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Sensory exploitation and condition indication may explain red pelvic spines in the brook stickleback Culaea inconstans

Andrew Hodgson
Eastern Washington University

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SENSORY EXPLOITATION AND CONDITION INDICATION MAY EXPLAIN RED PELVIC SPINES IN THE BROOK STICKLEBACK *CULAEA INCONSTANS*

A Thesis

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In Partial Fulfillment of the Requirements

for the Degree

Master of Science

By

Andrew Hodgson
Advisor: A. Ross Black

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Thesis of Andrew Hodgson approved by:

____ Date_______________

Dr. A. Ross Black, Graduate Committee Chair

____ Date_______________

Dr. Camille McNeely, Graduate Study Committee

____ Date_______________

Dr. Ryan Sain, Graduate Council Representative

Master's Thesis

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ABSTRACT

Background: Sensory bias models of the evolution of sexually selected traits predict that trait preferences evolve in a nonsexual context such as prey selection. Indicator models predict that sexually selected traits indicate mate condition. I investigated the potential for sensory exploitation and condition indication models to explain the evolution of what appears to be a recently evolved sexually selected trait.

Question: Did red pelvic spine coloration in male Turnbull National Wildlife Refuge (TNWR) brook stickleback (*Culaea inconstans*) evolve to exploit a preexisting sensory bias for red prey, thus helping males draw females to the nest? Or, did it evolve as an intersexual signal indicating male condition to females?

Methods: I recorded the frequency of red pelvic spine coloration in males versus females and breeding versus non-breeding males. I measured the condition factor of males with and without red coloration on their pelvic spines. I presented fish with a paired choice between a red versus an orange, yellow, green, blue, or purple bead, and recorded the proportion of bites at each color. I tested for sexual dimorphism in pelvic spines and made observations on their use by territorial males in comparison to dorsal spines.

Results: Red coloration is significantly more common in males than females and in breeding than nonbreeding males. TNWR brook stickleback prefer red to other colors in a predation context. Males with strongly red pelvic spines have a significantly higher mean condition factor than those with plain spines. Pelvic spine size is similar in males and females. Males tend to extend their dorsal spines more often than their pelvic spines during agonistic encounters.

Conclusions: Red pelvic spine coloration of TNWR brook stickleback is a secondary sexual character which may exploit a preexisting sensory bias for red prey while also indicating condition to females.

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INTRODUCTION

Darwin's original observation that sexual ornaments and behaviors evolve and persist despite reducing survival is widely supported (e.g. Cade 1975, Endler 1980, Tuttle and Ryan 1981, Promislow *et al.* 1992, Basolo and Alcaraz 2003, Hebets 2005). However, the ultimate mechanisms driving mate choice remain controversial despite much theoretical work and experimental investigation (for reviews see Ryan 1990, Kirkpatrick and Ryan 1991, Andersson 1994, Basolo 1995, Møller and Alatalo 1999, Kokko et al. 2003, Fuller and Noa 2010).

Models currently used to describe the evolution of mate choice include "direct benefits," "indicator mechanisms," and "Fisherian runaway sexual selection." Thus far, none of these have emerged from empirical studies with a clear claim of greater importance than the others (Basolo 1996). Direct benefit sexual selection models hold that females evolve a preference for cues in males that relate directly to offspring chances of survival (e.g. Künzler and Bakker 2000). This is the least controversial of mate preference hypotheses. It applies when males provide females with direct benefits such as food, territory, shelter, or parental care. It does not apply to the many cases in which females choose between males on the basis of characters not directly related to offspring survival (e.g. courtship dances, songs, conspicuous coloration patterns). Therefore, the major point of contention in sexual selection theory is the origin of apparently indirectly beneficial, intersexually selected mating preferences, such as that for red coloration in sticklebacks.

Darwin (1871) believed that intersexually selected traits were arbitrarily attractive, evolving because many animals simply possess an aesthetic sense (Prum 2010, 2012). In the same way that pigeon fanciers have added beauty to domesticated breeds, females in nature may select appealing qualities in males. He did not provide an explicit hypothesis for the evolution of female mate preferences (Darwin 1871, Kirkpatrick 1982).

Wallace (1891) dismissed female choice. He explained sexually dimorphic ornaments and behaviors as means of protection, conspecific recognition, or the release of "superabundant nervous energy." Fisher (1915) disagreed with Wallace, but admitted the most convincing of his arguments was the lack of an explanation for the origin of an aesthetic sense. He suggested an individual might increase the success of its offspring by choosing mates with certain attributes indicative of health (e.g. physiological or immunological), and that sexually selected traits may provide such information. This "good genes," or "indicator mechanism" argument for the origin of female preference developed throughout the 20th century (see Williams 1966, Zahavi 1975, Hamilton and Zuk 1982) and is now a leading theory of mate preference evolution with empirical support (Møller and Alatalo 1999).

Another leading theory, Fisherian runaway sexual selection (Fisher 1930, O'Donald 1967, Lande 1981, Kirkpatrick 1982) assumes an initial period in which those possessing a trait favored slightly by natural selection are also favored by certain mates, causing genes for the trait and those for the preference to increasingly occur together in succeeding generations. Over time the frequency of the preference and the advantage of the preferred trait come to reinforce each other by positive feedback. The process is thought to continue until positive sexual selection and negative natural selection of the trait are in balance.

Any model of sexual selection must explain both the origin of a female preference as well as the evolution of the preferred male trait (Schlupp *et al.* 1999). The current models do so in different ways (Ryan and Keddy-Hector 1992, Endler and Basolo 1998, Fuller *et al.* 2005): indicator and direct benefits models predict that the preferred trait evolves prior to or along with the preference. The Fisherian runaway model predicts the preference and the trait evolve together.

Empirical support for both indicator mechanisms and Fisherian runaway selection (Bakker 1993, Boughman 2007, McLennan 2007) in the evolution of male stickleback red breeding coloration imply that it coevolved with a female preference for red. However, there is also evidence that the preference for red arose by natural selection before the trait, in the form of a sensory bias for red prey items (Smith *et al.* 2004).

The sensory bias model of sexual selection is a recently developed (West-Eberhard 1979, 1984; Ryan 1990, Ryan and Keddy-Hector 1992, Basolo 1996) alternative to indicator and Fisherian runaway selection hypotheses. Sensory bias predicts that naturally selected adaptations of sensory and/or cognitive systems cause a preference for certain traits in some mates over others, generating selection for those traits and causing them to increase in frequency despite potentially reducing survival. Sensory bias can be distinguished from other models in that it predicts the preference for a trait evolves before and/or in a different context than the preferred trait, for instance the context of prey selection. However, it should be emphasized that any or all of the above mentioned potential mechanisms could act on the same population and contribute to the evolution of mate preferences (Andersson 1994).

Smith *et al.* (2004) showed that ninespine stickleback *Pungitius pungitius* (a representative of threespine stickleback *Gasterosteus aculeatus* ancestry with no red breeding coloration) direct significantly more bites at red-colored plastic strips than strips of other colors, providing evidence for a sensory bias origin of the more derived red breeding colors of the threespine stickleback, which females of the species find attractive (Bakker and Mundwiler 1994, Östlund-Nilsson *et al.* 2007). In this scenario red body color evolved because it exploited an ancestral sensory bias for red, which itself may have evolved through fitness advantages provided by the consumption of red/orange prey.

I test for evidence that sensory bias and/or condition indication have played a role in the evolution of what appears to be a recently evolved sexually selected trait: red pelvic spine coloration in the Turnbull National Wildlife Refuge population of brook stickleback (*Culaea inconstans*). A preference for the color red in this population in a nonsexual context (*i.e.* predation) would suggest a sensory bias origin for the trait. Superior physical condition in individuals with the trait over those without would suggest the trait could be selected by females to increase their fitness, and lend support to the condition indication sexual selection model. I also asked whether pelvic spine size differs between the sexes. Larger spines in males would be consistent with their use as agonistic and/or sexually attractive signals. Finally, in an effort to determine how red pelvic spine signals may be used I made observations comparing their extension to that of the dorsal spines during various common brook stickleback behaviors.

