

# FEMALE RED THROAT COLORATION IN TWO POPULATIONS OF THREESPINE STICKLEBACK

by

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## Summary

In a population of stream-resident stickleback from British Columbia, females frequently have orange-red throats which are conspicuous to the human eye and, according to two straightforward physical measures of coloration, are more intensely red than the throats of anadromous females. Stream and anadromous males from these populations, however, do not differ for a reflectance-based index of red chroma. This suggests that exceptional female red coloration in the stream population has not evolved as a byproduct of the evolution of exceptional coloration in males. In contrast to results for female lateral barring in another threespine stickleback population, red is not strongly associated with reproductive readiness and unlikely to function strictly as a signal of readiness to mate. Larger stream females

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have more intensely red throats though this pattern was significant only in one year and according to one technique. With these findings and the extensive literature already available for this species, the threespine stickleback becomes a promising model system for studying the evolution of female secondary sexual characters in species with conventional sexual dimorphism.

## Introduction

The evolution of conspicuous secondary sexual characters has been intensively studied for at least two decades. Most work has focused on males but in animal species with sexual role-reversal, female ornamentation has also received considerable attention (Andersson, 1994). Female secondary sexual characters have been less well studied in conventionally dimorphic species, in which males are the more conspicuous sex. Some work has been done of late, however, mainly with birds (*e.g.* Cuervo *et al.*, 1995; Amundsen *et al.*, 1997; Tella *et al.*, 1997). This research is noteworthy because female ornamentation in conventionally dimorphic species provides an instance in which both non-adaptive and adaptive explanations are readily testable. In the simplest scenario, female ornamentation may not be adaptive for females because it evolves as a correlated response to selection on males. Alternatively, it may be functional in its own right (Lande, 1980; Møller, 1993).

Here we present the first results of an ongoing study of female coloration in the threespine stickleback, a species with conventional dimorphism for coloration. The gaudy colors of the male threespine stickleback provide one of the classic examples of extravagant male ornamentation (*e.g.* Krebs & Davies, 1993; Anderson, 1994), whereas the less conspicuous (at least to the human eye) coloration of female stickleback has been relatively little studied (McLennan, 1996). In a noteworthy exception to this pattern, females of an Eastern US anadromous population have been reported to develop vertical barring on their flanks as their eggs mature and they become ready to mate. Further, males have been shown to court such females preferentially (Rowland *et al.*, 1991). This female pigmentation, however, is not obviously related to comparable display characters in males. In the related brook stickleback, *Culaea inconstans*, female nuptial coloration has been more extensively studied. This work has tended to emphasize the contrasting courtship coloration of males and females and the strong

relationship between ovulation and the appearance of a distinct female color pattern (McLennan, 1994); in the brook stickleback too, female pigmentation is the subject of a male preference (McLennan, 1995).

In the current study we present the first quantitative investigation of the expression of a typically male trait, orange-red throat coloration, in female threespine stickleback. The study population resides permanently in freshwater, in the upper reaches of the Little Campbell River, Southwestern British Columbia. In our principal analysis, we compare the throat coloration of both males and females to their counterparts from the geographically adjacent anadromous population, found in the lower reaches of the same small river/stream (Hagen, 1967). Anadromous and marine populations appear to be more morphologically conservative than freshwater-resident populations and the apparently weaker throat coloration of Little Campbell anadromous females may be representative of the more typical situation in this species. Because the ancestral form of threespine is thought to have been anadromous or marine, character states in such populations may also indicate ancestral states (Bell & Foster, 1994; but see Schluter *et al.*, 1997; Losos, 1999, concerning the difficulties in estimating ancestral states). In addition, we investigate two obvious possible correlates of female throat coloration, body size and reproductive state, to see if they provide any insights concerning the evolution of reduced chromatic dimorphism in the upper Little Campbell.

