THE EVOLUTION OF BODY COLOUR IN THREESPINE STICKLEBACKS
(GASTEROSTEUS ACULEATUS)

by

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ABSTRACT

This thesis addresses questions concerning the evolution of body colour in threespine sticklebacks (Gasterosteus aculeatus). Chapter 2 examines natural selection of colour in sticklebacks by investigating the possible divergence of cryptic colouration between a species pair. I determined that the upper body colour of benthics matched the littoral background (benthics’ habitat) colour more closely than did the upper body colour of limnetics, suggesting that in their own habitat benthics are more cryptically coloured than the limnetic species. Furthermore, I found that benthics exhibited a greater degree of colour plasticity and consistency in this plasticity than limnetics, which is likely an adaptive response to the greater spectral heterogeneity of the littoral zone.

Chapter 3 examines sexual selection of colour in sticklebacks by investigating whether UV is a secondary sexual character on the abdomen of four stickleback populations. Using colour measurements taken from reproductive males and females during the breeding season and individuals from the non-breeding season, I found that UV did not exhibit striking patterns of sexually dimorphism or seasonality on the abdomen, suggesting that UV is not a secondary sexual character on this part of the body in these populations. The Priest benthic population, however, exhibited significant sexual dimorphism and borderline significant seasonality, leaving open the possibility that UV may be a secondary sexual character in this population.
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CO-AUTHORSHIP STATEMENT

Chapters 2 and 3 were the result of a collaboration between my supervisor Dolph Schluter, and myself. Dolph helped me with the experimental design, data analysis and editing the manuscripts, and I collected the data and wrote the manuscripts.
CHAPTER 1: General Introduction

Animal colour patterns have been the focus of natural and sexual selection studies for decades (Dice and Blossom 1937; Endler 1978; Bennett et al. 1994; Ruxton et al. 2004), in large part because they are relatively easy to identify and measure (Endler 1983). In recent years, the accuracy of colour measurement has increased with the use of reflectance spectrometry (Endler 1990; Andersson and Pragge 2006) and high quality digital photography (Stevens et al. 2007; Bergman and Beehner 2008), allowing researchers to rapidly and reliably quantify animal colouration in natural populations. Despite the use of these advanced tools and the accumulated wealth of information on animal colour patterns, numerous questions about the evolution of body colour in natural populations remain unanswered.

Sympatric species pairs of threespine stickleback (Gasterosteus aculeatus) are useful systems for examining how colour patterns evolve in natural populations. Each species pair in southwestern British Columbia evolved independently from the marine stickleback 10,000-12,000 years ago following the retreat of the glaciers (McPhail 1994), and in each lake the benthic and limnetic species differ in terms of their ecology and morphology. The small limnetic species feeds primarily on zooplankton in the pelagic zone and the large benthic species feeds mainly on invertebrates in the littoral zone (Schluter and McPhail 1992).

The benthic and limnetic species also differ in their body colour patterns. During the non-breeding season, limnetics are more heavily melanized than benthics (Miller et al. 2007). For the most part, however, colour patterns outside of the breeding season have not been extensively quantified. As a result, very little is known about how body colour might be
shaped by divergent natural selection in these sticklebacks, and whether the species have evolved different cryptic colouration patterns.

Sexual selection of body colour in these species pairs, on the other hand, has been studied more extensively (e.g. Boughman 2001; Boughman et al. 2005; Albert et al. 2007). During the breeding season, males of both species generally exhibit nuptial colouration in the form of a red throat, blue iris, and a blue or blue-green body. Limnetic males typically have more intense red colouration than benthic males, which is displayed over a larger area (Boughman 2001), and limnetic females display stronger preferences for red males than benthic females do (Boughman 2001). Despite this wealth of knowledge, important questions regarding the sexual signals of sticklebacks remain unanswered. For instance, sexual selection of ultraviolet (UV) colouration has only recently been considered in these fish (Rick et al. 2004; Rowe et al. 2004) and as such, the role of UV wavelengths (300-400nm) in the sexual communication of sticklebacks remains largely a mystery.

This thesis addresses questions concerning the evolution of body colour in threespine sticklebacks. The second chapter examines natural selection of body colour in sticklebacks by investigating the possible divergence of cryptic colouration between a species pair. Pigmentation differences have been characterized between the benthic and limnetic species in two species pair lakes (Paxton and Priest) and a major gene responsible for these differences has been identified (Miller et al. 2007), but the adaptive significance of this colouration pattern has yet to be determined. In Chapter 2, I present an experiment utilizing digital photography designed to answer some fundamental questions regarding the evolution of background matching and colour plasticity in the Paxton Lake stickleback species pair. Namely, (1) does each species match its own habitat background colour better than the other
species, and (2) does one species exhibit greater colour plasticity than the other, and if so, is it adaptive?

The third chapter of this thesis addresses sexual selection of body colour in sticklebacks by examining whether UV is a secondary sexual character in two stickleback species pairs. Recently, male UV colouration has been linked to female preference in a number of stickleback populations (Boulcott et al. 2005; Rick et al. 2006; Rick and Bakker 2008a) and there is evidence that UV reflectance is intense on the abdomen region of sticklebacks (Rick et al. 2004; McLennan 2007; Rick and Bakker 2008b), but it is still unknown whether UV is a secondary sexual character among breeding males on this part of the body. Using red on the throat as a gauge for how a sexually selected colour should behave in these populations, I expected UV to exhibit (1) sexual dimorphism, and (2) seasonality on the abdomen. In addition, testing these predictions in species pair populations allowed me to investigate whether sexual selection of UV colouration may differ between closely related stickleback species.
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CHAPTER 2: Adaptive Colour Plasticity and Background Matching in a Threespine Stickleback (Gasterosteus aculeatus) Species Pair

INTRODUCTION

Animal pigmentation is rapidly becoming a key study system for examining the genetics of adaptation (Hoekstra 2006; Mundy 2007; Steiner et al. 2007). The growing wealth of knowledge of the genes involved in pigment production and motility, in combination with studies that quantify colour variation in natural populations and test for the selective advantages of such variation, allow researchers to explore the genetic basis of fitness-related traits (Mundy 2007). Despite these recent advances, however, the adaptive significance of many of the genes underlying pigmentation is not known.

Threespine sticklebacks (Gasterosteus aculeatus) are an ideal system for investigating selection on colour patterns. Populations of sticklebacks in the wild generally vary in pigmentation and one major gene responsible for this variation has been identified. Miller et al. (2007) found that in two sympatric species pairs of stickleback inhabiting small lakes (Paxton and Priest) in southwestern British Columbia, the benthic species have reduced gill and ventral skin pigmentation relative to limnetics, which are darker like the ancestral marines. The difference is caused by a divergent regulatory allele of the Kit ligand (Kitlg) gene (Miller et al. 2007). Specifically, cis-acting regulatory changes at the Kitlg locus cause reduced gene expression of Kitlg in benthics, resulting in reduced pigmentation in the gills and ventral skin (Miller et al. 2007). Although not directly mapped, the gene probably affects pigmentation over much of the body surface. For example, in addition to the gills and

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1 A version of this chapter will be submitted for publication. Clarke, J.M. and Schluter, D. Adaptive colour plasticity and background matching in a threespine stickleback (Gasterosteus aculeatus) species pair.
ventral skin, the benthic allele of Kitl is down-regulated in the dorsal skin of the tail region (Miller et al., unpublished data), and there is evidence from zebrafish (Danio rerio) that adult Kit mutants exhibit a lightening of dorsal body pigmentation (Parichy 2006), suggesting that Kitl is likely responsible for dorsal skin lightening in addition to ventral lightening. Despite the recent genetic findings, the adaptive significance of these pigmentation patterns is still unknown in threespine sticklebacks and warrants further attention.

Based on the ecological theory of adaptive radiation, phenotypic differentiation is mainly due to divergent natural selection between environments, which may stem from differences in habitat characteristics (Schluter 2000). One possible driver of phenotypic pigmentation differentiation is habitat-specific background colour. In habitats with high levels of predation, cryptic body colouration in the form of background matching is common among prey species and has been extensively studied for many years (Endler 1978; Ruxton et al. 2004). Background matching can be described as a prey’s visual resemblance to the background (in colour, brightness and pattern) as a means to decrease the risk of detection by predators (Endler 1978). There are numerous examples of habitat-specific body colour variation found in natural populations across a wide variety of taxa, including turtles (McGaugh 2008), freshwater fish (Whiteley et al. 2009), frogs (Wente and Phillips 2003), salamanders (Storfer et al. 1999), snails (Reed and Janzen 1999), isopods (Hargeby et al. 2004), lizards (Macedonia 2001; Stuart-Fox et al. 2004; Rosenblum 2006), and mice (Dice and Blossom 1937), among others. The authors of these studies have argued that the habitat-specific variation in prey colour is primarily due to selection by visual predators, resulting in adaptive background matching (Hargeby et al. 2004).
In this study, we tested the hypothesis that the observed pigmentation differences between the benthic and limnetic species of stickleback are the result of habitat-specific background matching. Each stickleback species pair in southwestern British Columbia evolved independently from the marine stickleback 10,000-12,000 years ago following the retreat of the glaciers (McPhail 1994), and in each lake the benthic and limnetic species differ in terms of their ecology and morphology. The small, limnetic species feeds primarily on zooplankton in the pelagic (open-water) zone, and the large, benthic species feeds mainly on invertebrates in the littoral zone (Schluter and McPhail 1992). In addition, limnetics and benthics have differential predation regimes: limnetics are likely preyed upon primarily by diving birds and predatory fish (Reimchen 1994), and benthics by large invertebrate predators (Reimchen 1980; Reimchen 1994). Colour differences also exist between the two habitats (littoral and pelagic zone) in a given lake (Boughman 2001). Based on background irradiance measurements in Paxton Lake, it is evident that the benthic species inhabits a light environment that is more variable and of a different colour than the limnetic species’ habitat (Fig. 2.1). We investigated how the Paxton benthic species has adapted to this novel habitat by examining whether its upper body colour is better matched to an extreme sample of its own habitat colour than the limnetic species.