MATERIALS AND METHODS

Fish Collection

I collected brook stickleback for all studies using un-baited mesh-walled minnow traps and dip nets from the outflow of Winslow Pool into Middle Pine Lake, Turnbull National Wildlife Refuge, Spokane Co., WA (47°24′45.45″N, 117°32′19.16″W). I set traps for one hour or less. The fish at this location live in thick shoals of several dozen individuals. They were most likely introduced to the watershed in the late 1990's by bait bucket transfer (Scholz *et al.* 2003). Macrophytes, algae, and mud provide shelter. Invertebrate prey is abundant. The only likely substantial predators are diving beetles and dragonfly larvae. I collected under a permit from the U.S. Fish and Wildlife Service (# TBL-11-017r). The Eastern Washington University Institutional Animal Care and Use Committee approved my methods.

Red Pelvic Spines and Condition

I collected fish for pelvic spine observations in November of 2011, and April, May and October of 2012. My estimate of the breeding season was based on the presence of gravid females and nuptially-dressed (dark black) males, which were observed in April and May but not present in samples from October through February. Fish were killed (using cerebral percussion) and examined within 30 minutes of collection immediately before processing. It is possible that my results underrepresent coloration due to color fading during the time elapsed between collection and processing. I scored pelvic spine coloration by eye through a dissection microscope. Coloration was confined to the posterior portion of the spines. Coloration scores included: none (plain spines), mild (small patch of reddish-orange pigment droplets at the base of the spines), moderate (continuous coloration extending approximately halfway up the spines), or strong (continuous coloration extending almost the full length of the spine). I did not use image analysis software for spine color measurement because I could not obtain a sufficiently high-resolution image of the colored areas of the spines due to their small size. For condition measurements I weighed each fish to the nearest 10th of a gram, measured total length to the nearest millimeter, I calculated the condition factor (K) for each fish as:

$$K = 100 \times \frac{weight}{length^b}$$

where b is the slope of the linear least squares regression of $\log_{10}$(weight) on $\log_{10}$(length) (Milinski and Bakker 1990, Bakker and Mundwiler 1994, Bolger and Connolly 1989). I then judged the level of coloration of the pelvic spines. The sex of all fish was determined by the presence of eggs or testes.

Color Preferences

I collected fish for prey color preference tests in January, February, November, and October of 2011 and January of 2013. They were transported in pond water and kept in groups of about 50 in a 200 l tank filled with tap water for one to four weeks of training to feed in captivity. The training tank was covered on three sides with pond-like scenery. It included brown aquarium rocks and thick plastic macrophytes. A chiller held water temperature at 16±2 °C. Photoperiod was set to match the natural photoperiod. I fed fish brown-colored dried chironomids (Penn-Plax® Pro Balance™ Blood Worms) or white-colored frozen mysid shrimp (San Francisco Bay Brand®) several times a day. The food was soaked in tank water and injected into the center of the tank through a modified transfer pipette. This procedure facilitated color preference testing.

For color preference trials I transferred three fish at a time to a 38 l testing tank. I covered the testing tank sides with scenery like that of the training tank, but on the back with a green shower curtain, which made beads and fish easier to distinguish. The testing tank included brown aquarium rocks but no artificial vegetation. Lighting for both tanks included a full spectrum Coralife® 14,000 K HQI lamp to emulate natural sunlight and the Sylvania Ecologic® 32 W fluorescent lighting of the room. After transfer to the testing tank I immediately presented fish with a pair of colored beads suspended on 32 cm long fishing line from a Tiny Love® Take-Along Mobile™ for babies. Bead pairs hung 41 mm apart and revolved at three rpm's around the tank's center. Beads measured 4.21 mm in height and 2.34 mm in width. From videos taken behind a black shroud with a Nikon D5100 camera I recorded the color of the first bead bitten by each fish. In order to quantify bead colors, I averaged red, green and blue (RGB) values for each bead using the selection tools and the "average" blur filter in Adobe Photoshop® (Table 1). Color measurements came from a single photograph taken with flash (Nikon D5100, exposure: 0.05 sec, aperture: f/18.0, ISO speed: 640) of all the beads used in the experiment. Stickleback vision corresponds to human vision (McKinnon 1995) with a few exceptions (Rush *et al.* 2003).

I conducted at least 12 trials for each color pair (red vs orange, red vs yellow, red vs green, red vs blue, red vs purple). Lone brook stickleback are rarely bold enough to bite beads. Therefore, I used three fish in each trial. From among the three fish, I recorded the color of the bead that was first bitten by the first fish to make a selection. All subsequent selections from this fish the others were ignored. Thus, each individual three-fish trial produced a single color selection and was considered a single trial replicate.

Pelvic Spine Size

I collected fish for pelvic spine size observations in November and December of 2012. I killed them by cerebral percussion. A digital caliper was used to measure standard fish length to the nearest tenth of a millimeter. I cleanly removed the spines at the joint, removed the membrane from the spine, and measured its total length at the anterior end from the joint to the tip with an ocular micrometer to the nearest 100th of a millimeter. I centered the spine in the field of view of the microscope and measured the width perpendicular to the midpoint of its length (mid-spine width), also to the nearest 100th of a millimeter. Relative spine length (spine length divided by the standard length of the fish), and relative mid-spine width (spine width divided by spine length) were calculated to correct for size differences of the fish. These values were used in the statistical analyses.

I calculated relative spine area as the product of relative mid-spine width and relative spine length. This calculation assumes the shape of the whole spine is approximated by an isosceles triangle that is similar and twice as tall as that delineated at the corners by endpoints of the line described by the mid-spine width measurements and the tip of the spine.

Spine Use

I collected fish for spine use observations in early July 2012. Three males kept in a large 200 l tank began exhibiting territorial behavior after about three weeks in captivity. The other three fish in the large tank were females who remained near the tank's surface and did not defend territories. Three other males established territories over the same period of time in a 100 l tank. Both tanks were planted with thick bundles of *Elodea* at the two back corners and center and were covered on the back and sides with photographs of vegetation. I considered a male territorial if he possessed nuptial colors (dark black and with a black vertical bar over his pupil), spent almost all of his time in one spot near a bundle of elodea and attacked other fish who came near. Behavioral observations lasted three days. An initial focal fish was chosen at random and observed for 10 minutes. After this I rotated through focal fish until all of them had been observed at least once per day. Brook stickleback have five dorsal spines and two spines extending from their pelvis. The spines normally lie flat against the body but are extended when they are threatened by predators and during agonistic encounters with conspecifics and courtship. I recorded the extension (or lack of extension) of dorsal and/or pelvic spines during four common stickleback behaviors including: (i) yawns, which are an apparently non-agonistic behavior, perhaps a reflex, in which the fish fully opens his mouth and stretches his body (see Morris 1958); (ii) Sigmoid or S-displays, where the fish bends laterally into the shape of an "S" (Burks *et al.* 1985, McLennan and Ward 2008); (iii) circle fights, where fish chase each other head to head in a circle (Burks *et al.* 1985, McLennan and Ward 2008); and (iv) charges/bites, where one fish charges directly at another, sometimes biting. I also recorded the use of dorsal and/or pelvic spines when fish were not engaged in any of these behaviors.

Statistical Analyses

I used binomial tests (Howell 2007) with an alpha of 0.05 to determine male versus female, and breeding versus non-breeding season differences in the frequency of red pelvic spine coloration. Data from fish sampled during the breeding season were pooled for analysis, as were those from outside the breeding season.

I used ANOVA with Tukey HSD (Quinn and Keough 2002) post-hoc comparisons to test for condition factor differences between groups with different degrees of red pelvic spine coloration during the breeding season.

I analyzed the frequencies of bites at beads of different colors using binomial tests in which expected bite frequency at each color was 0.5 and alpha varied according to the Holm-Bonferroni sequentially reflective multiple test procedure (Holm 1979). For inspection times and latencies to bite I ran Wilcoxon rank sum tests to determine differences because the data could not be normalized. I checked for trends over time in latency to bite using least-squares regression.

I tested for differences in relative pelvic spine length using t-tests and for differences in relative pelvic spine width and relative spine area using Mann-Whitney U tests because this data was nonparametric.

