios where the efficacy of melanic nuptial expression would likely be quite high. Firstly, the nesting sites of some of the Queen Charlotte lakes are in littoral regions where the substrate is highly reflective. Melanic males nesting in microhabitats where ambient light levels are elevated might very well appear quite conspicuous. Secondly, differences in the spatial geometry of courtship in dystrophic systems may influence the conspicuousness of melanic nuptial colour. As far as the author is aware, this possibility has not been addressed. Here it is significant to note that male ninespine sticklebacks (*Pungitius pungitius*) possess melanic nuptial colour and that females view courtship behaviour largely from a position below the male (Morris 1958). A melanic male silhouetted against the downwelling light would certainly provide a highly conspicuous visual stimulus.

In conclusion, it is evident that the behavioural results presented here indicate that signal masking can occur and that signal design is likely largely determined by parameters related to the generation of visual contrast. These findings are consistent with the sensory drive hypothesis (Endler 1992, Ryan and Keddy-Hector 1992). However, it is also evident that the conspicuousness of red nuptial colour in dystrophic habitats must be quantified to determine if signal masking does indeed occur in nature.

CONCLUDING DISCUSSION

The electrophysiology experiments showed that spectral sensitivity of the ON and OFF pathways in the Quamichan Lake fish differed, with the OFF response exhibiting peak sensitivity to medium wavelength light and the ON response exhibiting sensitivity to both medium and long wavelengths. Differential spectral sensitivity of the ON and OFF pathways provides a basis for coding of colour opponency (i.e. colour discrimination). The presence of colour opponent spectral mechanisms in the Quamichan Lake fish (Chapter 2) suggests that colour discrimination between a red throat patch and a spectrally offset visual background is distinctly possible. In the throat colour preference experiments, hue discrimination was constrained by manipulating the visual background such that its spectral characteristics closely matched that of the throat patch (Chapter 3). This manipulation resulted in a decrease in female response frequency to red throat colour. This finding suggests that if an epigamic signal were effectively masked by the ambient spectra, preference for it would decline, leaving the possibility for the evolution of a novel, more conspicuous signal.

Colour discrimination for stickleback resident to dystrophic habitats does not seem likely for two reasons. Firstly, the spectral distribution of light in dystrophic systems is very narrow (Chapter 1). Hence, all visual targets will reflect similar wavelengths, regardless of their intrinsic spectral character. Secondly, the electrophysiological experiments suggest that populations from heavily stained systems (e.g. the Drizzle Lake population) may not be capable of colour discrimination. Since both the ON and OFF pathways of the Drizzle Lake fish were found to exhibit similar spectral sensitivity, it is unlikely that coding of colour opponency between spectral mechanisms can occur. Thus, there is a distinct possibility that stickleback from dystrophic systems possess only luminance sensitivity. Given this possibility, it is evident that only an optical signal which provides luminance contrast would be effective in dystrophic habitats. However, without data on the spectral characteristics of red and black nuptial colour and the spatial geometry of courtship interactions, one cannot establish which hue, red or black, would be more effective in generating luminance contrast. Laterally positioned red nuptial colour would appear quite bright against the low backwelling irradiance characteristic of dystrophic systems, while black nuptial colour may appear conspicuous if males were to establish breeding territories in microhabitats where ambient light levels are elevated (Chapter 3). It is evident that one must determine which nuptial signal is perceived by female threespine sticklebacks to be more conspicuous if we wish to establish whether signal masking occurs in natural habitats.

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CURRICULUM VITAE

Craig George McDonald

Place of Birth: San Joseph, British Columbia, Canada

EDUCATION

M.Sc. in Biology: University of Victoria
B.Sc. in Biology: University of Victoria

RESEARCH EXPERIENCE

September 1991-February 1994: Graduate Researcher. UVic.
May-August 1991: Research Assistant. UVic.

TEACHING EXPERIENCE

September 1991 - April 1994: Teaching Assistant. UVic.

FELLOWSHIPS AND AWARDS

1994 Gerald Fitzgerald Award - Best Student Poster Presentation. 9th Biennial Conference on the Ecological and Evolutionary Ethology of Fishes.
1993 King Platt Memorial Award. UVic.
1993 Graduate Teaching Fellowship. UVic.
1992 King Platt Memorial Award. UVic.
1991 King Platt Memorial Award. UVic.
1991 Graduate Teaching Fellowship. UVic.
1991 NSERC Summer Research Scholarship.

PUBLICATIONS/ABSTRACTS

CG McDonald and CW Hawryshyn (in press) Intraspecific variation of spectral sensitivity in threespine stickleback (*Gasterosteus aculeatus*) from different photic regimes. Journal of Comparative Physiology A.

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Title of Thesis: The influence of the spectral quality of underwater light on visual sensitivity and the perception of optical signals in the threespine stickleback (*Gasterosteus aculeatus*).

Author [redacted]

CRAIG G. MCDONALD

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