In addition to general pigmentation differences, sticklebacks also have some capacity to lighten or darken their upper body colour in relation to their background (Hogben and Landgrebe 1940; Huntingford and Coyle 2007; J. M. Clarke, pers. obs.), but this has not been formally tested in the species pairs. Although colour change has been recognized in many taxa, this behaviour in fish has been studied for over 150 years (Sugimoto 2002) and the physiological details of these responses still garners much attention by scientists today (Fujii
2000), making them ideal organisms for background matching studies. Pigment cells have a number of different functions in animal skin, and one of the most important functions for skin chromatophores (pigment cells) in teleost fish is crypsis (Sugimoto et al. 2005). Furthermore, it is recognized that melanosome (melanin granule) motility in the melanophore layer of the skin, in particular, appears to be a common component of rapid (physiological) cryptic colour change in fish (Fujii 2000). On a black background, dispersion of these melanosomes causes a rapid darkening of the skin, while on a light background aggregation causes lightening of the skin (Bagnara and Hadley 1973). Based on this information, it is worth testing whether natural populations of stickleback have evolved the capacity to change colour over a short period of time to adjust to their current background. Depending on the number of different backgrounds a prey is seen against, the best strategy for colouration may not be one that provides crypsis against one background, but one that provides crypsis against a variety of backgrounds (Ruxton et al. 2004). With colour change, an individual has the ability to respond to a spectrally heterogeneous environment by expressing a phenotype that is optimally cryptic against a suite of background colours (Wente and Phillips 2003). We investigated whether the more variable colour background of the littoral habitat has favored greater colour plasticity in the benthic species relative to the limnetic species.

In this study, we tested using digital photography whether the upper body colour (from a dorsal perspective) of each species from Paxton Lake is better matched to a sample of its own habitat colour than the other species, in terms of RGB colour score (within the human-visible spectrum). In addition, colour change was examined to determine if any observed plasticity is indeed an adaptive (cryptic) response. In combination with background irradiance measurements from Paxton Lake, these results allowed us to assess whether the
observed pigmentation differences between the species are due to habitat-specific background matching.

Figure 2.1. The distribution of sidewelling $\lambda P_{50}$ values from the pelagic and littoral zone in Paxton Lake measured at 10cm depth. The $\lambda P_{50}$ value is the wavelength that halves the area under the irradiance spectrum and is an index of the dominant water colour. The range of $\lambda P_{50}$ values along the y-axis align with the wavelengths depicted on the colour bar to the far left.

MATERIALS AND METHODS

Measurement of Lake Water Colour

The background colour of the water was measured in Paxton Lake in both the littoral zone and the pelagic zone to quantify colour differences between these two habitats. In June 2008, the sidewelling light was measured (as relative irradiance) in three areas of the lake: open water (pelagic zone), 1m depth (littoral zone) and 0.3m depth (littoral zone). The irradiance spectrum sums the radiances from all light sources (Endler 1990), so underwater sidewelling irradiance provides a measure of the background colour of the habitat that the
fish is seen against (Cummings 2004). Upwelling and downwelling light were also measured at this time and exhibited similar trends as sideward light, however sideward light was primarily used in this study because it incorporates components of the upwelling and downwelling light fields as well as any vegetation colour, in addition to its primary measure of sideward light (Cummings 2004), making it suitable for a general measure of overall background irradiance. Forty-three sampling locations were chosen randomly across the lake and comprised twenty open water locations, twenty 0.1m depth locations and three 0.3m depth locations.

At each of these 43 sampling locations, sideward irradiance was measured at the following depths (in order of measurement): 10cm, 30cm, 50cm, 1m and 2m. For sampling locations less than 2m deep (all littoral zone samples), irradiance was measured at as many of these depths as possible. Irradiance was measured using an Ocean Optics USB2000 spectrometer and a 200µm UV/VIS fiber-optic cable attached to a CC-3-UV cosine corrector (acting as an irradiance probe which collected light from a 180° field of view), all of which were connected to a laptop computer running the spectrum-analyzing software OOIBase32 by Ocean Optics. The end of the cable was modified to a 90° angle so that the cosine corrector was oriented parallel to the surface. The weighted probe was lowered vertically into the water at each sampling location and the irradiances were recorded at the predetermined depths using OOIBase32. All of these measurements were taken on the same day within a 4-hour period. Notes were taken on the amount and type(s) of vegetation found at each sampling location, as well as the time of sampling and the current weather conditions.

The $\lambda_{P_{50}}$ value of each sideward irradiance spectrum recorded by OOIBase32 was then calculated. $\lambda_{P_{50}}$ is the wavelength that divides the area under the irradiance curve in...
half and is considered to be the dominant wavelength of the sidewelling spectrum (Boughman 2001; Albert et al. 2007). These values were used to determine if the background colour differed significantly between habitat zones in Paxton Lake at similar depths and to examine the degree of background colour variation in each habitat zone.

**Background Matching Experiment**

To determine whether Paxton Lake sticklebacks match their respective habitat colours and have the ability to rapidly change colour based on their current background, an experiment was performed during the non-breeding season that involved placing individual fish against extremes of a “littoral” colour background and a “pelagic” colour background in an alternating fashion. Using digital photography, each fish was photographed from above against one of the backgrounds after a predetermined acclimation period, and then transferred to a different coloured background (if they were against a “littoral” background first they were transferred to a “pelagic” background, and vice versa). After an identical acclimation period against this new background, digital photographs were taken of the fish once again. Using a pixel sampling technique that measured the RGB (Red, Green, Blue) colour scores of the upper body region of each fish (from a dorsal perspective) and its background, we were able to examine the degree of human-visible background matching in the benthic and limnetic sticklebacks and whether they could rapidly change colour when placed against a new background.

The use of digital photography for measuring animal colour has become increasingly popular in recent years (Gerald et al. 2001; Stevens et al. 2007; Bergman and Beehner 2008). Although spectrophotometry is considered to be the most reliable and objective method for measuring colour and can measure wavelengths beyond the human-visible spectrum (e.g.
UV), it is not ideal for all types of animal colour measurements (Gerald et al. 2001; Bergman and Beehner 2008). Spectrophotometry is limited to small, localized points of measurement, which do not adequately capture heterogeneity across the colour patch (Stevens et al. 2007). In addition, this method typically requires handling the animal in some fashion because the probe needs to be extremely close to or touching the subject (Stevens et al. 2007). Not surprisingly, this can disrupt the natural behaviour of the animal being measured (Stevens et al. 2007). As a result, digital photography was used in this study because it allowed us to sample colour (via pixels) from a much larger body region at a single moment in time without interfering with the animal’s behaviour. Despite the restriction to human-visible wavelengths, this noninvasive method proved ideal for studying such subtle colour differences in small fish.

*Selection of Representative Habitat Colours*

In order to experimentally test habitat background matching, representative colours from the littoral habitat and pelagic habitat needed to be selected. This was done by selecting pixels from photographs taken of each habitat (or habitat-specific vegetation) from Paxton Lake. To represent the pelagic zone, a photograph was taken of the surface water in the middle of the lake; to represent the littoral zone, chara (*Chara* spp.) was extracted from the experimental ponds at the University of British Columbia and photographed outdoors in an empty aquarium (Fig. A1.1). Chara is an abundant and dominant form of vegetation in the littoral zone of Paxton Lake and thus accurately represents the colours found in this habitat. Both of these photos were taken using a Nikon D1H digital SLR camera set to “Daylight” white balance and saved as uncompressed Tagged Image File Format (TIFF) files, as
recommended by Stevens et al. (2007). The pelagic zone photo was cropped to 362 x 888 pixels while the littoral zone photo remained uncropped at 1312 x 2000 pixels.

These photos were imported into Adobe Photoshop CS3 Extended where they were converted to grayscale, with each pixel in the photo being represented by a grayscale value between 0 and 255 (0 = black, 255 = white). The photos were then converted to a text file so that each pixel was replaced by its grayscale value in numerical form. A frequency distribution of the grayscale values for each of the photos was then created, providing a graphical representation of the pixel brightness distribution in the photograph.

In choosing the representative colour from each photograph, two primary criteria formed the basis for this selection process: 1) the colour should be of a brightness value that is relatively common in the photograph, and 2) the colours from the two habitats should be highly contrasted from each other with respect to brightness. Selecting “extremes” (in terms of brightness) from the range of colours in each habitat was done in an attempt to improve the chances of detecting subtle colour changes by the fish as they were introduced into the different backgrounds. As a result, “background matching” in our study refers to an individual’s ability to match an extreme sample of the habitat colour. Based on these criteria, 20 random pixels were chosen from the 5-10% of the brightest pixels in the littoral photo, and 20 random pixels were chosen from the 5-10% of the darkest pixels in the pelagic photo. The brightest and darkest 0-5% of the pixels were avoided to prevent the inclusion of pixels resembling white or black, since these colours do not accurately represent the colours of the habitats. Following a visual inspection of the 20 littoral photo pixels that ensured the exclusion of such pixels, one was randomly chosen as the representative littoral zone colour (RGB = 168, 179, 74). Similarly, after visual inspection of the 20 random pixels taken from
the pelagic photo, one pixel was randomly chosen as the representative pelagic zone colour (RGB = 25, 35, 44). All 20 of the littoral pixels were comparable in colour, as were all 20 of the pelagic pixels. The representative pixel colours qualitatively correspond to the actual background irradiance colours of the Paxton Lake habitats (Fig. 2.1): a green-yellow for the littoral zone and a blue for the pelagic zone.