## Methods

This study was conducted in two breeding seasons using distinct methodologies. Stream and anadromous stickleback used in the first set of analyses were collected with baited minnow traps from the upper and lower reaches, respectively, of the Little Campbell River (Hagen, 1967) in April and June of 1995. Upon capture they were transported by car to the University of British Columbia (Vancouver, Canada) where they were subsequently held and studied. Fish were maintained on approximately natural photoperiods at 17-21°C, initially in 93 l stock aquaria and later in 10 l aquaria for a behavioral study (McKinnon & deMayo, unpubl.).

In July, as reproductive activity was declining, nonovulated females of both populations were photographed under standardized conditions (following Frischknecht, 1993). A small sample of non-territorial stream males was also included. Slides were analyzed using an X-Rite 310 Photographic Densitometer (Frischknecht, 1993; Bakker & Mundwiler, 1994). We measured optical density at five 0.5 mm sites on a ventral view of each fish, roughly evenly spaced along the axis of bilateral symmetry on the lower jaw and throat. For each point we calculated a red index, designed to be independent of brightness, based on dividing optical density for red (700 nm) by the sum of optical densities for red, green (546.1 nm) and blue

(435.8 nm; again following Frischknecht, 1993, with only slight modifications to account for the use of a neutral grey card rather than a white card in photographs). Although this is a crude quantification of color (Endler, 1990) it correlates strongly with other methods of evaluating red intensity/chroma (Frischknecht, 1993; Bakker & Mundwiler, 1994). We present results based on analyses of the maximum red index for each individual (Frischknecht, 1993); nearly identical results, not presented in the interest of brevity, were obtained for mean values for each individual.

The second set of experimental subjects was collected from the same sites in April and June of 1998. They were briefly held in Vancouver then transported by air to Whitewater, Wisconsin where they were held at 17-21°C on approximately natural photoperiods. In Whitewater, as in Vancouver, fish were fed a mixture of frozen bloodworms and frozen brine shrimp. Experimental females were measured for standard length and moved into 113 l tanks, each divided into 3 compartments by perforated opaque dividers. They were held one per compartment so they could be tracked individually without difficulty. Each female had her throat reflectance taken in up to two sessions, once ovulated and ready to breed (with clear eggs readily visible and ready to extrude, upon light pressure to her abdomen) and once non-ovulated (and not obviously gravid). Prior to the non-ovulated reflectance-collection, each stream female was transferred overnight to a 113 l test tank. The next day she was presented for fifteen minutes with a female of approximately the same size enclosed in a jar for a related study of female aggression (McKinnon & Weggel, unpubl.). Afterward her reflectance was collected. The order in which reflectances were taken, ovulated first or second, was randomized and roughly balanced. Females were also used occasionally, though not on the same day as reflectances were collected, as stimuli in jars in the female aggression study or to bring males into breeding condition. Anadromous females were not included in the aggression study; they were held in the isolation compartments except when used to stimulate males. All data were collected in June, July and early August of 1998, with the stream stickleback data being collected earlier in the research season, consistent with their earlier breeding season in nature (Hagen, 1967). In both 1995 and 1998, any fish for which gender was uncertain were sacrificed, dissected, and their gonads inspected.

Reflectances were taken using an Ocean Optics S2000 spectroradiometer coupled to a microcomputer running Ocean Optics OOIBASE software. The system was calibrated using a mercury-argon lamp. Illumination for data collection came from a deuterium-halogen lamp through the illumination fibers of an Ocean Optics 200  $\mu\text{m}$  probe. This was attached to a small plexiglass apparatus built to hold the illumination probe at a fixed angle (anterior to the subject, 55° from the angle of the light-gathering probe) relative to the light gathering probe while positioning both as close to the experimental subject as possible. The light gathering probe sat normal to the experimental subject. A small aperture containing no plexiglass was built into the apparatus; light reached the subject and reflected off it through this hole. The experimental subject (after light dabbing with tissue to remove excess moisture) or the reflectance standard (a disc of Spectralon<sup>TM</sup>, which reflects greater than 99% of light in the visible spectrum) were placed against this aperture. The 200  $\mu\text{m}$  light gathering fiber optic possessed an acceptance angle of 28° and was held 1.2 mm from the experimental subject or standard, resulting in light being captured from a 0.8 mm diameter spot. Three to eight samples were taken from the throat area of each individual. Spots which appeared to have the highest chroma orange-red coloration (*i.e.* 'purest' color, with strong reflectance at longer wavelengths and rapid drop off in reflectance at shorter wavelengths) were deliberately

sampled preferentially and subsequent analyses focused on the highest chroma spot (defined below) sampled from each individual in each session.