Relative use of dorsal and pelvic spines during different behaviors was analyzed with t-tests when individual fish were treated as the units of replication and with binomial tests when individual behaviors were treated as the unit of replication.

RESULTS

Red Pelvic Spine Coloration

Frequency of pelvic spine coloration peaked in males during May and was lowest in mid-winter (Fig. 1). Frequency of red/orange pelvic spine coloration was greater ($Z=18.5$, $p<0.0002$) in males ($mean=0.61$, $SE=0.02$, $n=429$) than females ($mean=0.03$, $SE=0.01$, $n=440$) during the breeding season. Frequency of red spines with mild, moderate, or strong coloration was greater ($Z=4.5$, $p<0.01$, $n=369$) among males during the midsummer breeding season ($mean=0.29$, $SE=0.03$) than in non-breeding males ($mean=0.09$, $SE=0.04$) sampled in November 2011 and October 2012.

Level of Spine Coloration and Condition

Condition increased with the level of pelvic spine coloration (ANOVA, $F_{3,148}$ =3.47, p=0.017, Table 2, Fig. 2). Males with strong coloration had a higher condition factor (mean=1.23, SE=0.03, Tukey HSD, p=0.026) than those with plain spines (mean=1.15, SE=0.02). All other post-hoc pairwise comparisons were nonsignificant.

Color Preferences

Fish bit red beads significantly more frequently than yellow, green, blue and purple but not orange beads (Table 2, Fig. 3). First bites by individuals (i.e. the initial choice of each fish) were similarly directed at red beads significantly more often than any other color except for orange (Table 2, Fig. 4). When the analysis is run using only the first bite per trial, which eliminates the possibility of a lack of independence between replicates, fish bit red beads significantly more frequently than other colors except for orange (Table 3, Fig. 5). Throughout all trials and all paired comparisons, the frequency of bites at red beads by females (0.79±0.04, n=98) was statistically indistinguishable (Z=1.125, p=0.26) from that of males (0.84±0.04, n=185). Median length of inspection did not differ significantly between inspections of red beads and beads of other colors (Table 5, Fig. 6). Median latency to bite red beads was lower than latency to bite every other color except green and was significantly lower for bites at red than blue (Table 6, Fig. 7).

Pelvic spine size

Females had longer relative mean pelvic spine lengths on both the right and the left side (Table 7). For left-side spines the difference was not significant (t=1.759, p=0.08). For right-side spines the difference was significant (t=2.733, p=0.007). Males had significantly greater relative median spine width on the right side (U=897.5, p=0.017) but not on the left (U=1,030.0, p=0.097). Relative median pelvic spine area did not differ between males and females (males: median=0.0280, range=0.0282, females: median=0.0276, range=0.0244; U=1123, p=0.31).

Spine Use

Territorial males extended pelvic spines significantly fewer times in 10 minute periods than dorsal spines (pelvic spines: mean= 2.00, SE=0.93; dorsal spines: mean=15.33, SE=3.27; t=3.93, p=0.003). Pelvic spines were also extended significantly fewer times than dorsal spines specifically during agonistic encounters (pelvic spines: mean= 2.00, SE=0.93; dorsal spines: mean=6.83, SE=1.92; t=2.26, p=0.047). Dorsal spines were most commonly extended when fish were not executing any other behavior, while pelvic spine extension was most common during yawns (Table 8). Fish were more likely to extend dorsal spines than not during all behaviors and never extended their pelvic spines except when also extending dorsal spines (Table 8). In the foregoing analyses individual fish were treated as the unit of replication. When behaviors are treated as replicates dorsal spine use is significantly more common than the use of dorsal+pelvic spines for all behaviors except yawns (Table 9, Fig. 8).

DISCUSSION

Red Pelvic Spine Coloration

To My knowledge red pelvic spine coloration in brook stickleback has not been previously reported. With the possible exception of fifteenspine stickleback *Spinachia spinachia*, (Östlund-Nilsson 2000), all stickleback genera exhibit some kind of breeding coloration. The red nuptial dress of the threespine stickleback typically includes red pelvic spines as well as a red jaw and belly (Bigelow and Schroeder 1953). Nonterritorial male threespine stickleback (McLennan and McPhail 1988) as well as females in some populations (Bigelow and Schroeder 1953, Nordeide 2002) develop red coloration in their pelvic spines. Blackspotted stickleback (*Gasterosteus wheatlandi*) males have red pelvic spines (Bigelow and Schroeder 1953, Östlund-Nilsson and Mayer 2007). Breeding fourspine, or “bloody” stickleback (*Apeltes quadracus*) males have bright red pelvic spines (Bigelow and Schroeder 1953, Reisman 1963, Rowland 1974, Bayer 1980). Breeding male ninespine stickleback *Pungitius pungitius*, the sister species to the brook stickleback, have no red coloration (but see Bigelow and Schroeder 1953). They develop a black ventral surface that contrasts with bright white pelvic spines. Reisman and Cade (1964) in a study of a brook stickleback population from New York report that in breeding males, the body is black and the ventral spines are white as in *P. pungitius*.

McLennan (1993a) leaves red pelvic spine membrane presence/absence criteria out of her behavior-based Gasterosteid phylogenetic reconstruction citing a lack of information on the subject at the time for the brook stickleback. McLennan (2011, personal communication) has indicated that red pelvic spines in the brook stickleback would represent a return to the ancestral stickleback condition. This, along with a general lack of reports in the literature of red pigment being expressed at all in this species, suggest that the Turnbull National Wildlife Refuge brook stickleback (hereafter TNWR stickleback) are unusual in this respect.

Red pelvic spine coloration in breeding males, which was evidently lost in brook stickleback ancestry, has reappeared at least in this population. While the trait was lost, the female preference for it may have persisted as a side effect of sensitivity to red prey. Brook stickleback females might be swayed into spawning with a red-spined male over a plain-spined male, all else being equal, simply because she is attracted to red.

The pigment or pigments making up the red color in TNWR stickleback are not known. The hue appears to vary from strongly red to neon orange. Spines without red coloration can be transparent to mildly yellowish. The specific hue may depend on the relative amounts of carotenoid pigments (e.g. red astaxanthin and yellowish tunaxanthin/lutein) as in threespine stickleback (Wedekind *et al*. 1998).

Level of Spine Coloration and Condition

My results are consistent with other findings regarding stickleback red coloration and preferences for red. In threespine stickleback there is evidence that both the preference and the trait have become exaggerated through an indicator mechanism (Boughman 2007). The magnitude of red expressed is often positively condition dependent (e.g. Milinski and Bakker 1990), but this varies among populations. Boughman (2007) found that red color was strongly condition dependent in limnetic stickleback, weak in anadromous forms, and nonexistent in benthic forms. Bakker and Mudwiller (1994) found that in males from two sites with limited intermigration, males with more intense red received more eggs from females, but redder males were in better condition at only one of the sites. Candolin (1999) reports a curvilinear relationship with condition as measured by lipid stores. Red coloration was most intense in males of high and low condition, and lower for males of intermediate condition. Frischknecht (1993) found red coloration correlated with current, short-term condition while intensity of blue eye color indicated long-term, overall condition and development. Barber *et al.* (2001) found offspring of redder stickleback fathers grew more slowly than those of dull fathers, but also that they were more resistant to parasitic infection. Red coloration in stickleback is often, but not always, associated with greater condition.

In TNWR stickleback females choosing higher-condition males with strong red spine coloration over lower-condition plain-spined males may benefit either directly or genetically or both. High condition males could be better parents (males care for the eggs and fry), better territory-holders, more resistant to disease (Milinski and Bakker 1990, Barber *et al.* 2001), and/or possess genes for any of these traits and/or better condition itself.