These two colours were then printed out on paper using a high-quality laser jet printer. Based on these colour samples, paint was created that faithfully recreated these habitat colours. The paint used in this study was a water-borne, 100% acrylic, interior-exterior “EcoLogic” brand (#70654) paint with eggshell finish from Cloverdale Paint and was not harmful to the fish.

Fish Collection

Juvenile benthic and limnetic sticklebacks (less than 1 year old) sampled from the experimental ponds facility at the University of British Columbia were used for this experiment. Juveniles were used in this study so that colour would not be confounded by sexual display. These fish were born in the experimental ponds and were the offspring of parents taken directly from Paxton Lake, Texada Island, British Columbia in the spring of 2008 (the “benthic” and “limnetic” ponds were both stocked with 20 adult sticklebacks during this time). Each pond is 25x15m² and contains a shallow (littoral) zone at one end, and a 6m deep (pelagic) zone at the other end. The shallow littoral zone contains chara vegetation and a layer of sand and limestone gravel extracted from surface mines near Paxton Lake. In August 2008, juvenile fish were sampled from both the “benthic” pond and the “limnetic” pond.
As fish were collected from the ponds, they were separated into labeled white and yellow buckets based on source pond and collection depth. Bucket colour was random with respect to species and depth. The fish were processed immediately after collection. To minimize overheating and exposure to direct sunlight, the fish were placed in as much shade as possible following the collection and throughout the experimental procedure. A black hand-held umbrella provided additional shade when necessary.

*Experimental Set-Up and Procedure*

Forty-eight transparent plastic cups (16 oz.) were cut to a height of 5cm and painted on the inside (to minimize the number of barriers between the fish and the background colour) using either the littoral paint or the pelagic paint described above. An array of 24 painted cups (6 rows of 4) was placed in equally sized circular holes within a cardboard frame with the cups arranged in an alternating littoral/pelagic pattern such that 12 littoral and 12 pelagic cups were used (Fig. A1.1). In the second frame, 12 littoral cups and 12 pelagic cups were placed in a similar alternating pattern as the first frame, but in opposite positions (such that wherever a pelagic cup was placed in the first frame, a littoral cup was placed in that position in the second frame, and vice versa). A GretagMacbeth Mini ColorChecker chart (Manufactured by Munsell Colour) was positioned beside the plastic cup in each shot and thus appeared in every photograph taken (Fig. A1.2). The ColorChecker chart is commonly used in photography and video and consists of an array of 24 coloured squares (including a 6-step grayscale), and served as a technical reference for both grayscale and colour. Using this chart, all of the photographs could be faithfully standardized later on (Bergman and Beehner 2008). In addition to the ColorChecker chart, a white sticker identifying each fish was placed in each shot.
Each fish was then placed in a cup with a small amount of water from the source buckets (approximately 2cm deep). This was done in a staggered fashion such that every fish was in a cup for 15 minutes prior to being photographed. During this acclimation period, human disturbances were kept to a minimum. Fifteen minutes was chosen as an acclimation time because real-time photography (every 20 seconds) over a 20-minute test period revealed that this was an adequate length of time to observe the physiological colour changes in these fish (see Appendix 1 for a detailed description of this time course experiment and results).

Two photos of each fish were taken using manual focus. Manual focus proved to be faster and more reliable than automatic focus and is considered by Stevens et al. (2007) to be the best choice for animal colouration studies that utilize digital photography. Photographs were taken in the shade under natural light conditions using the Nikon D1H digital SLR camera set to “Shadow” white balance and were saved as uncompressed TIFF files. After the first photo was taken, the lens was unfocused and refocused on the same fish for the second photo. This ensured that identical photos of the same fish were not taken and increased the chances of having usable photos for sampling. Each photograph captured the stickleback in the cup, the ColorChecker chart and the identification tag (Fig. A1.2).

Immediately after being photographed, each fish was transferred to a cup of the opposite colour in Frame 2. As in Frame 1, each fish was given 15 minutes to acclimate to its new background before being photographed, as described above. In total, 96 fish were used in this experiment (48 benthics and 48 limnetics), with 4 photos taken of each fish (2 against the littoral background and 2 against the pelagic background).

In order to examine whether coloured reflections from the sides of the painted cups had any effect on the observed upper body colour of the fish, we placed a single piece of
white chalk (acting as a white standard) under water in each of the cups and photographed it in the same manner as the fish.

*Sampling of Pixels*

One photograph was selected for each fish on each background (littoral and pelagic) and used for subsequent pixel sampling. Selection was based on the following criteria: 1) the fish was not obscured by any shadows in the cup, 2) the fish was not obscured by glare on the water surface, and 3) the fish was in focus.

After the best quality photographs were standardized for colour and brightness (see Appendix 1 for a more detailed description of this procedure), a circular sample of pixels along the dorsum of each fish was selected using the circular marquee tool provided by Adobe Photoshop CS3 Extended. This circle was positioned over the dorsal region of the fish directly between the pectoral fins. The size and position of the circle was determined by the width of the fish between the pectoral fins such that the edges of the circle reached the edges of the fish (at the point where the pectoral fins meet the body). A second circle of pixels having an area equal to 75% that of the first circle was then centered within the first circle, extracted, and saved to a file. This reduced circle ensured that reflections on the extreme outer edges of the fish were not sampled. The number of pixels sampled on a fish ranged from 421 to 2828, depending on the size of the fish and magnification of the photo. A circle of pixels of equivalent reduced area was then used to sample the background colour of each fish’s cup. The circle was positioned over an area of uniform colour of the cup as close to the fish’s body as possible. This sample of pixels was also saved in a file. The average R, G and B colour scores of both the dorsal sample and the background sample were measured using the ‘Eyedropper’ tool with the ‘101 by 101 average’ setting in Photoshop. Pixels were
sampled from the white chalk standard in the same fashion to investigate any possible cup reflection effects on the upper body colour of the fish.

Data Analysis

Colour analysis in this study focused primarily on the R:B and G:B ratios. The difference in the controlled R:G ratio between backgrounds (i.e. the paint colours) was the smallest of all three ratios used in this study, making it the least informative of the three for measuring background matching and plasticity in the fish. Analysis focused on RGB ratios rather than the absolute colours (R, G, B) because the value in each colour channel is only informative relative to the values in the other channels (Bergman and Beahner 2008).

Background matching was measured by taking the difference between the dorsal colour sample and the local background sample, which corrected for any slight variation in illumination between images. The degree of colour change (plasticity) was measured by calculating the difference in dorsal colour between the two different backgrounds (littoral minus pelagic). In addition to plasticity, the consistency of colour change for each species was also measured. Consistency (an intraclass correlation coefficient) provides an estimate of the reproducibility of “pre” and “post” variation in measurement (Shrout and Fleiss 1979), or in our case, the similarity of upper body colour change among individuals of the same species.

Based on the pixel samples from the white chalk photos, a small effect of cup reflection on dorsal colouration was found. Between backgrounds, the R:B and G:B ratios of the white chalk exhibited a mean change of 0.15 and 0.09, respectively. In comparison, benthics exhibited a mean change of 0.96 and 0.55 in these ratios between backgrounds, while limnetics exhibited a mean change of 0.55 and 0.33. Rather than correct for this effect,
we focused solely on relative comparisons between benthics and limnetics in this study, as both species experienced the identical effect between backgrounds. All statistical analyses were performed in R version 2.6.0 (R Development Core Team 2007).

RESULTS

Lake Water Colour

In general, the dominant wavelength of the pelagic zone was blue, while the littoral zone exhibited a dominant wavelength that was more greenish-yellow (Fig. 2.1). At a lake depth of 10cm, the dominant wavelength of sidewelling light was shorter (more blue) in the pelagic zone (mean $\lambda_{P_{50}} \pm SE = 498.7 \pm 2.24$) than in the littoral zone (529.9 $\pm 4.65$) ($t_{41} = 5.77, P < 0.00001$; Fig. 2.1). Additionally, the littoral zone had more variance in $\lambda_{P_{50}}$ values than the pelagic zone at 10cm depth ($F_{19,22} = 0.203, P = 0.0009$), revealing that the littoral zone is more spectrally heterogeneous than the pelagic zone in Paxton Lake. These trends remained the same at additional depths. Even though the $\lambda_{P_{50}}$ values of both habitats increased with depth due to an expected redshift in wavelength (Boughman 2001; Albert et al. 2007), the means remained significantly different at each measured depth, as did the variance. This strongly suggests that the littoral zone and pelagic zone have different background colours, with the littoral zone exhibiting more variation in colour than the pelagic zone, regardless of depth (up to 50cm).

Background Matching

Benthics and limnetics matched the colour of the pelagic background equally well (Table 2.1). However, benthics matched the littoral background better than did the limnetics (Table 2.1). Benthics and limnetics did not differ significantly in their deviation from the
pelagic background in either the R:B or G:B ratio (Table 2.1). Limnetics deviated significantly more than benthics from the littoral background colour R:B and G:B ratios (Table 2.1).

Table 2.1. T-test results comparing the species upper body colour deviations from each background (pelagic and littoral; degrees of freedom = 62 and 86, respectively). Bold P-values indicate significance.