To enable collection of reflectance data it was necessary to anaesthetize experimental subjects briefly. 2-phenoxy ethanol was used for this purpose because it may affect stickleback coloration less than MS-222 and result in less incidental mortality (Baube, pers. comm.). 0.5 ml of 2-phenoxy ethanol was suspended in 500 ml of water and fish were placed in this until unable to right themselves. Their reflectances were then taken. Although handling likely reduces coloration intensity somewhat, it was minimized and fish were processed as rapidly as possible, almost always in less than five minutes in total.

Male stickleback of both populations were held in 113 l stock tanks until they were transferred individually to 113 l aquaria provided with trays of fine sand and the aquarium plant java moss for nest-building. Males were exposed to both restrained and free-swimming females until they had complete nests and would unequivocally court a free-swimming female. They were then used in a male choice study involving dummy females (McKinnon *et al.*, unpubl). After the behavioral test each male had his reflectance taken within 24 hours. The same anaesthetization and reflectance-collection protocol was used with males as with females.

Red 'Chroma' values were calculated from reflectance data using one component of the segment classification method of Endler (1990). This method is intended to describe color in a manner general to animal visual systems but not specific to any particular species. Thus it should be less biased toward the human visual system than the slide-based method. Visual models based on the sensory system and light environments of our particular stickleback populations would be informative here but such models are not available or feasible at present. Although some data on the spectral properties of the cones of stickleback have been collected (Lythgoe, 1979; Baube *et al.*, 1995), there is clearly considerable variation in color vision among populations (McDonald & Hawryshyn, 1995) and we currently have neither vision data nor data on the properties of the local light environments of our study populations.

Our measure of red chroma was the 'LM' value of Endler (1990) for the 400-700 nm (Baube *et al.*, 1995) range. In this calculation, reflectance is first summed for each of the four 75 nm segments into which this 300 nm range is divided; each summed segment is divided by total reflectance over this range to adjust for overall brightness. LM is the adjusted summation for the long wavelength segment (625-700 nm) minus one of the medium segments (475-550 nm). LM was calculated for each of up to eight reflectances collected in each session with each individual. The maximum value of LM obtained in a session was then used in subsequent analyses. Prior to collection of reflectance data a human estimate of red intensity (modified for the less extensive coloration of females from Rowland, 1984), using a 0 (no red)-4 (intensely red) scale was estimated for all females sampled by one of us (JSM).

## Results

### *Sex and population differences*

In both portions of the study, stream-resident females displayed much more intense orange-red throat coloration than anadromous females (Figs 1-3). For the slide data, the maximum red index (Fig. 1) differed significantly

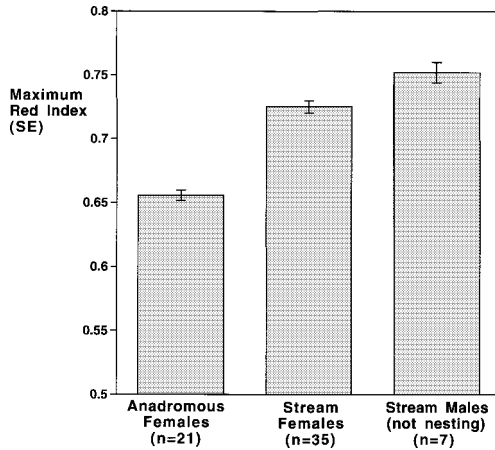


Fig. 1. Mean maximum values ( $\pm$  SE) of red indices (based on densitometer readings from slides) for five sites on the throats of female and male stream-resident threespine stickleback from the upper Little Campbell River (British Columbia) and female anadromous stickleback from the lower Little Campbell. Significant differences were present between all groups (details in text).