Color Preferences

Sexual signals can evolve to indicate condition even if they evolved originally because they exploit the sensory system of mates (Garcia and Ramirez 2005, Arnqvist 2006). This scenario has been suggested for threespine stickleback (Boughman 2007) based on findings that the color red is preferred in non-mating contexts. For example threespine stickleback bit red plastic strips at a higher rate than other colors (Smith *et al.* 2004). Ninespine stickleback have been observed to do the same (Smith *et al.* 2004), even though this species does not exhibit any red coloration. Ibrahim and Huntingford (1988) found that threespine stickleback use the color red as a cue in predation. Rowe *et al.* (2004) showed that the spectral sensitivities of the threespine stickleback are optimized for representing its red breeding colors. They found similar spectral sensitivities in ninespine, blackspotted, and fourspine stickleback. Based on these results the authors suggest a sensory bias origin of red sexual colors (Rowe *et al.* 2004). Morris (1958) investigated the use of differently colored objects by males when building nests. He offered ninespine stickleback red, green, blue, and yellow cotton strings for nest building and found that yellow was strongly preferred. Threespine stickleback initially used green cotton but developed a preference for red cotton as they worked. This led to a red ring around the nest entrance. Östlund-Nilsson and Holmlund (2003) found similar results using red and green algae. The nest-building males also chose to decorate with red sticks over green, silver, blue, or yellow sticks and blue spangles. Females showed a strong preference for males with decorated over non-decorated nests. Östlund-Nilsson and Holmlund (2003) suggest conspicuous nest decorations may indicate low levels of nest predation or male dominance to females, and that the male use of red reflects a preference for their own nuptial colors. However, it also seems possible that a preexisting bias for red has played a role in male and female nest material preferences.

One prediction of sensory bias models is that preferences arise ancestrally to the traits they favor (Proctor 1992, Endler and Basolo 1998). My results do not address this prediction; nor do those of Smith *et al.* (2004). This is because red breeding colors, in the form of red pelvic spines, originated in an ancestor of all the sticklebacks thus far tested for color preferences in a nonsexual context. Ancestral representatives such as the fourspine stickleback and the yellow tubesnout *Aulorhynchus flavidus* (Hart 1973, Akagawa *et al.* 2004) express red pigment in their pelvic spines or fins, therefore the red prey color preferences of threespine, ninespine and brook stickleback could be a side effect of ancestral preferences for mates with red coloration. In this scenario, females would be predicted to more strongly favor red prey than males, and in species with no red coloration they should be expected to prefer in their prey the color that is most prominent in the sexually displaying males of their species. The former prediction is not upheld in prey color preference studies of threespine or ninespine stickleback (Smith *et al.* 2004). From my study there is also no evidence for a sex difference in prey preference in brook stickleback. The later expectation is not upheld in ninespine stickleback (Smith *et al.* 2004). Breeding males exhibit black (body) and white (pelvic spine) coloration (Smith *et al.* 2004, Morris 1958), yet both sexes favor red prey. Cronly-Dillon and Sharma (1968) report a higher spectral sensitivity at the red end of the spectrum in female than male threespine stickleback during the breeding season. This result has been refuted in a more recent study which found a similar positive breeding season increase in red sensitivity in both sexes (Boulcott and Braithwaite 2007). Also, it seems unlikely a bias for red prey as strong as I found in TNWR stickleback could have arisen from a preexisting preference for a sexual signal as subtle as red pigmentation on the back of the pelvic spines, which is usually not visible (the spines are held up against the body most of the time), only occurs in about half of breeding males, and may not exist at all in most populations.

Color sensitivity can depend upon water clarity (Levine and MacNichol 1979, Endler 1992). Species residing in shallower waters tend to be relatively sensitive to red, and those in more colored waters to blue. The ponds at TNWR are shallow and the water is tea-colored due to high concentrations of dissolved organic compounds. Thus environmental light conditions may in part explain the apparent sensitivity to red in TNWR stickleback, as they do in threespine stickleback (Boughman 2001). This assumes that the fish either experienced similar conditions before their introduction in the late 1990's or adapted visually to the new conditions by way of phenotypic plasticity or evolution. Fish with a greater sensitivity to red may have been at an advantage because red coloration is common in invertebrate prey, or due to the health benefits of consuming carotenoids. The TNWR ponds contain numerous blood-red chironomids, pink and red branchiopods, red copepods and pink amphipods. These taxa are a good source of carotenoids (Tanaka *et al.* 1976, McLennan 2007), which cannot be synthesized by animals but are important for maintaining health (Smith *et al.* 2004 and references therein).

Pelvic Spine Size

TNWR stickleback show slight sexual dimorphism in spine dimensions but no overall difference in area. These findings do not support my prediction that males would have larger spines because they are being used as agonistic and/or sexual signals.

Spine Use

The red pelvic spines in TNWR stickleback meet Darwin's suggested criteria (Andersson 1994) for sexual selection in a visual display in that it is developed in males almost exclusively and only during the mating season. Another of Darwin's criteria for invoking sexual selection is that the trait is used in displays directed at potential mates or same-sex rivals. Pelvic spines are important in both male-male agonistic and male-female sexual interactions in all the stickleback genera with the possible exception of the fifteenspine stickleback, for which I found no information on the subject.

Territorial male threespine and blackspotted stickleback erect their pelvic spines during charges at rivals, head-down and broadside threat displays (Van Iersel 1953, McInerney 1969). In fourspine stickleback (see Rowland 1974), territorial males erect their red pelvic spines in response to the approach of an intruder. The intruder then typically assumes a head-down position from which the defending fish's pelvic spines are conspicuous. The pelvic spines are also raised in lateral displays. Attacking fish charge into a neighbor's territory with all spines depressed but may raise them afterward and almost always flee with dorsal spines erect and pelvic spines depressed. In ninespine stickleback pelvic spines are associated with threats. They are held erect when threatening to attack and depressed in fish more likely to flee (Morris 1958).

Brook stickleback commonly erect their pelvic spines during S-displays and flare them during broadside displays, head-down displays, and circle fights (McKenzie 1969, McLennan 1993b, Ward and McLennan 2005). In the TNWR brook stickleback population, I observed the occasional use of pelvic spines in S-displays, broadside displays and circle fights. I did not see head-down displays. Only the posterior of the pelvic spines is colored in this population and the pigment is barely visible (through the transparent portion of the spine membrane) when looking at the erect spines from the front, but is strikingly conspicuous when viewed from directly behind and below the fish. In the agonistic interactions described above males would rarely perceive each other from behind and below. Thus, while the red pigmentation may have evolved in part as a signal between rival males I suggest that it functions mainly as a signal to potential mates.

Direct interaction of the female with the male pelvic spines during courtship has been reported in four of the six stickleback genera. Blackspotted stickleback males hold their pelvic spines fully erect during courtship. The female follows close behind and below while fixating on the pelvic spines and nudging the male between them. The female drags her abdomen on the substrate in order to remain in contact with the pelvic region of the male. Such contact appears to be necessary for the courtship to proceed even though it can be difficult to maintain due to variation in substrate topography and the circuitous route the pair takes to the nest (McInerney 1969). Fourspine males also hold their pelvic spines erect throughout thier attempts to attract a female to the nest. When a male leads a female to the nest he assumes a head down posture and she a head up posture below him. Thus, both fish seem to facilitate the interaction as the female fixes her gaze on his bright red pelvic spines. She follows him to the nest in this orientation and frequently nudges between the pelvic spines (Reisman 1963, Rowland 1974). Ninespine stickleback male nest-leading behavior is similar to that described in the fourspine in terms of the orientations of the fish and the females strong interest in the pelvic spines (Morris 1958), but the pelvic spines are bright white instead of red.

In McKenzie's (1969) description of courtship in brook stickleback from Ontario, CA, the male vibrates his fins with both dorsal and pelvic fins fully erect in his initial response to a ripe female. He lunges and strikes her on the top of the head. She may respond by turning head-up and sinking to the bottom (see also McLennan 1993b). The male then swims toward his nest with exaggerated side-to-side movements. If she so chooses the female follows him back to the nest from behind and below, a position from which she has a clear view of his pelvic spines, which are held erect during this display.

Conclusions

Darwin's (1871) assumption that animals possess a "taste for the beautiful" could be construed as an early form of a preexisting sensory bias hypothesis of mate preference evolution. The idea that preferences could evolve independently of favored traits was not taken seriously until a century after his death. Today, it is clear that sensory biases will need to be integrated with the more traditional mate choice explanations (e.g. direct benefits, indicator mechanisms and Fisherian runaway) in order to explain the form of preferences in addition to their origin and persistence (see Ryan and Keddy-Hector 1992, Kokko *et al.* 2003).