<table>
<thead>
<tr>
<th></th>
<th>Deviation from Pelagic Background (Benthic vs Limnetic)</th>
<th>Deviation from Littoral Background (Benthic vs Limnetic)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ben. mean</td>
<td>Lim. mean</td>
</tr>
<tr>
<td>R:B</td>
<td>0.530</td>
<td>0.526</td>
</tr>
<tr>
<td>G:B</td>
<td>0.309</td>
<td>0.327</td>
</tr>
</tbody>
</table>

**Colour Plasticity and Consistency**

Even though both species have the ability to change colour, benthics exhibited significantly greater plasticity than the limnetics in the R:B and G:B ratios (R:B: \(t_{58} = 5.86, P < 0.00001\); G:B: \(t_{58} = 4.81, P = 0.00001\)) (Fig. 2.2; Table 2.2). The degree of plasticity was measured as the difference in upper body colour between the littoral background and the pelagic background. All 30 benthics used in this analysis exhibited a positive plasticity value for the R:B and G:B ratios (Fig. 2.2), adjusting their upper body colours from a green-blue in the pelagic background to a green-yellow in the littoral background (or in the opposite direction, depending on which background they were introduced to first), while 28 of 30 limnetics displayed positive plasticity values for the same ratios (Fig. 2.2).

Benthics also exhibited greater consistency in this colour change than limnetics for both colour ratios considered (Table 2.2). Consistency is an intraclass correlation coefficient
that measures the similarity of upper body colour change among individuals of the same species.

Figure 2.2. Change in upper body colour between the pelagic and littoral background. Individuals are depicted by a line connecting their colour in the pelagic background to their colour in the littoral background (when possible) in the R:B (A & B) and G:B (C & D) ratios. Benthics are represented in the left panel (A & C) and limnetics in the right panel (B & D). The mean and 95% confidence interval for the littoral background ratios are illustrated by a dashed line and pale green band, respectively. Similarly, the mean and 95% confidence interval for the pelagic background ratios are represented by a dashed line and pale blue band, respectively.
Table 2.2. Plasticity and consistency values for benthics and limnetics. Plasticity is the mean difference in the colour ratio between backgrounds. Consistency is the intraclass correlation coefficient for the change in colour ratio between backgrounds and measures the similarity of upper body colour change among individuals of the same species.

<table>
<thead>
<tr>
<th></th>
<th>Plasticity (mean difference between backgrounds ± SE)</th>
<th>Consistency (intraclass correlation coefficient)</th>
<th>Benthics (n = 30)</th>
<th>Limnetics (n = 30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R:B</td>
<td>0.96 ± 0.04</td>
<td>R:B</td>
<td>0.65</td>
<td>0.28</td>
</tr>
<tr>
<td>G:B</td>
<td>0.55 ± 0.03</td>
<td>G:B</td>
<td>0.50</td>
<td>0.25</td>
</tr>
</tbody>
</table>

DISCUSSION

In this study we examined whether upper body colouration has diverged between the benthic and limnetic species of stickleback in association with different background colours in their habitats, and whether colour plasticity plays a role in this cryptic colouration pattern. Given that a major gene underlying pigmentation differences between benthics and limnetics has been identified, investigating the adaptive significance of these colour patterns could provide valuable clues to the genetics of adaptation in this species pair. The results revealed that the upper body colour of benthics matched the littoral background colour more closely than did the upper body colour of limnetics, suggesting that in their own habitat (littoral zone), benthics are more cryptically coloured than the limnetic species. There was no difference between species, however, in the resemblance of their upper body colouration to the pelagic background (limnetics’ habitat). This is because benthics exhibited greater colour plasticity than the limnetic species, which enabled them to resemble both backgrounds. Benthics also displayed greater consistency than limnetics in their colour changes. These
results suggest that the greater degree of colour plasticity observed in the benthics is likely an adaptive response to the greater spectral heterogeneity of the littoral zone. Altogether, the results of this study indicate that the upper body colouration and colour plasticity observed in the benthic and limnetic species are likely adaptive (cryptic) responses to the spectral qualities of the littoral and pelagic habitats, respectively.

In the littoral background, benthics exhibited a more green-yellow upper body colour than did the limnetics (Fig. 2.2), suggesting that benthics have adapted to the colour of the littoral zone by chromatic background matching. A stickleback possessing body colouration that contrasts sharply with the colour of its habitat background will presumably have lower fitness (due to predation by visual predators) than a stickleback that is chromatically matched to its background (Endler 1978). As a result, the green-yellow background irradiance of the littoral zone likely favored background matching in the benthic species. Cryptic colouration in the form of background matching has been documented in other systems (Hoekstra 2006; Rosenblum 2006; Whiteley et al. 2009), but to our knowledge this is the first evidence of background matching in a stickleback species pair.

The results of this study also suggest that the greater degree of colour change observed in the benthics evolved as an adaptive response to the greater spectral heterogeneity of the littoral zone (Fig. 2.1). Environmental heterogeneity is believed to be what selects for adaptive plasticity (Buskirk 2002; Doughty and Reznick 2004; Schlichting 2004), so our result is the first key step towards demonstrating this strategy as an adaptation (Doughty and Reznick 2004; Berrigan and Scheiner 2004). Doughty and Reznick (2004) outline six indirect lines of evidence to support the hypothesis that plasticity is an adaptation: (1) the production of different phenotypes in different developmental environments, (2) environmental
heterogeneity, (3) reversal of fitness of alternative phenotypes in different environments, (4) cue reliability, (5) genetic variation for plasticity that evolves in response to selection, and (6) comparative evidence that plasticity is correlated with environmental heterogeneity. Stickleback meet three of these criteria directly (1, 2 and 6) and two indirectly (4 and 5).

Benthics produced alternative phenotypes in different environments (Fig. 2.2), fulfilling criterion 1. Specifically, their upper body colouration changed to become more littoral-like (in terms of RGB scores) when placed in the littoral background, and became more pelagic-like when placed in the pelagic background. This was less clear in the limnetics, which matched the pelagic background colour comparably to the benthics but displayed relatively inconsistent changes in their colouration (Table 2.2), suggesting that a more fixed colour strategy may be favoured in the pelagic zone, which is an expected response in a spectrally homogenous habitat (Wente and Phillips 2003).

Secondly, the sidewelling irradiance measurements from Paxton Lake provide strong evidence that the littoral zone is spatially heterogeneous in background colour compared to the pelagic zone (Fig. 2.1), fulfilling criterion 2. Demonstrating environmental heterogeneity (either spatial or temporal) in natural habitats is considered an important criterion for determining whether plasticity is adaptive because experiments that attempt to represent heterogeneity might elicit a plastic response even if they do not adequately re-create the heterogeneity encountered in the wild (Doughty and Reznick 2004).

The reversal of fitness of alternative phenotypes in different environments (criterion 3) was not formally tested in this study. Such examinations will provide the necessary test of this particular criterion, as will studies that thoroughly investigate any costs of colour change.
According to the fourth criterion put forth by Doughty and Reznick (2004), reliable cues that predict the future selective environment are necessary for adaptive plasticity to evolve. Similarly, the rate of the plastic response must also correspond to the time frame that the organism experiences the environmental heterogeneity in order to enhance fitness (Doughty and Reznick 2004). In fish, the cue for rapid colour change is light information collected by the eye from the environment, which is then sent to the central nervous system where it is processed and the appropriate hormones that trigger colour change are released by the endocrine system (Fujii 2000; Sugimoto 2002). The most rapid colour changes may even result from a direct connection between the nervous system and chromatophores, bypassing the endocrine system altogether (Fujii 2000). Benthics in our study likely used light information from the background as a cue to change colour (and predict the future selective environment), since the only feature that differed between the littoral and pelagic treatment was the background colour of the cups. In addition, based on Figures A1.3 & A1.4, benthics exhibited the most rapid colour change within approximately 2.5 minutes of being introduced into a new background, but it is unknown whether this corresponds to the rate at which they encounter background colour variation in their habitat.

Limnetics, on the other hand, encounter much less colour variation in their habitat (Fig. 2.1), and as a result, their fitness is likely not enhanced (relative to benthics) by plasticity in upper body colouration. In support of this, limnetics in our study exhibited inconsistent colour change relative to benthics, particularly in the littoral background (Figs. A1.3 & A1.4), suggesting that their use of background colour as a cue for colour change is not as accurate as that of benthics. The fact that limnetics could still rapidly change their
upper body colouration (albeit in an inconsistent fashion), however, leaves open the possibility that they rely on other cues in their environment not tested in this study.

Documenting the presence of genetic variation in plastic traits is the fifth criterion for establishing that a plastic response is adaptive (Doughty and Reznick 2004). This was not directly examined in our study. However, as described by Doughty and Reznick (2004), differences in plasticity among species are evidence for genetic variation in plasticity, indirectly fulfilling criterion 5. For instance, a correlation between phenotypic plasticity and ecological variation among species suggests that genetic variation for plasticity was “shaped” by selection in each species’ habitat (Doughty and Reznick 2004). Additional studies that quantify genetic variance for this plastic trait will need to be performed in order to confirm this. The sixth and final line of evidence is that plastic phenotype expression across species is correlated with environmental heterogeneity (Doughty and Reznick 2004), which is supported by our findings. Specifically, benthics exhibited greater phenotypic plasticity of upper body colouration than the limnetics and utilize a habitat that is more spectrally heterogeneous than that of the limnetics.