among anadromous females, stream females and stream males (ANOVA:  $F_{2,60} = 67.24, p < 0.0001$ ). Stream females had a significantly higher mean red index than anadromous females ( $t_{54} = 10.12, p < 0.0001$ ); the index for stream-resident males was significantly higher than for stream females ( $t_{40} = 2.43, p < 0.02$ ; both results remain significant after application of a sequential bonferroni correction). Red coloration also appeared to have a more extensive distribution in freshwater females than in anadromous females. Red pigmentation in anadromous females was mainly limited to a relatively narrow strip along the axis of bilateral symmetry on the throat, whereas in freshwater females it often covered most of the throat area and associated membranes. Although the extent of coloration was not quantitatively analyzed, the red coloration of stream females was clearly not so extensive as we have observed on territorial stream males. On females it was confined almost exclusively to the throat region, never extending more than trivially onto lateral portions of the head or body. Red pigmentation on the latter areas, particularly from the pectoral fins forward, is typical of stream males and of males of the species in general (Bell & Foster, 1994).

Reflectance data were collected from territorial males and females of both populations. For each female the overall maximum LM value, whether ovulated or non-ovulated (or whichever was available — just one score was

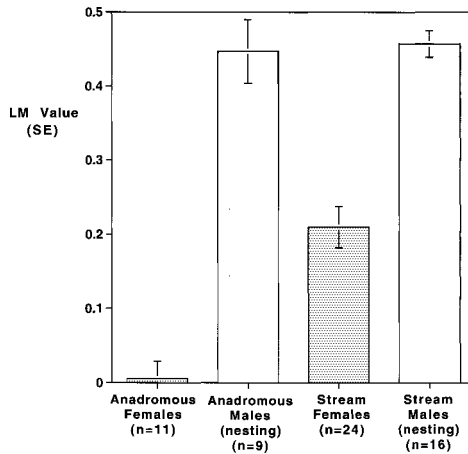


Fig. 2. Mean maximum LM values ( $\pm$  SE), calculated from reflectance data, for the throats of female and male stream-resident threespine stickleback from the upper Little Campbell River and anadromous stickleback from the lower Little Campbell. Significant differences were present between sexes and between females of the two populations (details in text).

recorded for 11 of 24 stream females and 4 of 11 anadromous females, which either failed to become ovulated or died before a second scanning), was used in these analyses. In a two way ANOVA of LM values in which gender and population are the independent variables, both had significant effects (Fig. 2;  $F_{1,56} = 123.53, 11.96, p < 0.0001, 0.001$ , respectively). However there was also a significant interaction between population and gender ( $F_{1,56} = 9.84, p < 0.003$ ), a result of the weaker dimorphism between males and females of the stream population compared to the anadromous population. There was no significant difference between LM values for males of the two populations ( $t_{23} = 0.25, p = 0.80$ ), whereas there is a highly significant difference between females ( $t_{33} = 4.60, p < 0.0001$ ). Although the difference in LM is greater between genders in the anadromous population than in the stream population, the difference between genders is highly significant in each case (stream:  $t_{38} = 6.65, p < 0.0001$ ; anadromous:  $t_{18} = 9.38, p < 0.0001$ ; significant results in the preceding four  $t$ -tests remain significant after application of a sequential bonferroni correction). It is noteworthy that there is some overlap for LM between genders in the stream population but not in the anadromous population. Nevertheless, some anadromous females do possess red throat pigmentation readily apparent to the human eye, and comparable in LM value to females of the stream population with weak-intermediate red throats.