Sensory bias appears to have played a role in the evolution of "courtship trembling" behavior in *Neumania papillator* water mites (Proctor 1991, 1992), complex mating calls in the Tungara frog *Physalaemus pustulosus* (Ryan and Rand 1990), complex swords in swordtail fishes (Basolo 1990, 1995, 1996), long tails in widowbirds (Pryke and Andersson 2002), orange spots in the guppy *Poecilia reticulata* (Rodd *et al.* 2002), song structure in Costa's hummingbird *Calypte costae* (Clark and Feo 2009), song repertoires in birds (Collins 1999), nuptial food gifts in insects (Sakaluk 2000), "egg-spots" in Cichlids (Egger *et al.* 2011), mud pillar (Christy 1995) and sand hood (Christy 2003) building in fiddler crabs *Uca beebei* and *Uca musica*, red pelage and skin colors in primates (Fernandez and Morris 2007), pollinator attraction in orchids (Schiestl and Cozzolino 2008), Anoline lizard head bobbing patterns (Fleishman 1992) and mate color preferences in birds (Møller and Erritzøe 2010). Preferences for entirely novel signals (e.g. red leg bands in zebra finches *Taeniopygia guttata*, Burley *et al.* 1982; an orange dorsal fin tumor in Poeciliids, Schlupp *et al.* 1999; white crests in finches, Burley and Symanski 1998; gene transfer-induced red coloration in zebrafish, Owen *et al.* 2012) also indicate that preferences can exist independently of favored traits. Other studies have found a lack of support for the sensory bias model (Tobias and Hill 1998, Borgia and Keagy 2006, Fuller and Noa 2010).

Indicator mechanism ideas of female mate preference evolution have dominated sexual selection theory since its inception. Darwin (1871) allowed that secondary sexual traits could indicate condition (see Prum 2012), but must have felt this was not a sufficient explanation, given the prevalence and complexity of such traits. For example, the 300 distinct notes and 66 types of songs of the winter wren *Troglodytes troglodytes* (Kroodsma 1980), or the 58 elements, 15 separate behaviors and 10 plumate ornaments described by Scholes (2006) in the dance of Carola's parotia (*Parotia carolae*) seem much more than sufficient as condition-conveying signals. There are other theoretical reasons to consider sensory bias models. Pre-existing biases should be common. Females must respond appropriately to stimuli such as prey, predators, and mates in order to succeed. This should give them a wide variety of positive and negative biases that could potentially be exploited in courtship (Arnqvist 2006). In indicator and Fisherian models, preferences would need to evolve independently with each male trait, and happen to come about at the same time in the population (Arnqvist 1996). Thus sensory exploitation models can more parsimoniously explain the high variation in sexually dimorphic traits (both intra- and interspecifically), as well as the fact that that many organisms use multiple traits, in multiple sensory modalities, to attract a partner.

Preexisting sensory biases may be either adaptive, in that they evolved by direct selection, or "hidden," in that they are an incidental side-effect of sensory system structure (Ryan 1990, Enquist and Arak 1993, Endler and Basolo 1998, Arnqvist 2006). The preference for red in stickleback is a clear example of an adaptive preexisting bias, evolving directly by selection for the detection of prey and/or other ecological functions. Adaptive bias-based sexual selection studies may be limited to explaining relatively simple and specific preference-trait systems, because simple preferences such as those for a particular color will vary with the ecological situation of organisms. Hidden preferences might be more likely to explain broad patterns and complex traits. For instance the fact that organisms so widely separated ecologically, taxonomically and neurologically as insects, humpback whales, humans, about 5,000 species of birds, bats (Behr and Helversen 2004), and mice (Holy and Guo 2005) have an apparent preference for song-like aural stimuli suggests a side-effect of sensory structure.

Red pelvic spines in the TNWR population of brook stickleback may represent an opportunity for research into the mechanisms behind the evolution of a derived sexually selected trait. However, I do not know whether the trait or the preference are currently under selection or whether males with redder spines receive more eggs and sire more offspring than those without. If so, it remains to be determined whether this occurs by way of female choice or competition among males. The importance of direct and/or genetic benefits and Fisherian runaway sexual selection have also not yet been established. The bias for red may extend back to or beyond the common ancestor of the sticklebacks. The closest extant stickleback relative is most likely the yellow tubesnout (McLennan 1991, McLennan 2004, Kawahara *et al.* 2008, Mattern and McLennan 2004). Breeding male yellow tubesnouts exhibit bright red pelvic fin coloration (Hart 1973, Akagawa *et al.* 2004), which might be homologous to the red in pelvic spines of sticklebacks. Yellow tubesnouts would be a good subject for a color preference study, as would the Japanese tubesnout, *Aulichthys japonicas*, which does not express red coloration (Akagawa *et al.* 2004). In general, my results are consistent with a scenario in which red breeding colors in stickleback arose in response to a preexisting sensory bias for red and are also maintained as an honest signal of condition.

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TABLES AND FIGURES

Table 1. Mean levels of red, green and blue measured in colored beads.

	Hue		
Bead color	Red	Green	Blue
Red	136	24	31
Orange	210	68	24
Yellow	178	155	4
Green	83	143	87
Blue	29	38	138
Purple	74	52	80

Table 2. Numbers of female and male *Culaea inconstans* sampled from Turnbull National Wildlife Refuge, Spokane Co., Wash., USA, with plain spines or spines with mild, moderate or stong coloration. The mean condition factor ±SE for males is given in parentheses for samples with condition data. Condition factor was calculated as 100 x weight/length^b, where b is the slope of the linear least squares regression of $\log_{10}$(weight) on $\log_{10}$(length).

	Female Coloration				Male Coloration			
Date	Plain	Mild	Moderate	Strong	Plain	Mild	Moderate	Strong
7/12/11	36	2	1	0	28	6	11	3
7/21/11	34	3	2	0	31	5	0	0
11/14/11	17	0	0	0	16	0	0	0
11/16/11	49	3	0	0	37	11	1	0
11/28/11	8	0	0	0	7	0	0	0
4/11/12	34	0	0	0	24	7	4	0
4/18/12	40	0	0	0	14 (1.13±0.03)	2 (1.12±0.25)	3 (1.20±0.15)	1
4/25/12	31	0	0	0	18 (1.15±0.04)	19 (1.19±0.02)	10 (1.22±0.06)	3 (1.25±0.09)
4/30/12	57	1	1	0	13 (1.18±0.06)	14 (1.17±0.04)	12 (1.30±0.06)	7 (1.30±0.07)
5/7/12	52	1	0	0	9 (0.97±0.05)	11 (1.02±0.02)	12 (1.11±0.06)	5 (1.10±0.03)
5/11/12	40	0	0	0	0	6 (1.12±0.04)	7 (1.14±0.04)	2 (1.22±0.01)
5/18/12	104	0	1	0	44 (1.17±0.02)	23 (1.22±0.03)	41 (1.20±0.02)	28 (1.21±0.04)
10/6/12	78	0	0	0	53	13	1	1

Table 3. Sample sizes, p-values and Holm-Bonferroni-corrected alpha values for differences between observed and expected frequencies of all bites per comparison and first bites per fish directed at differently-colored beads by *Culaea inconstans* from Turnbull National Wildlife Refuge, Spokane Co., Wash., USA.

		All bites		First bites	
Paired Comparison	Alpha	n	p-value	n	p-value
Orange vs red	0.0500	110	0.3169	22	0.0669
Yellow vs red	0.0100	198	>0.0001	31	0.0004
Green vs red	0.0125	118	>0.0001	20	0.0013
Blue vs red	0.0250	156	>0.0001	20	0.0207
Purple vs red	0.0167	71	>0.0001	21	0.0133

Table 4. Sample sizes, p-values and Holm-Bonferroni-corrected alpha values for differences between observed and expected frequencies of first bites per trial directed at differently-colored beads by *Culaea inconstans*, Turnbull National Wildlife Refuge, Spokane Co., Wash., USA.