Animal colour change may represent a trade-off between camouflage, communication and thermoregulation (Stuart-Fox and Moussalli 2009). In our study, it is unlikely that the colour plasticity observed was directly related to sexual signaling as we sampled reproductively immature individuals in the non-breeding season and measured their colour change in complete social isolation. In addition, thermoregulation likely did not play a role in this observed plasticity because benthics exhibited rapid colour change towards the background colour when introduced into a new cup, even though they never left the source water (Figs. A1.3 & A1.4). Presumably during this brief transfer the temperature of the
water did not change so dramatically to elicit such a rapid thermoregulatory response in colour. As a result, our findings suggest that colour plasticity in these sticklebacks evolved primarily due to crypsis and are consistent with the general hypothesis that if adaptive phenotypic plasticity evolves due to natural selection arising from environmental variation, then species that experience a relatively more variable habitat should exhibit greater levels of plasticity (Day et al. 1994; Buskirk 2002). As described by Price et al. (2003), exploitation of new habitats may be associated with a loss of plasticity and the evolution of specialization when there is no selection to maintain the plastic response, leading to phenotypic differentiation between populations. Accordingly, it is possible that the differential spectral characteristics of the Paxton Lake habitats may be driving the selection for a generalist (plastic) and a specialist (fixed) strategy in terms of cryptic colouration.

Considering that a patch of colour on a fish is a complex, multicomponent trait (Grether et al. 2004), understanding the physiological underpinnings of the observed colour differences between the limnetic and benthic species is not a simple task. The basic ‘dermal chromatophore unit’ of fishes (as well as reptiles and amphibians) consists of three adjacent cell layers of chromatophores: melanophores at the basal surface (which contain melanins), followed by iridophores (which contain crystalline platelets) and xanthophores in the outermost layer (which contain carotenoids and pteridines) (Grether et al. 2004). Since changes in any one component can drastically alter the colour produced, different morphs or species may exhibit variation in the components of the chromatophore unit they possess (Grether et al. 2004). Adding to this complexity is the fact that dispersion and aggregation of pigment-containing organelles or reflective platelets in all three layers (not just the melanophore layer) are responsible for colour changes, and thus also play a role in the overall
colour of the fish. Colour change may be coordinated by altering all three chromatophore layers independently, as with cryptic colouration in tree frogs (Nielsen and Dyck 1978), but this has yet to be studied in much detail in fish. A detailed physiological investigation of stickleback chromatophore units in combination with a genetic mapping study would help disentangle how these colour responses evolved and diverged in this species pair.

In summary, the results of this study suggest that the observed upper body colour differences between the benthic and limnetic species are due to background matching. Selection studies that directly test the adaptive significance of upper body colouration in this species pair using live predators will provide greater insight into this result, as will studies that examine the parallel evolution of cryptic colouration in the Priest Lake species pair. In addition, a genetic mapping study is required to test whether the Kit ligand gene is directly involved in the observed benthic-limnetic difference in upper body colouration. The results of this study also suggest that the greater degree of colour plasticity observed in the benthic species likely evolved as an adaptive response to the relatively greater spectral heterogeneity of the littoral zone. Overall, this research highlights how divergent natural selection arising from habitat-specific spectral characteristics can shape cryptic colouration differences between species.
REFERENCES


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CHAPTER 3: Does UV Exhibit Patterns of a Secondary Sexual Character on the Abdomen of Threespine Sticklebacks (*Gasterosteus aculeatus*)?

INTRODUCTION

Fish colour patterns involving ultraviolet (UV) wavelengths (300-400nm) have been discovered in many species (Losey et al. 1999), and this spectral range has been found to play a role in many life history events, including mate choice (Boulcott et al. 2005). It has even been suggested that UV may be a private communication channel for some species, as these wavelengths are strongly scattered in water relative to long wavelengths (Lythgoe 1979), potentially allowing social communication over short distances without the risk of predator detection at long distances (Cummings et al. 2003). In most cases, however, there is still little evidence that UV is relatively more important than other colours in the spectrum (Kevan et al. 2001), and its role as a sexual signal continues to remain largely a mystery in most fish species.

The function of colour as a sexual signal in threespine stickleback has garnered much attention, in large part because visual signaling is an important contributor to the evolution of this species (Milinski and Bakker 1990; Braithwaite and Barber 2000; Boughman 2001; Boughman et al. 2005). During the breeding season, most populations of male stickleback display nuptial colouration consisting of a red throat, blue iris, and in some cases, a blue or blue-green body (McLennan 2007). Red colouration, in particular, plays a major role in sexual selection within this species (Milinski and Bakker 1990; Boughman 2001; Boughman et al. 2005). Produced by carotenoid pigments that can only be obtained through the diet,

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2 A version of this chapter will be submitted for publication. Clarke, J.M. and Schluter, D. Does UV exhibit patterns of a secondary sexual character on the abdomen of threespine sticklebacks (*Gasterosteus aculeatus*)?
redness has been linked to male body condition (Milinski and Bakker 1990; Barber et al. 2000; Boughman 2007), mating success (Bakker and Mundwiler 1994), and nest defense (McKinnon 1996).

Considering the significance of human-visible nuptial colours in these fish and the importance of UV signals in many vertebrates (Tovée 1995), it is likely that UV wavelengths also play a role in the life history events of the threespine stickleback. There is growing evidence that this is indeed the case. Threespine sticklebacks have UV-reflective body patterns, particularly on the abdomen region (Rick et al. 2004; McLennan 2007; Rick and Bakker 2008a), and possess a fourth photoreceptor that is sensitive to ultraviolet light, with a maximal absorption peak at 360nm (Rowe et al. 2004). These fish use UV perception when shoaling (Modarressie et al. 2006) and when locating foraging patches (Boulcott and Braithwaite 2005); however, Modarressie and Bakker (2007) have suggested that UV may be more essential during social interactions than in foraging tasks. Indeed, male sticklebacks viewed by females in full spectrum conditions (UV-inclusive) were more attractive to females than males viewed under human-visible conditions (400-700nm) (Boulcott et al. 2005; Rick et al. 2006). Furthermore, UV and red appear to be the most important wavelengths (relative to green and blue) in the visual attractiveness of males, as individuals lacking these wavelengths were least preferred by females (Rick and Bakker 2008b). There is also evidence that UV wavelengths make females more attractive to males (Rick and Bakker 2008c), and males tend to exhibit more aggression towards male opponents who exhibit UV colouration than males who do not (Rick and Bakker 2008a). From these studies it is evident that UV plays some role in the social communication of sticklebacks, but the details remain unclear.
Despite the uncertainty regarding the role of UV wavelengths as a sexual signal in stickleback, numerous hypotheses have been formulated. It has been proposed that UV colouration may be signaling honest information to the receiver (female stickleback), which is suggested by the condition-dependence of UV contrast on the anal fin region of one male stickleback population (Rick et al. 2004). It has even been suggested that UV may be acting as a private communication channel, since UV light is transmitted over such short distances (Cummings et al. 2003), potentially allowing sticklebacks to highlight specific informative body regions during courtship without being seen at a distance by predators (McLennan 2007). In addition, evidence that UV colouration is involved in aggressive interactions suggests that UV may play a direct role in male-male competition (Rick and Bakker 2008a).

In this study, we test whether UV is a secondary sexual character on the abdomen of male threespine sticklebacks, an area of the body that exhibits particularly intense UV reflectance (Rick et al. 2004; McLennan 2007; Rick and Bakker 2008a). Using red on the throat as a gauge for how a sexually selected colour should behave in these populations, simple hypotheses can be formulated regarding the pattern of UV body colour. First, if UV is indeed a secondary sexual character on the abdomen, we expect to see sexual dimorphism of UV. Although sexual dimorphism is considered a basic indicator of whether sexual selection is present or absent in a population or species (Boughman 2007), it appears to be a good signature of sexual selection in many stickleback populations when examining colour signals. Redness on the throat, for example, is well known to be a sexually dimorphic colour exhibited by males in stickleback populations (Boughman 2001; Boughman et al. 2005; Albert et al. 2007). Sexual dimorphism of UV in threespine sticklebacks has yet to be examined.
Second, we expect to see UV change between the non-breeding season and the breeding season. This change is expected to take the form of an increase in male UV between seasons accompanied by no change in female UV, or an increase in male UV between seasons accompanied by a decrease in female UV. Like sexual dimorphism, seasonality of UV has yet to be examined in any threespine stickleback population.

MATERIALS AND METHODS

Study Populations

Limnetic and benthic sticklebacks from two freshwater lakes (Paxton and Priest) on Texada Island, British Columbia were used as study populations. Paxton and Priest are two of five known lake systems that currently support sympatric species of stickleback in southwestern British Columbia (Schluter and McPhail 1992), and were colonized independently by the marine stickleback after the glaciers retreated 10,000-12,000 years ago (McPhail 1994). The small limnetic species feeds primarily on zooplankton in the pelagic zone and the large benthic species feeds mainly on invertebrates in the littoral zone (Schluter and McPhail 1992). The benthic and limnetic species also tend to differ in their expression of male human-visible body colour and female sensitivity to these colours (Boughman 2001; Albert et al. 2007). Limnetics typically have more intense red colouration than benthics which is displayed over a larger area (Boughman 2001), and limnetic females display stronger preferences for red males than benthic females do (Boughman 2001). UV body colour, however, has not been specifically examined in these species pairs.
Fish Collection

The colours of adult threespine sticklebacks from Paxton Lake and Priest Lake were measured during the non-breeding season (February 2008) and the breeding season (April and May 2008). Fish were collected using minnow traps and placed as a group in a dark, covered container prior to measurement. The same collection method was employed each time the lakes were sampled.