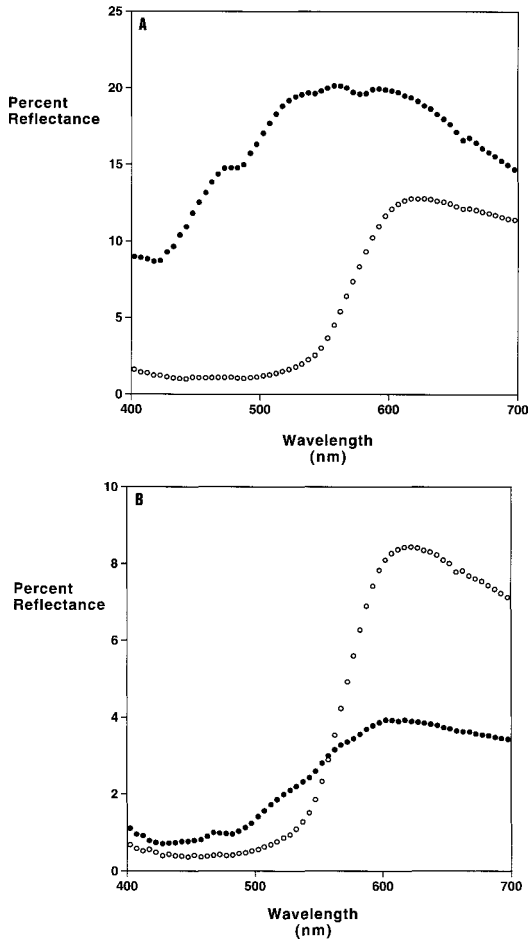


Fig. 3. (a) Mean maximum reflectance from throats of male (open circles) and female (filled circles) anadromous threespine stickleback from the lower Little Campbell River. (b) Mean maximum reflectance from throats of male (open circles) and female (filled circles) stream-resident threespine stickleback from the upper Little Campbell River. Standard errors are omitted in the interests of clarity. 5 nm increments are used because they correspond to the approximate resolution of the spectrophotometry apparatus. Note the difference in scales for reflectance.

Inspection of the plots of mean reflectance (Fig. 3) reveals differences not only in the shape of the spectrum but also in overall mean reflectance, or brightness. Anadromous females possess little or no mean bias toward stronger reflectance of long wavelengths, unlike all other population-sex classes, and overall show higher reflectance, consistently above eight per

TABLE 1. Mean LM (a measure of relative red 'chroma') values calculated from reflectance data for ovulated and non-ovulated females from two populations of stickleback native to the Little Campbell River

Population	LM ovulated	LM non-ovulated
Stream-resident	0.21 (0.043, 13)	0.19 (0.039, 13)
Anadromous	0.015 (0.032, 7)	-0.01 (0.022, 7)

SE values,  $N$  in brackets.

cent. Stream females, stream males and anadromous males show a varying tendency toward stronger reflectance of long wavelengths and lower reflectance at most wavelengths. Nevertheless, anadromous males exhibit a higher peak reflectance than either sex of the stream-resident population.

#### *Ovulation and throat coloration*

Female red throat coloration does not appear to be determined decisively by short-term reproductive readiness (Table 1). The maximum LM of stream-resident females was higher when ovulated than non-ovulated but the difference was non-significant (paired  $t$ -test:  $t_{12} = 0.90$ ,  $p = 0.39$ ). A similar non-significant pattern was observed in anadromous females (paired  $t$ -test:  $t_6 = 1.21$ ,  $p = 0.27$ ). In addition, the LM values of both stream and anadromous females varied consistently among individuals in different stages of reproduction. Ovulated and non-gravid LM values were strongly correlated within each population (stream:  $r = 0.83$ ,  $N = 13$ ,  $p = 0.0004$ ; anadromous:  $r = 0.75$ ,  $N = 7$ ,  $p = 0.051$ ), though with a sample size of seven the results for the anadromous population are only marginally significant. For the stream females LM was also correlated with the human assessment of color (maximum overall LM vs maximum overall human red score:  $r = 0.78$ ,  $N = 21$ ,  $p < 0.0001$ ).