Paired Comparison	alpha	n	p-value
Orange vs red	0.0500	14	0.7905
Yellow vs red	0.0100	12	0.0004
Green vs red	0.0167	12	0.0063
Blue vs red	0.0250	16	0.0212
Purple vs red	0.0125	16	0.0041

Table 5. Sample sizes, Wilcoxon rank sum test statistics (W) and p-values for differences in median length of inspections of red beads versus beads of other colors in *Culaea inconstans*, Turnbull National Wildlife Refuge, Spokane Co., Wash, USA.

Paired Comparison	n (red)	n (other*)	W	p-value
Orange vs red	19	21	189	1.000
Yellow vs red	27	9	139	0.546
Green vs red	31	26	410	0.923
Blue vs red	30	17	225	0.506
Purple vs red	28	11	108	0.155

*other refers to the color of th bead that is not red

Table 6. Sample sizes, Wilcoxon rank sum test statistics (W) and p-values for differences in latency to bite red beads versus beads of other colors in *Culaea inconstans*, Turnbull National Wildlife Refuge, Spokane Co., Wash., USA.

Paired Comparison	n (red)	n (other*)	W	p-value
Orange vs red	35	25	404	0.444
Yellow vs red	45	15	144	0.164
Green vs red	26	9	148	0.255
Blue vs red	42	26	296	0.002
Purple vs red	10	7	23	0.179

*other refers to the color of th bead that is not red

Table 7. Sample sizes (n) and relative mean or median spine lengths and widths (see text for definitions) of male and female *Culaea inconstans* from Turnbull National Wildlife Refuge, Spokane Co., Wash., USA. L=Left, R=Right.

		Male	Female
Length (L)	n	66	71
	Mean	0.071	0.074
	SE	<0.0001	<0.0001
Length (R)	n	61	71
	Mean	0.070	0.074
	SE	<0.0001	<0.0001
Width (L)	n	49	52
	Median	0.128	0.120
	Range	0.388	0.065
Width (R)	n	46	54
	Median	0.128	0.121
	Range	0.077	0.064

Table 8. Mean number of times pelvic and/or dorsal spines were extended in ten minute observation periods (n=27) of *Culaea inconstans* (n=6) from Turnbull National Wildlife Refuge, Spokane Co., Wash., USA.

Behavior	Spines extended			
	None	Dorsal	Pelvic	Dorsal+Pelvic
None	—	8.50	0.00	0.17
Yawns	0.50	2.67	0.00	2.17
Sigmoids	0.50	3.67	0.00	1.67
Circle Fights	0.33	1.17	0.00	0.00
Charges/Bite	1.00	4.17	0.00	0.33

Table 9. Sample sizes (n) and p-values for differences between the frequency of dorsal spine extension and extension of both dorsal and pelvic spines during different behaviors of male territorial *Culaea inconstans* from Turnbull National Wildlife Refuge, Spokane Co., Wash., USA.

	n	p-value
Yawns	31	0.360
Sigmoids	35	0.020
Circle fights	8	0.004
Charges/Bite	28	<0.001
Spines alone	55	<0.001

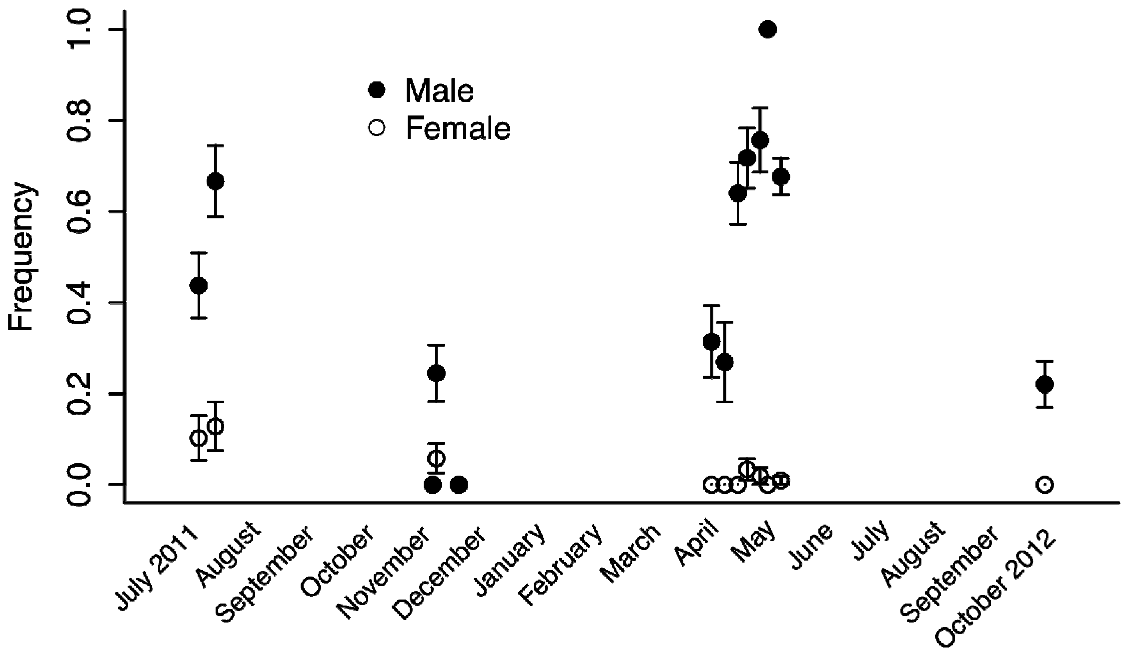

Fig. 1. Frequency (±SE) of red coloration in male and female *Culaea inconstans* from November 2011 to October 2012 at Turnbull National Wildlife Refuge, Spokane Co. Wash., USA.

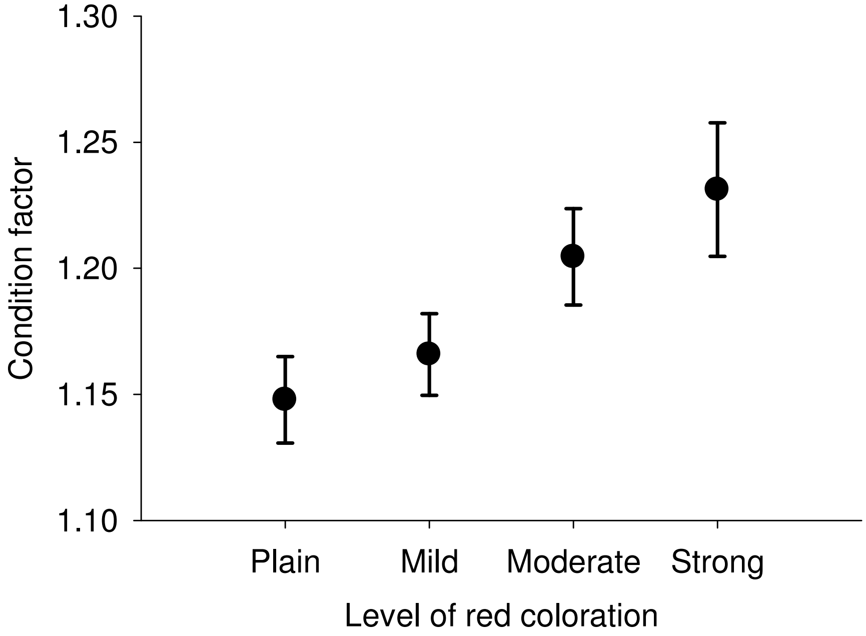

Fig. 2. Mean condition factors (±SE, calculated as 100 x weight/length^b, where b is the slope of the linear least squares regression of $\log_{10}$(weight) on $\log_{10}$(length)) of male *Culaea inconstans* from Turnbull National Wildlife Refuge, Spokane Co. Wash., USA, with different levels of red coloration of their pelvic spines during mid-breeding season April-May 2012.