Colour Measurement

Fish were selected one at a time from the covered container, and the sex and species (benthic or limnetic) was recorded. The sex of the fish was not recorded during the non-breeding season because sexual dimorphism in the form of nuptial colouration is the most conspicuous observational method of distinguishing between the sexes. Prior to colour measurement, each fish was briefly placed in a small container of dilute MS-222, an anaesthetic that safely and temporarily sedated the fish.

Reflectance measurements of each fish were taken using a USB2000 spectrometer (by Ocean Optics), a UV/human-visible light source, a bifurcated fibre-optic probe (encased in a custom made sheath to minimize ambient light effects and to keep the probe at a constant distance from the body surface), and the OOIBase32 software from Ocean Optics. Prior to measurement, the right side of the fish was lightly dried using a Kimwipe to avoid any unwanted water reflectance effects. The probe was then placed at a 90° angle on four predetermined locations along the right side of the fish’s body: the throat, anterior abdomen, posterior abdomen, and posterior ventral-lateral abdomen (Fig. 3.1). These specific abdomen areas were chosen because together they encompass the general location of particularly intense UV reflectance found in previous studies (Rick et al. 2004; McLennan 2007; Rick
and Bakker 2008a). However, our subsequent analyses focused solely on the anterior abdomen location because it exhibited the most intense UV reflectance of all three measured abdomen areas.

Reflectance was measured over the range 300-700nm relative to a WS-1 Spectralon manufactured by Ocean Optics. At each of the body points, a spectral reading was recorded (displaying percent reflectance vs. wavelength) using the OOIBase32 software and imported into Excel and compiled using CLR 1.05 (Montgomerie 2008). After colour measurement, the fish were revived in a bucket of water and returned to the lake.

![Throat Abdomen](image)

Figure 3.1. The body areas of the stickleback that were measured using reflectance spectrometry. On the right side of each fish, four areas of the body were measured: the throat, anterior abdomen (A), posterior abdomen (B) and posterior ventral-lateral abdomen (C). Data analyses were only performed on the throat and anterior abdomen (A) locations.

*Selection of Prime Breeding Individuals*

To ensure that our dataset consisted of individuals in prime breeding condition, we selected the males with the reddest throats and the females who were the most gravid at the time of measurement. These are regarded as key characteristics of breeding condition in the stickleback populations used in this study. The top 50% of males with the highest red chroma values from each population (Paxton benthic, Paxton limnetic, Priest benthic, Priest limnetic) were selected from the April/May dataset. Red chroma was calculated by dividing the area
under the reflectance curve between 600 and 700nm by the area under the entire curve (300-700nm), giving a measure of red intensity/saturation. Similarly, the top 50% of females from each population with the most distended abdomens were chosen (based on visual inspection of photographs). The creation of a dataset consisting of individuals presumably in the best relative breeding condition acted as a baseline for which we could examine the sexual role of UV in these individuals.

Quantifying Colour

We quantified reflectance by taking into account the sensitivities of the stickleback photopigments. Sticklebacks have four cone photopigments: a UV-wavelength sensitive (UVS), short-wavelength sensitive (SWS), medium-wavelength sensitive (MWS) and long-wavelength sensitive (LWS) photopigment with absorption peaks around 360, 445, 530, and 605nm, respectively (Rowe et al. 2004). Based on colour space models, a particular hue as perceived by a stickleback corresponds to the relative output of these four cone types (Rick and Bakker 2008b).

In the UV-B portion of each spectrum (300-320nm) there was substantial noise in the form of high-peaked oscillations. To control for this noise, the reflectance values of this section were replaced by the median reflectance between 300 and 320nm. To quantify reflectance in units relevant to stickleback vision, each individual’s reflectance spectrum was multiplied by the spectral sensitivity of each cone type (based on stickleback photopigment absorption spectra from Rowe et al. 2004 and β-peak positions calculated from a formula from Palacios et al. 1998), and then summed over the entire visual spectrum (300-700nm). This calculation was performed for each cone type (UVS, SWS, MWS, LWS), producing 4 values for a given reflectance spectrum. Each of these values was then multiplied by a
scaling factor (one for each cone type) which was determined by inputting a flat (achromatic) reflectance spectrum into the calculation and scaling the values such that there was equal stimulation of all cones (Vorobyev and Osorio 1998; Rick and Bakker 2008b). This step assumes that the stickleback eye is adapted to viewing an achromatic background, which is necessary when using a model involving photopigment sensitivities (Vorobyev and Osorio 1998; Hunt et al. 2001; Rick and Bakker 2008b). Relative values for each cone type were then calculated by dividing each of the absolute values by the sum of all four values. This step removed the brightness component of the colour, allowing us to focus solely on hue (Hunt et al. 2001). These four values thus represent the coordinates of the hue in stickleback colour space (Neumeyer 1992; Rick and Bakker 2008b).

This method quantified reflectance in each of the four regions of the colour spectrum (UV, SWL: “blue”, MWL: “green”, LWL: “red”) using a weighted average of observed reflectance values, where the weights were the respective photopigment sensitivities. It is similar to the quantal catch method described by Vorobyev et al. (1998) with the exception that we did not incorporate an irradiance spectrum into our calculations, nor did we incorporate the absorption and scatter properties of water or ocular media transmission properties. Because our calculations do not represent relative quantal catch rates as they are typically calculated due to the omission of an irradiance spectrum (Hunt et al. 2001; Rick and Bakker 2008b), they will be referred to simply as hue components in this study.

Data Analysis

Across populations, paired t-tests were used to compare the red hue component on the throat between the sexes, as well as the UV hue component on the abdomen, using the population as the replicate (n = 4). To examine sexual dimorphism at the population level,
separate two-sample t-tests were performed for each stickleback population. Similarly, paired t-tests using the population as the replicate (n = 4) were used to compare the red hue component on the throat between breeding and non-breeding individuals (seasonality), as well as the UV hue component on the abdomen. Separate two-sample t-tests were used to examine seasonality within populations. All statistical analyses were performed in R version 2.6.0 (R Development Core Team 2007).

RESULTS

Red on the Throat

The red throat of breeding males is well established as a secondary sexual characteristic in these stickleback populations (Boughman 2001; Boughman et al. 2005; Albert et al. 2007). In our study red colouration on the throat was used as a gauge for how a sexually selected colour should behave. Because we chose the reddest males for our dataset, examining sexual dimorphism and seasonality did not provide any new information about red on the throat. Instead, the examination was primarily a confirmation of our method of testing whether a colour represents a secondary sexual character in males, so that this same method could be applied to testing the role of UV reflectance on the abdomen.

Males had significantly redder throats than females (Paired t-test, P = 0.021, df = 3, mean of differences ± SE = 0.034 ± 0.012; Fig. 3.2). At the population level, Paxton benthics, Paxton limnetics and Priest limnetics all exhibited significant sexual dimorphism for the red hue component on the throat (PXB: t_{22} = 3.14, P = 0.0047; PXL: t_{24} = 2.10, P = 0.047; PRL: t_{10} = 2.73, P = 0.021), while that of the Priest benthic population was borderline significant (PRB: t_{27} = 1.91, P = 0.067).
Males exhibited a trend toward a higher red hue component on the throat than non-breeding individuals (Paired t-test, P = 0.084, df = 3, mean of differences ± SE = 0.028 ± 0.018). At the population level, both the Paxton benthics and Priest limnetics exhibited significant seasonality of red on the throat (PXB: $t_{30} = -4.45$, $P = 0.00011$; PRL: $t_{15} = -3.45$, $P = 0.0035$). The Paxton limnetic and Priest benthic populations also exhibited trends toward seasonality, but these were not statistically significant (PXL: $t_{29} = -0.915$, $P = 0.368$; PRB: $t_{30} = -1.45$, $P = 0.156$). Females did not differ from non-breeding individuals in their red hue component (Paired t-test, $P = 0.363$, df = 3). Overall, red on the throat was sexually dimorphic and seasonal in the stickleback populations.

Figure 3.2. Mean red (LWL) hue component on the throat based on the reflectance spectra of breeding males, breeding females and non-breeding individuals of the four stickleback populations. Error bars denote standard error.
UV on the Abdomen

UV on the abdomen did not exhibit clear patterns of sexual dimorphism and seasonality as seen with red on the throat. Following Hunt et al. (2001) and Rick & Bakker (2008b), mean reflectance spectra were calculated for each of the populations, with separate spectra for each sex (with the exception of the non-breeding spectra, where only one mean spectrum was calculated for each population). Relative hue components were calculated from these mean spectra. This provided a qualitative examination of overall abdomen colour among populations. Generally, abdomens exhibited a peak in the UV in most populations and life history stages, with the exception of the non-breeding individuals of the Priest benthic population (Fig. 3.3). In addition, abdomens were also often “brown” (high red, medium green, low blue) in the human-visible part of the spectrum, though this varied among populations and life histories (Fig. 3.3).
Figure 3.3. Relative hue components calculated from the mean abdomen spectra of breeding males, breeding females, and non-breeding individuals of the four stickleback populations (A-D). This provided a qualitative examination of overall abdomen colour among populations and life histories.