#### *Body size and throat coloration*

In the red index data derived from the 1995 slides there was a clear positive correlation between standard length and maximum red index for the stream-resident females ( $r = 0.497$ ,  $N = 35$ ,  $p = 0.0024$ ; Fig. 4a). Stream males exhibited a similar pattern but with a very small sample size it was not significant ( $r = 0.666$ ,  $N = 7$ ,  $p = 0.103$ ). Despite their relatively weak

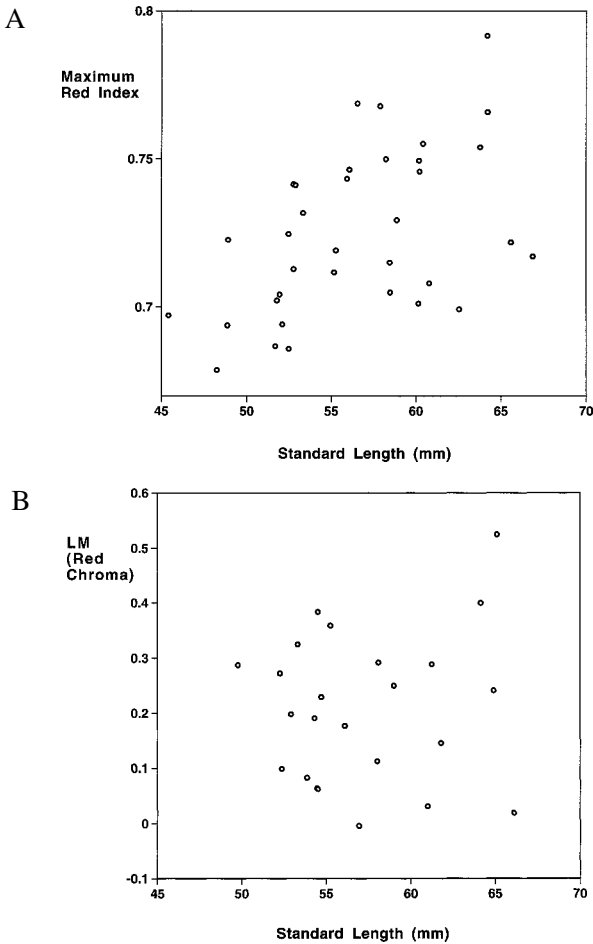


Fig. 4. A. Relationship between standard length and maximum value of throat red index, based on densitometer readings from slides, of stream-resident female threespine stickleback from the upper Little Campbell River (British Columbia). B. Relationship between standard length and maximum throat LM value, calculated from reflectance data, for stream-resident female threespine stickleback from the upper Little Campbell River (British Columbia).

throat coloration, anadromous females too exhibited a positive relationship between size and red index ( $r = 0.448$ ,  $N = 21$ ,  $p = 0.042$ ).

Correlations between body size and throat coloration were generally weaker and nonsignificant in the 1998 data, although positive in all three sex-population classes in which they were positive in 1995 (stream females:  $r = 0.109$ ,  $N = 24$ ,  $p = 0.61$ , Fig. 4b; stream males:  $r = 0.482$ ,

$N = 16$ ,  $p = 0.059$ ; anadromous females:  $r = 0.291$ ,  $N = 11$ ,  $p = 0.39$ ). The relationship was weakly negative for anadromous males ( $r = -0.116$ ,  $N = 8$ ,  $p = 0.79$ ) but far from significant. It is noteworthy that for the sex-population class of most interest, stream females, the pattern in size variation was somewhat different in the 1998 data set than in the 1995 data set. Specifically, very small individuals of less than 50 mm in length, which tended to have especially weak coloration, were absent from the 1998 data set.

## Discussion

The principal result of the present study is the quantitative documentation of red throat coloration in a population of threespine stickleback, a species usually viewed as possessing strictly dimorphic coloration. Upper Little Campbell stream-resident females possessed throat pigmentation markedly different from that of females from a nearby anadromous population. The throats of many females possessed an orange-red coloration which sometimes overlapped in 'LM', a simple measure of red chroma, with the throats of territorial males. Red pigmentation, however, was less extensively distributed over the body in females than in males.