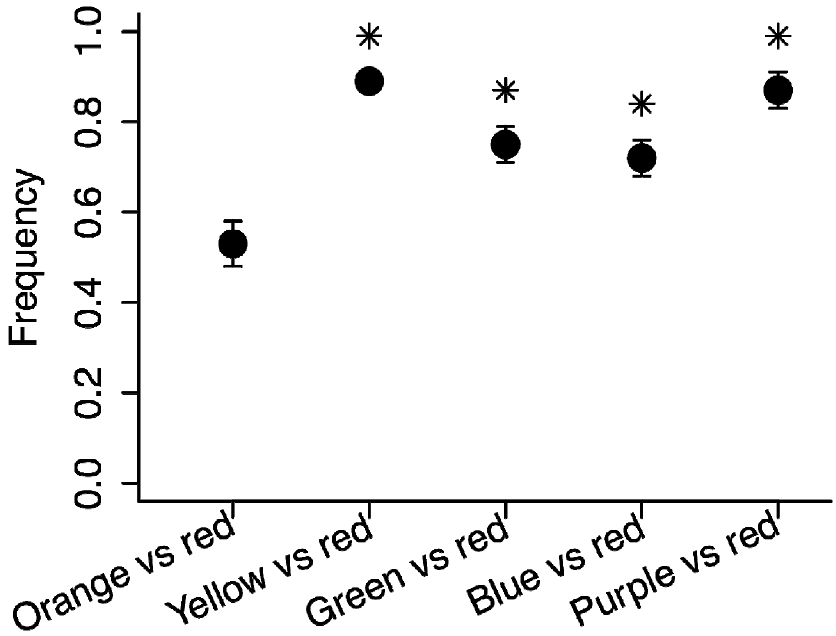

Fig. 3. Frequencies (±SE) of all bites by all fish in all trials directed at red beads versus beads of other colors by *Culaea inconstans* from Turnbull National Wildlife Refuge, Spokane Co. Wash., USA.

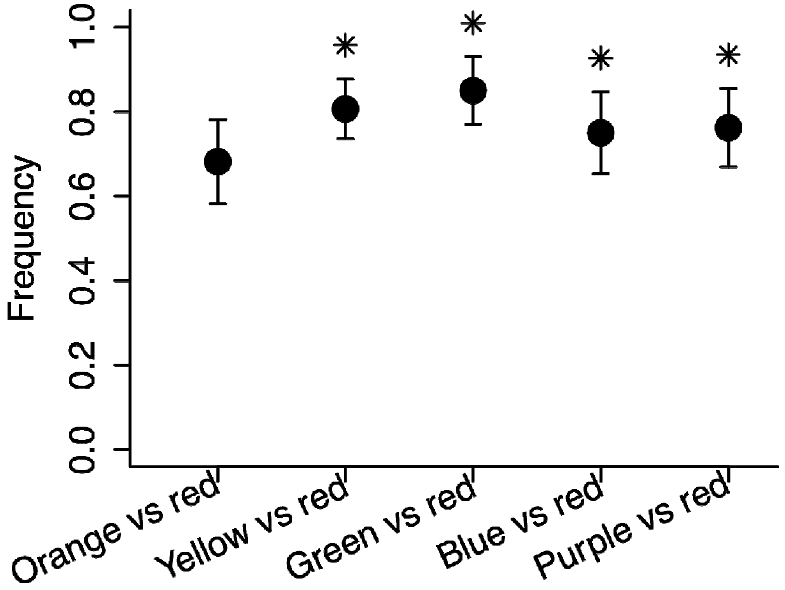

Fig. 4. Frequencies (±SE) of first bites per fish directed at red beads versus beads of other colors by Culaea *inconstans* from Turnbull National Wildlife Refuge, Spokane Co. Wash., USA.

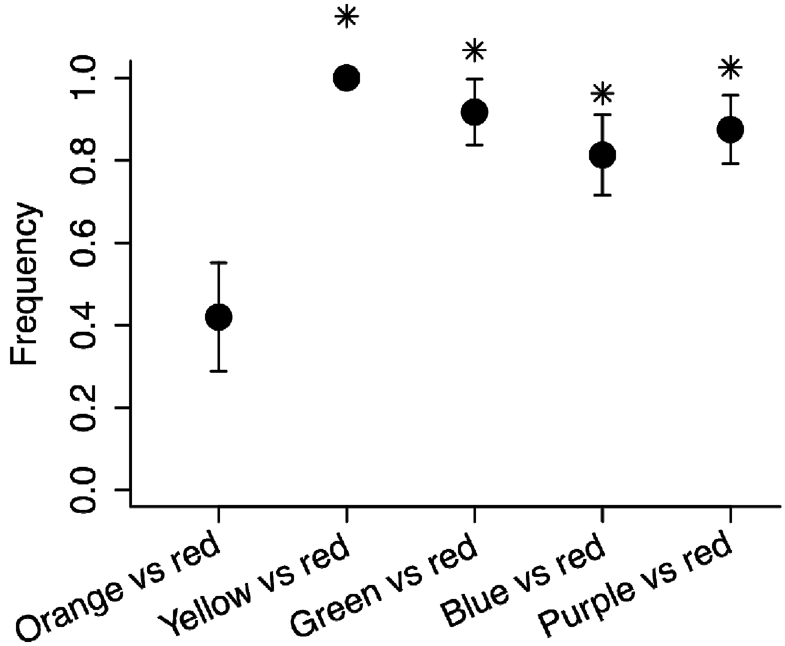

Fig. 5. Frequencies (±SE) of first bites per trial directed at red beads versus beads of other colors by *Culaea inconstans* from Turnbull National Wildlife Refuge, Spokane Co. Wash., USA.

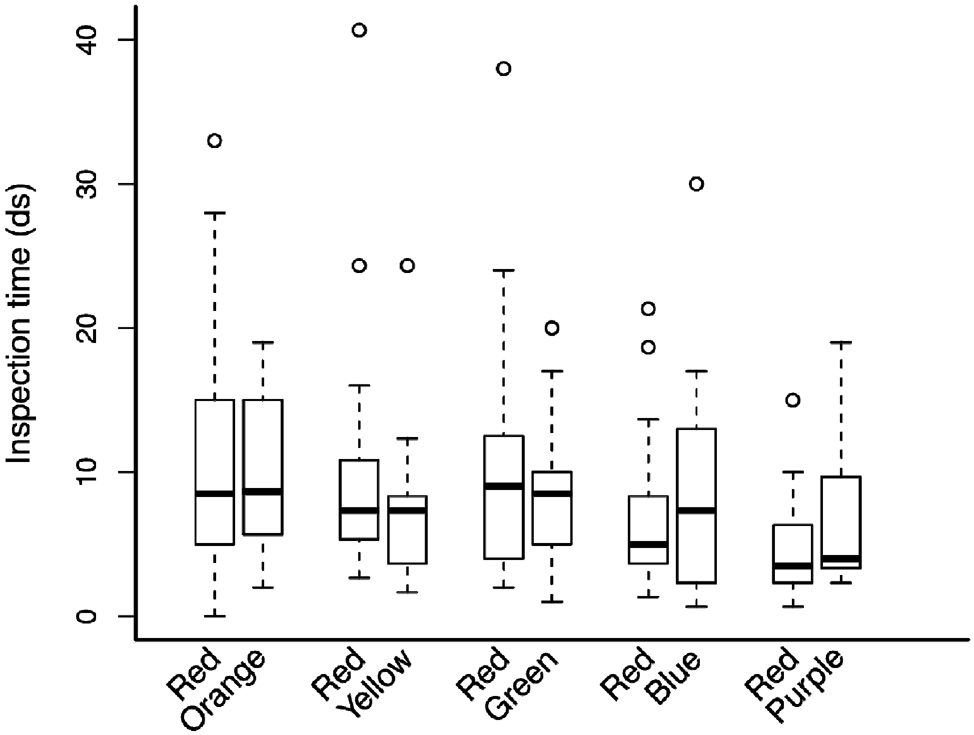

Fig. 6. Box and whisker plot of inspection time of red beads versus beads of other colors by *Culaea inconstans* from Turnbull National Wildlife Refuge, Spokane Co. Wash., USA. The figure shows median latency to bite (lines), 1st and 3rd quartiles (boxes) and ranges (whiskers). Open circles are values that exceed 1.5 times the interquartile range of the boxes.