To quantify these patterns in UV, relative hue components were calculated from each individual’s abdomen reflectance spectrum. In terms of sexual dimorphism, there was a trend towards higher UV in males than females, but this was not statistically significant (Paired t-test, \( P = 0.071, \text{df} = 3, \text{mean of differences} \pm \text{SE} = 0.017 \pm 0.010 \)). At the population level, only the Priest benthic population exhibited sexual dimorphism for UV, but the significance level was borderline (\( t_{24} = 2.09, P = 0.048; \text{Fig. 3.4} \)).
In terms of seasonality, the UV hue component on the abdomen of breeding males did not significantly differ from that on the abdomen of non-breeding individuals (Paired t-test, P = 0.407, df = 3, mean of differences ± SE = 0.011 ± 0.019). At the population level, only the Priest benthic population exhibited seasonality for UV (t_{29} = -2.87, P = 0.0075; Fig. 3.4). Females of this same population also exhibited a higher UV hue component than non-breeding individuals, but this relationship was not significant (t_{30} = -1.77, P = 0.086).

![Graph](image_url)

**Figure 3.4.** Mean UV hue component on the abdomen based on the reflectance spectra of breeding males, breeding females and non-breeding individuals of the four stickleback populations. Error bars denote standard error.
DISCUSSION

Overall, the patterns of sexual dimorphism and seasonality of UV on the abdomen were not as strong as those exhibited by red on the throat, suggesting that UV is not a secondary sexual character on the abdomen of these populations of threespine sticklebacks. In addition, the magnitude of the differences in red on the throat between sexes was generally larger than that of UV on the abdomen (Figs. 3.2 & 3.4). At the population level, only the Priest benthic population exhibited significant seasonality and borderline significant sexual dimorphism of UV on the abdomen. However, these significant results from the Priest benthic population should be interpreted with caution; considering the number of statistical tests that were performed, statistical significance could have arisen solely by chance in this population as 1 out of 20 t-tests will exhibit a significant p-value (p < 0.05) even if the null hypothesis is true. Thus, our results do not provide strong evidence that UV is a secondary sexual character on the abdomen of sticklebacks, but leave open this possibility in the Priest benthic population.

The general abdomen region of sticklebacks exhibits intense UV reflectance (Rick et al. 2004; McLennan 2007; Rick and Bakker 2008a), but our results provide very little evidence that UV on this area of the body is a secondary sexual character. Behavioural experiments have demonstrated a general role for UV in stickleback mate choice (without isolating any particular body areas) (Boulcott et al. 2005; Rick et al. 2006; Rick and Bakker 2008b), so it is possible that UV may play a social communication function on the abdomen without being an exaggerated secondary sexual character. For instance, UV on the abdomen could just be part of the overall colour “mosaic” (red throat, blue iris, and green flanks; Milinski and Bakker 1990) of the breeding male stickleback, in which case it wouldn’t
necessarily need to be an exaggerated secondary sexual character. The presence of UV on the abdomen could increase the total contrast (and thus attractiveness) of the entire male nuptial signal. In fact, there is some evidence that suggests that female preference for a male’s sexual signal is related more to visual contrast than the colour itself. For instance, Baube et al. (1995) found that “dummy” male sticklebacks that exhibited some degree of visual contrast on their bodies were preferred more by females than male dummies that were uniformly red or tan. Furthermore, both red and UV colours likely contrast with the habitat background colour, which is primarily blue-green in Priest Lake (Albert et al. 2007), increasing the overall visual contrast of this already high-contrast signal.

It is also possible that our method of colour measurement was not sensitive enough to detect UV as an exaggerated secondary sexual character. For instance, threespine sticklebacks have the capacity to rapidly change their body colour in response to their background (Hogben and Landgrebe 1940; Huntingford and Coyle 2007; J. M. Clarke, pers. obs.), which may have reduced the intensity of UV on the abdomen at the time of measurement. In addition, the direct effect of the anaesthetic (MS-222) on stickleback colouration is not known, so it is also possible that exaggerated UV colouration in males was attenuated after this procedure. Patterns of sexual dimorphism and seasonality of red on the throat were successfully detected using these methods, suggesting that our colour measurement technique was sensitive enough to detect similar patterns of UV colouration on the abdomen, but it is unknown whether structural (UV) and pigment-based (red) colours respond differently to physiological colour change and/or anaesthetic.

Our results do not rule out the possibility that UV may be a secondary sexual character on the abdomen of the Priest benthic population, but this will need to be tested
through mate choice experiments that isolate the abdomen region. UV as a secondary sexual character may be ideal for social communication in these lakes, as UV wavelengths are only effective over short distances and in shallow water because UV scatters strongly in water relative to other wavelengths (Lythgoe 1979; Losey et al. 1999). Furthermore, the background irradiance of these lakes lacks a UV component (Albert et al. 2007), so UV on the abdomen of these sticklebacks would likely stand out against the background and be easily detected, much like redness on the throat (Boughman 2001). This makes UV ideal for social communication at short distances while at the same time minimizing predator detection at long distances (Cummings et al. 2003). Thus, the hypothesis that UV on the abdomen may act as a private communication channel in the Priest benthic population cannot be ruled out.

As demonstrated in our study, the patterns of UV as a potential secondary sexual character on the abdomen were not universal across all stickleback populations. This suggests that UV as a secondary sexual character on the abdomen may be limited to certain populations during the breeding season. It is difficult to speculate why one population/species displayed this secondary sexual character and not another. Differences in the amount of available UV in the light environments of each lake or habitat, for instance, could have favoured UV as a sexual signal in one population and not another, as signals are expected to evolve in response to the features of the signaling environment (‘sensory drive’; Endler 1992; Endler 1993; Boughman 2001). However, irradiance measurements taken in these lakes during the breeding season (data not shown), suggest that these lakes and habitats do not significantly differ in their relative UV light availability near the surface. Additional work that addresses this question in more detail, such as the examination of possible
differences in UV photopigment sensitivities between the species, will provide greater insight into how UV patterns may be shaped by sexual selection in sticklebacks.

It is unclear what information UV on the abdomen may be providing to discriminating females if it is indeed a secondary sexual character in the Priest benthic population. In another study, UV contrast was related to male body condition on a more posterior abdomen position than our focal area (Rick et al. 2004), but we did not find that UV (or any other hue component) was related to our measure of condition, which was based on residuals taken from a linear regression of log standard length on log weight. This finding does not rule out the possibility that UV may be providing some other information about male quality not examined in this study. For instance, it has been suggested that UV may provide information to females only during male courtship behaviours such as the “zigzag” dance or nest fanning (McLennan 2007). Future studies that examine UV male colouration as an honest signal will shed light on what information UV may be sending to receivers.

In addition to the possible role of UV colouration in female mate choice, Rick and Bakker (2008a) found that male sticklebacks displayed significantly greater levels of aggression towards male opponents exhibiting a UV component to their colouration than male opponents lacking this component, suggesting that UV wavelengths are also used in male-male competition. This aggression is similar to the intrasexual behavioural response of male sticklebacks to red nuptial colouration (Bakker and Sevenster 1983; Rowland 1984). Thus, UV may be a signal to rival males and could be indirectly providing honest information about aggression and territoriality to females through male-male competition (Candolin 1999).
To our knowledge, this is the first study to examine both sexual dimorphism and seasonality of UV body colour in natural fish populations. In summary, our results suggest that UV does not exhibit striking patterns of a secondary sexual character on the abdomen of threespine sticklebacks, but do not rule out this possibility in the Priest benthic population. UV may be well suited for social communication at short distances such as in courtship behaviour, as these wavelengths scatter rapidly in water and the irradiance colour of Priest Lake suggests that UV body colour likely stands out against the background. Although behavioural studies focusing on this specific body area will need to be performed in order to determine the role of UV in female mate choice and/or male-male competition, our study suggests that UV on the abdomen may not be important in sexual communication. Future work that examines UV as an honest signal on the abdomen and other body areas will provide greater insight into the role of UV in sexual communication, as will studies that investigate possible species differences in UV wavelength perception.
REFERENCES


Montgomerie, R. 2008. CLR, version 1.05. Queen’s University, Kingston, Canada. (Available at: http://post.queensu.ca/~mont/color/analyze.html)


CHAPTER 4: General Conclusion

This thesis focused on research questions concerning the evolution of body colour in threespine sticklebacks. In Chapter 2, I examined whether upper body colouration has diverged between the benthic and limnetic species due to background matching and whether colour plasticity plays a role in this cryptic colouration pattern. I experimentally placed benthic and limnetic sticklebacks against each of the habitat background colours (littoral or pelagic) and digitally photographed them to examine the degree of background matching and whether they could change colour when placed against a new background. The upper body colour of benthics matched the littoral background (benthics’ habitat) colour more closely than did the upper body colour of limnetics, suggesting that in their own habitat benthics are more cryptically coloured than the limnetic species. There was no difference between species, however, in the resemblance of their upper body colouration to the pelagic background (limnetics’ habitat). Benthics were able to resemble both backgrounds by exhibiting consistent changes in their upper body colouration to adjust to the current background colour. Limnetics, despite having the capacity to change colour, did not exhibit as much consistent, directed change towards the background colour as benthics. These results suggest that the greater degree of colour change observed in the benthics is an adaptive response to the greater spectral heterogeneity of the littoral zone. Overall, this chapter highlighted how divergent natural selection arising from habitat-specific spectral characteristics can shape cryptic colouration differences between species.