This finding raises a series of questions. Initially, it is reasonable to ask whether the situation observed in the upper Little Campbell is unusual. This population has been extensively studied (Hagen, 1967; Hay & McPhail, 1975; McPhail & Hay, 1983) but the coloration of these females has not previously, to the best of our knowledge, been remarked upon in print. This is not necessarily because previous investigators did not notice the throat pigmentation of these females, since it was well known to J.D. McPhail (pers. comm.). Thus it is possible that female red throat coloration is not rare but has been ignored by investigators more interested in other aspects of stickleback biology. Indeed, Von Hippel (1999) has recently reported that females with red throats are present in a stream-resident population from Northern California. Red-throated females are also common in a Norwegian lake (S. Kraak & B. Mundwiler, pers. comm.) and occur at least occasionally in other populations (T.C.M. Bakker, pers. comm.; R.C. Sargent, pers. comm.). Clearly red-throated female stickleback are more common than has previously been indicated by the literature. Nevertheless, our impression from

informal observations of other populations and discussions with colleagues is that female coloration such as we have observed in the upper Little Campbell (and Kraak and Mundwiler have seen in Norway) is truly exceptional. Certainly the comparison with the Little Campbell anadromous population suggests this. A more systematic survey is required, however, before any authoritative generalizations about color-dimorphism in stickleback are possible.

Although knowledge of geographic variation in stickleback color dimorphism is in an early state, the present results suggest that the threespine stickleback may be an excellent model species for investigation of the evolution of diminished forms of male display traits in females of conventionally dimorphic species. Variation among populations, including independently evolving lineages in Europe and North America (Bell & Foster, 1994), provides a comparative perspective while the extensive research already conducted on male stickleback (Rowland, 1994) can inform experimental work with females.

Some results from the present study suggest tentative evolutionary conclusions and avenues for further work. The comparative results indicate that female coloration in the stream population is not a correlated response to the evolution of exceptionally strong coloration in stream males. The measure of red chroma used here, LM, was similar in stream and anadromous males. In fact, LM may underestimate chroma in the anadromous population given the higher mean for long wavelength reflectance of anadromous males. Also, red coloration appears to reach its greatest extent in some (though not all) males of the anadromous population, where it can cover extensive lateral portions of the male's body back to the anal fin. This is in contrast to the stream males in which coloration is more concentrated in the head and immediately adjacent areas. Clearly stream females are not more intensely red than anadromous females as an indirect effect of the evolution of especially intense or extensive red coloration in stream males. However, investigation of additional populations, especially freshwater populations with less conspicuously colored females, is required to assess patterns of dimorphism more comprehensively. It is also important to note that the pattern in dimorphism between populations does not eliminate the possibility that female coloration is generally caused by a correlated response to the evolution of male coloration. Reduced selection against such coloration in females of the stream population, perhaps due to differences in predation, may permit

expression of this correlation and thereby account for that population's more intense female coloration.

The presence of red coloration in the throats of some anadromous females suggests that the throat coloration of the stream population in question is quantitatively rather than qualitatively different from anadromous or marine threespine stickleback. It is also noteworthy that red is expressed elsewhere on the body in both populations, on the membranes attached to the pelvic spines. The function of this pelvic coloration in females is unknown but it is possible that red throat coloration enhances the signal otherwise manifest only in the pelvic spine membranes.

Female LM tended to be higher in ovulated females of both populations than in non-ovulated females, but the pattern was not statistically significant. Nevertheless, these data do not show decisively that unovulated females possess throat coloration as intense as that of ovulated females. If female throat coloration is influenced by social interactions (Bakker, 1994; Candolin, 1999) it is possible that our failure to detect a significant difference between ovulated and nonovulated stream females is due in part to their different social experiences. Even so, it is still clear that female red coloration is not simply a signal of ovulation, or it could not be so elevated in nonovulated females. This is in contrast to the lateral barring pattern on females from Long Island, which apparently conveys just such information (Rowland *et al.*, 1991). In addition, female LM varied relatively consistently among individuals over the period of the study, as the LM values of ovulated and non-ovulated females were strongly correlated.