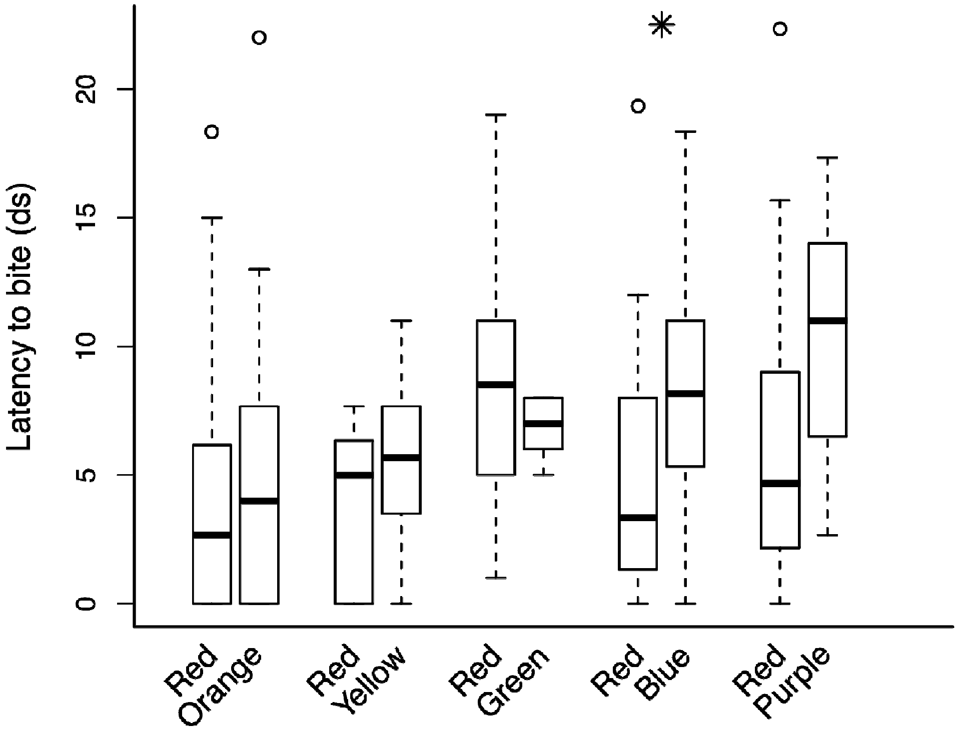

Fig. 7. Box and whisker plot of latency to bite red beads versus beads of other colors by *Culaea inconstans* from Turnbull National Wildlife Refuge, Spokane Co. Wash., USA. The figure shows median latency to bite (lines), 1st and 3rd quartiles (boxes) and ranges (whiskers). Open circles are values that exceed 1.5 times the interquartile range of the boxes. The asterisk represents statistically significant difference.

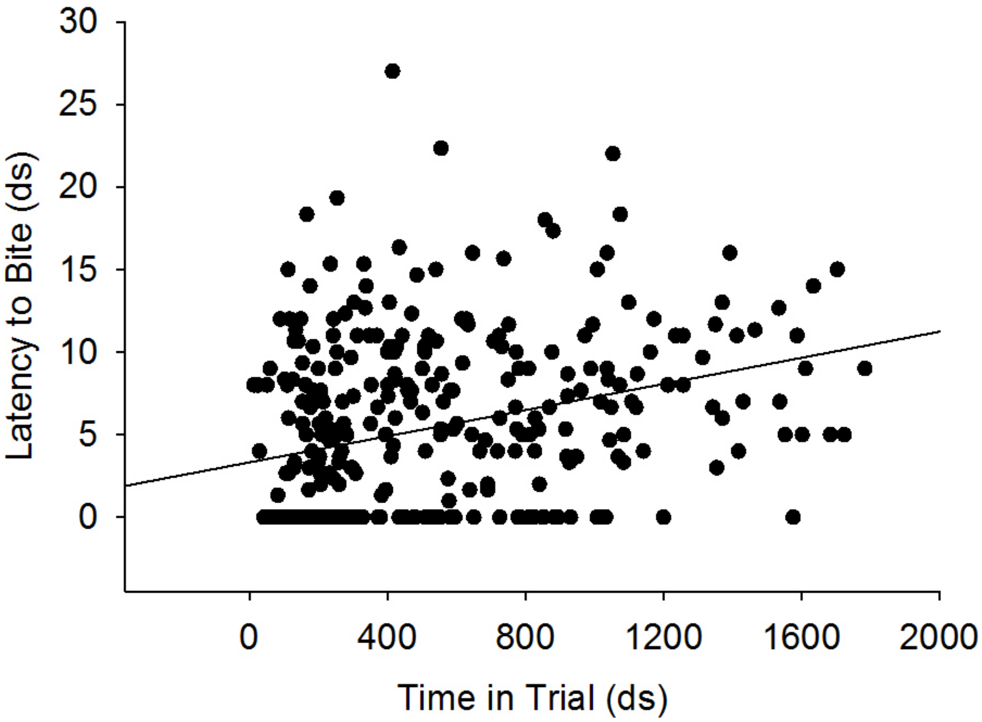

Fig. 8. Linear regression of latency to bite beads on time throughout trials by *Culaea inconstans* from Turnbull National Wildlife Refuge, Spokane Co. Wash., USA.

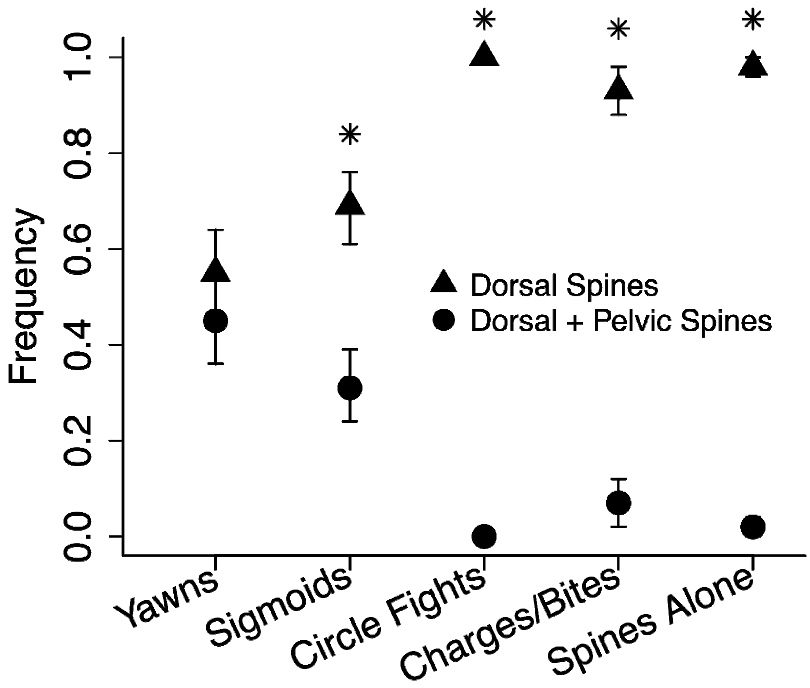

Fig 9. Frequency of the extension of dorsal and pelvic spines during yawns, sigmoid displays, circle fights and other behaviors (charges and bites), and in the absence of other behaviors (spines alone), based on 270 total minutes of observation of six territorial males of *Culaea inconstans* from Turnbull National Wildlife Refuge, Spokane Co. Wash., USA.

VITA

Author: Andrew G. Hodgson

Place of Birth: Spokane, Washington

Undergraduate Schools Attended: Spokane Falls Community College, Eastern Washington University

Degrees Awarded: Bachelor of Science, 2010, Eastern Washington University

Honors and Awards:

Graduated Magna Cum Laude, Eastern Washington University, 2010

Outstanding Graduating Senior, Eastern Washington University, 2011.

Biology Graduate Fellowship, Eastern Washington University, 2011.

Biology Graduate Fellowship, Eastern Washington University, 2012.

Travel Grant, for presentation at the Seventh International Conference on Stickleback Behavior and Evolution, 2012

Travel Grant, for presentation at the Ecological Society of America meeting, 2012.