In Chapter 3, I examined whether UV exhibits patterns of a secondary sexual character on the abdomen of four stickleback populations. During the non-breeding and breeding seasons, I measured the throat and abdomen colours of male and female
sticklebacks using reflectance spectrometry. Using red on the throat as a gauge for how a sexually selected colour should behave in these populations, I found that red was both sexually dimorphic and seasonal. Based on these red throat results, I also expected UV on the abdomen to be sexually dimorphic and seasonal if it was indeed a secondary sexual character. Overall, the patterns of sexual dimorphism and seasonality of UV on the abdomen were not as strong as those exhibited by red on the throat, suggesting UV is not a secondary sexual character on this body area. Behavioural studies have demonstrated a general role for UV in stickleback mate choice, so it is possible that UV may play a social communication function on the abdomen without being an exaggerated secondary sexual character. For instance, the presence of UV may increase the total contrast (and attractiveness) of the entire male nuptial signal. At the population level, only the Priest benthic population exhibited significant seasonality and borderline significant sexual dimorphism for this colour pattern, leaving open the possibility that UV on the abdomen may be a secondary sexual character in this population. This colour pattern may be well suited for sexual communication at short distances such as in courtship behaviour, as UV scatters rapidly in water and the irradiance colour of Priest Lake suggests that UV body colour likely stands out against the background. Overall, this chapter highlighted the apparent unimportance of UV on the abdomen as a sexual signal in threespine sticklebacks but suggested that this trend may not be universal across all populations.

In summary, I have investigated the evolution of body colour in threespine sticklebacks by addressing both natural and sexual selection of colour patterns in species pair populations. It is evident from the results presented in Chapter 2 that natural selection is a potent force that can shape body colour in threespine stickleback populations. Considering
that very little is known about natural selection of colour in sticklebacks, my results have the potential to provide motivation for studies focusing on the evolution of other cryptic colouration patterns such as disruptive colouration and countershading (Ruxton et al. 2004). In addition, future work that addresses the genetic and physiological underpinnings of cryptic colour patterns will provide greater insight into how body colour is shaped by natural selection in threespine stickleback populations. The results presented in Chapter 3 highlight the importance of examining UV colour patterns in order to better understand the overall nuptial colour repertoire of threespine sticklebacks. Although I did not find UV to be a striking secondary sexual character on the abdomen, these results will contribute to our general understanding of UV colouration in sticklebacks. Behavioural experiments that isolate particular body regions of the stickleback during mate choice will provide valuable insight into the role of UV in sexual communication.
REFERENCES

APPENDIX 1: Chapter 2 Supplementary Materials

MATERIALS AND METHODS

Standardization of Photographs

The photographs were standardized for colour and brightness using the program Picture Window Pro 4.0, which recognizes the GretagMacbeth brand of colour reference cards. This program provides a 6x4 frame that can be superimposed over the 24 squares of the ColorChecker chart. Each square of this superimposed frame is numbered, which corresponds to a specified colour square in the ColorChecker chart (as described in the GretagMacbeth manual). Once the frame is superimposed over the corresponding colour squares, the entire photo is then standardized based on the known reference colours of the ColorChecker chart and can be saved as a new file.

To validate whether this program correctly standardized the photos, linearity and RGB equality tests were performed on the corrected photos (Stevens et al. 2007; Bergman and Behehner 2008). A linearity test checks for a linear relationship between each of the RGB values and the % reflection values across the six-step grayscale on the ColorChecker chart (20%, 35%, 50%, 65%, 80% and 95%) (Bergman and Behehner 2008). An RGB equality test ensures that all of the grey values in the photo have equal values in all three colour channels. This was performed by examining whether R=G=B in each of the six grey squares on the ColorChecker chart (Bergman and Behehner 2008). The results of the linearity and equality tests were comparable to those described by Bergman and Behehner (2008) in their validation of this photo sampling method, providing strong evidence that the photos were correctly standardized. As an additional validation, we also performed correlation tests to examine whether the photo standardization process (both linearization and equalization) was
responsible for any of the variation in fish colour observed in our study. Based on these
tests, we did not find any evidence for such a relationship, providing additional validation of
the photo standardization procedure.

*Time Course Photographs*

According to Frankino and Raff (2004), measuring terminal phenotypes across
alternative environments is informative, but additional information is usually needed to fully
understand the evolution of a plastic response. Accordingly, to further investigate the change
in colour exhibited by these fish, an additional background matching trial was performed that
more closely examined this colour change as it was progressing in real time. As in the
previous experiment, digital photography was used to capture the upper body colouration of
the fish in each background, but in this case a photograph was taken every 20 seconds as the
fish was acclimating to its current background instead of after the acclimation period.

2 limnetics and 2 benthics were sampled from the same experimental ponds described
above and were held for a short period in white buckets, sorted by source pond. As in the
previous experiment, 2cm of water was placed in the plastic cups immediately prior to the
trial. Two fish were sampled at a time. The first fish was placed in a cup and time “0” began
as soon as the first photo was taken. The second fish was then placed in an adjacent cup and
time “0” for this fish began 10 seconds after time “0” for the first fish, at which time a
photograph was taken. A photograph was taken of each fish every 20 seconds (including
time zero) for 20 minutes. This alternated between the two fish such that a photo was taken
every 10 seconds. The photographs were taken in exactly the same way as described above in
the previous experiment and all shots included the ColorChecker card and an identification tag.

Following the 20 minute time period, the fish were then transferred to the oppositely coloured cup and time “0” for the first fish in this new background began as soon as the first photograph was taken. Photography then proceeded in the same fashion as described for the first background. In total, 120 high-quality photos were taken of each fish (60 per background) and all photos were standardized using Picture Window Pro 4.0 and pixels were sampled from each photo in Photoshop as described earlier. Based on the RGB scores across a 20-minute time series, the dynamics of colour change could then be examined more closely in these fish.

RESULTS

Time Course Experiment

As illustrated in Figures A1.3 & A1.4, both species have the ability to rapidly change their upper body colouration, but there were clear differences in this response between benthics and limnetics. In benthics, the most rapid rate of colour change occurred within the first 2 to 2.5 minutes of being introduced into a new background, regardless of the colour of the background. This change exhibited by the benthics was directed towards the colour of the background, suggesting that benthics were adjusting their upper body colouration to match the current background colour. Following this initial and rapid response, the rate of colour change plateaued and in some cases, a continued, gradual change towards the background colour was exhibited. These responses were comparable to those described by Hogben and Landgrebe (1940), in which colour change of *G. aculeatus* against alternate
backgrounds was examined over time more invasively using a specially designed apparatus in the laboratory.

Limnetics displayed strikingly different patterns of colour change over time from the benthics and between backgrounds. Despite the fact that limnetics have the ability to rapidly change their upper body colouration, there did not appear to be a directed change towards the background colour like that exhibited by the benthics. Furthermore, unlike the benthics, the response of the limnetics did not resemble the stickleback response described by Hogben and Landgrebe (1940). As illustrated in Figure A1.4, the upper body colouration of limnetics against the littoral background changed over time in an oscillatory fashion and did not ultimately result in a noticeable directed change towards the background colour by the end of the 20-minute test period. The amplitudes of these oscillations were dramatically larger than the fluctuations exhibited by the benthic individuals against the same background. In the pelagic background, the colour change exhibited by the limnetics was more comparable to that of benthics as there were dramatically smaller fluctuations over the time course (Fig. A1.3). Unlike the benthics, however, there was still no recognizable directed change towards the colour of this background.

Combined, these results suggest that limnetics may have a similar capacity for colour change as benthics, but this change does not appear to be directed towards the colour of the background. Moreover, the colour change of limnetics is more stable over time when they are against the pelagic background than when they are against the littoral background. Benthics, on the other hand, can rapidly direct their body colouration towards either background colour in a consistent fashion with only small fluctuations in colour over time.
REFERENCES


Figure A1.1. Photographs of the open water of Paxton Lake (A) and chara vegetation (B) used to sample representative pixels for the pelagic zone colour and littoral zone colour, respectively. (C) One of the two frames used in the background matching experiment. 24 coloured cups (12 pelagic and 12 littoral) were used in each frame.
Figure A1.2. Examples of standardized photographs from the background matching experiment. (A) and (B) illustrate the same benthic individual against the pelagic and littoral background, respectively. Similarly, (C) and (D) illustrate the same limnetic individual against the pelagic and littoral background, respectively.
Figure A1.3. Time series depicting the change in upper body colouration of benthic individuals (A & B) and limnetic individuals (C & D) against the pelagic background. At each time interval, the body colour deviation from the pelagic background colour is illustrated by a red, green and blue point, which represent the degree to which the body colour of the fish deviates from the red, green and blue channels of the background colour, respectively. Gaps in the time series indicate the fish was not sampled due to poor photograph quality.
Figure A1.4. Time series depicting the change in upper body colouration of benthic individuals (A & B) and limnetic individuals (C & D) against the littoral background. At each time interval, the body colour deviation from the littoral background colour is illustrated by a red, green and blue point, which represent the degree to which the body colour of the fish deviates from the red, green and blue channels of the background colour, respectively. Gaps in the time series indicate the fish was not sampled due to poor photograph quality.
APPENDIX 2: Animal Care Certificates

THE UNIVERSITY OF BRITISH COLUMBIA

Jason Clarke

has successfully completed the online training requirements of the Canadian Council on Animal Care (CCAC) / National Institutional Animal User Training (NIAUT) Program

Certificate #: 2647 - 07
Date Issued: December 14, 2007
APPENDIX 2: Animal Care Certificates

ANIMAL CARE CERTIFICATE

Application Number: A07-0237
Investigator or Course Director: Dalpi Schiester
Department: Zoology
Animals:

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Start Date: April 1, 2006  
Approval Date: September 17, 2007

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<td>Natural Sciences and Engineering Research Council of Canada (NSERC)</td>
<td>The genetics of adaptations to new environments</td>
</tr>
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Unfunded title: Ecology and genetics of adaptive radiation

The Animal Care Committee has examined and approved the use of animals for the above experimental project.

This certificate is valid for one year from the above start or approval date (whichever is later) provided there is no change in the experimental procedures. Annual review is required by the CCAC and some granting agencies.