The positive relationship between the red index and stream female standard length in the 1995 data set suggests that throat coloration may reinforce the perceived size of females to conspecifics. This might benefit a female, especially if she is large, either in a courtship or agonistic context; but one also could envision simple mechanistic explanations, such as slow accumulation of dietary carotenoids, which might account for more intense red coloration in larger, possibly older fish. In a bird, the Lesser Kestrel, expression of male traits increases with female age, leading Tella *et al.* (1997) to suggest that non-adaptive age-related patterns in hormone production lead to expression of typically male traits in older females. In any case, the size-chroma relationship in the present study was inconsistent among years and methodologies. However, the weaker results for 1998 could easily be due to a paucity of small, weakly colored females in that sample. In addition, the positive

(though inconsistently significant) relationship between size and red throat coloration observed in all sex-population samples except the anadromous males suggests that the correlation between size and red intensity may be a relatively general one in these populations. These patterns deserve additional attention and should be considered in the development of further hypotheses concerning the evolution of female throat coloration.

Red throat coloration was at one time argued to release aggression in male stickleback (Tinbergen, 1951) and although the current view of the effect of the male red signal is more complex (Rowland, 1994), certainly red throat coloration in most populations indicates to a male the presence of a likely rival. In this light it is noteworthy that the red throat coloration of upper Little Campbell female stickleback should be highly conspicuous to males when females perform the head-up behavior typical in courtship. Thus courting stream-males are likely to be exposed to a signal associated in most populations with an intruding competitor, a signal which frequently elicits aggression. It is perhaps surprising, then, that female throat coloration does not change substantially with ovulation. If upper Little Campbell males behave aggressively toward a flash of red in the middle of their territory, as previous studies suggest they should, one might predict that female red chroma should diminish in ovulated females preparing to spawn. Alternatively, the possibility remains open that a red throat on a gravid female elicits a very different male response than a red throat on a male body. Clearly evaluation of the male response to female red throat coloration is important; we have collected some data on this point, but this work is not yet complete. The red throat may also function as a signal to other females, and this possibility too deserves investigation.

Analyses of spectral data are still rapidly evolving and remain controversial (*e.g.* Cuthill *et al.*, 1999; Grill & Rush, 1999). Thus it is worth considering whether our analyses are appropriate and robust. We contend that they are sensible given the current state of knowledge in this field. We nevertheless acknowledge that as new methods are developed and further data collected on the stickleback visual system (especially if data are gathered for our study populations), revisions may be in order. Our central finding, that females of the stream population have exceptional throat coloration for this species while males of that population do not, is unlikely to change substantially — simple inspection of the reflectance curves reveals obvious patterns which are robust to different methods of analysis. Whether one analyzes

the pattern using the human eye, slides and a densitometer, models based explicitly on the stickleback visual system, or either segment classification or principal components analysis of reflectances, this pattern is unlikely to change greatly. The relationships between throat coloration and ovulation, however, could conceivably prove significant with a different analysis of the reflectance data, a larger sample and/or more consistent social experiences. But ovulation is clearly not the whole story since females with high chroma when ovulated also possess relatively high chroma when not ovulated. The body-size coloration relationship is our least robust finding. Our data and our experience with the stream population lead us to suspect that the correlation is a real one but not strong, important mainly at the extremes of body size. Thus this relationship may be most sensitive to choice of color-analysis method. In any case, we have made some preliminary comparisons between LM and a measure of red chroma based on data for the cones of the stickleback eye (using a model modified slightly from Baube *et al.*, 1995). For the ovulated stream female data set, the two measures were strongly correlated ( $r = 0.97$ ,  $N = 18$ ,  $p < 0.001$ ).

We conclude by noting that, although stickleback have been studied intensively for as long as almost any fish, the species continues to supply us with new phenomena to investigate. The relatively late discovery of the white stickleback (Blouw & Hagen, 1990) is another excellent example of such a finding. It is remarkable how ignorant we remain of the natural history of even the best studied creatures.

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