

Interpopulation Differences in Courtship and Spawning Behavior in the Brook stickleback (*Culaea inconstans*)

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ABSTRACT.—Although behavioral variability has been well documented in the threespine stickleback *Gasterosteus aculeatus*, very little is known about the extent of variability in the other members of the Gasterosteidae. We investigated the courtship and spawning repertoires of four allopatric populations of brook stickleback *Culaea inconstans* from Algonquin Park, central Ontario, Tooley Creek, southern Ontario, Two Mile Creek, New York and Sutherland Creek, Nebraska. The results indicated that the male courtship repertoire is quite conservative, with behavioral differences appearing primarily in the frequency and duration of headdown and broadside threat displays performed during courtship and the frequency and duration of male quivering bouts once females enter the nest. In addition to documenting male behavioral variability, data are presented showing that female *C. inconstans* spend a substantially longer duration of time in the nest than other gasterosteids, and that the length of time a female spends in the nest may be influenced by male behavior performed during egg deposition.

INTRODUCTION

Interpopulation differences in the structure and performance of behavioral displays can contribute to the development of behavioral syndromes (Sih *et al.*, 2004a,b) and to the existence of differentially adapted populations within the boundary of one species (Utter, 2004). Consequently, documenting behavioral differences across populations has been of interest to biologists for decades (reviewed in Foster, 1999; Foster and Endler, 1999). Such variability may be ephemeral, reflecting shifting environmental adaptive regimes (*e.g.*, predation, density, food availability, degree of cover: Huntingford, 1982; Lahti *et al.*, 2002 and references therein) or it may be a more stable part of the population's behavioral repertoire (*e.g.*, the loss of fin-erection, tail-lifting, s-threat, ramming, biting and circling in cave mollies: Parzefall *et al.*, 1997). Whatever the explanation for the underlying cause, variability determines the pathways that are accessible to each population for future evolutionary change, while at the same time reflecting the influences of selection and contingency in the past (Ryan *et al.*, 1996).

Within the family Gasterosteidae, researchers working with the three-spined stickleback *Gasterosteus aculeatus* have been documenting interpopulation variability for over 60 y. Data were collected in two stages: first, detailed laboratory investigations of a relatively few populations were conducted (Wootton, 1976 and references therein), followed by an increase in the number of populations examined and a combination of both laboratory and field studies. These efforts have produced a relatively large database documenting interpopulation differences in: (1) the frequency of the male zigzag dance and dorsal pricking (Foster, 1994a, 1995; Foster *et al.*, 1998; Ishikawa and Mori, 2000; Scott and Foster, 2007), (2) male diversionary displays in response to nest raiding (Foster, 1988, 1994b; Ridgeway and McPhail, 1988), (3) the male and female nuptial colour signal (reviewed in McLennan, 2006), (4) the correlation between boldness, aggression and predation

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(reviewed in Huntingford and Coyle, 2006) and (5) male courtship aggression (Ridgway and McPhail, 1987). To date however, such widespread investigations have not been undertaken for any other gasterosteids.

The brook stickleback *Culaea inconstans* is an intriguing species with which to begin building such a database because the specific designation “*inconstans*” means “changeable” in Latin; the term was initially chosen by Kirtland (1840) to reflect morphological variability in the frequency of dorsal spines (Nelson, 1969; reviewed in Mattern, 2006b). Investigations of behavioral variability in this species are rare, but so far interpopulation differences in both agonistic displays (Burks *et al.*, 1985; McLennan and Ward, *in press*) and the structure/cycling of the male and female nuptial colour signals (Ward and McLennan, 2006) have been described.

Recent phylogeographic analysis indicated that *Culaea inconstans* was subdivided into two lineages reflecting distinct glacial refugia at least 4.2 mya (Gach, 1996; Mattern, 2006a). Analysis based on three mitochondrial genes further postulates that the Algonquin Park fish have been separate from the Tooley Creek and New York populations for a minimum of 1.4 my (Mattern, 2006a). In this study we continue to build the brook stickleback database by systematically examining the courtship repertoires from four geographically disjunct populations belonging to the Mississippi lineage (Sutherland Creek, Nebraska) and the Atlantic lineage (Tooley Creek, Southern Ontario; Algonquin Park, Central Ontario and Two Mile Creek, New York).

Like all other gasterosteids, male brook sticklebacks defend territories, build nests and vigorously court passing conspecific females. *Culaea inconstans*' courtship repertoire is quite complex, consisting of at least twelve sexual and aggressive displays (Hall, 1956; Foster, 1973; Winn, 1960; Reisman and Cade, 1967; McKenzie, 1969a,b), so there is ample room for variability to exist. Sexual displays include pummeling (the male darts forward, striking the female with his snout), an exaggerated lead to the nest and a highly stereotypic nest show display in which the male positions himself with his mouth wide open at the top of the nest entrance and rapidly vibrates his pectoral fins. A receptive female responds to these courtship overtures by following the male during one of his lead/show bouts and pushing past him into the nest, where she is quivered by her mate until she deposits her clutch and flees. In this study, we divide the mating sequence into courtship (characterized by both sexual and aggressive behaviors) and spawning phases in order to answer two questions: what is the extent of interpopulation variability in: (1) the male courtship repertoire? and (2) the male-female dialogue during the act of spawning itself?

METHODS

Adult brook stickleback were collected using minnow traps in early May of 2004 and 2006 from Sutherland Creek, Nebraska (41°08'13"N, 101°07'28"W); in 2004 from Two Mile Creek, New York (42°05'52"N, 78°26'38"W); in 1993 from Ring-Neck Pond, Algonquin Park, Ontario (45°34'52"N, 78°23'19"W) and in 1992 and 2006 from Tooley Creek, Ontario (43°52'11"N, 78°46'53"W). Sutherland Creek runs between farmland and is characterized by clear water flowing gently over a soft silt and pebble substrate, whereas Two Mile Creek and Tooley Creek are more marsh-like, with slow-moving turbid water frequently interrupted by patches of grass. Ring-Neck Pond is large, shallow and heavily vegetated with clear water and a muddy substrate.

Fish were placed in day packers and transported back to the lab in chilled, continually aerated water. They were maintained under winter conditions (10–12 C; 12h:12h light-dark) in 200-l fiberglass tanks with continual flow-through of dechlorinated water. Reproductive

behavior was discouraged by the lack of suitable nesting materials, although large sticks and stones were provided for shelter. Individuals were moved as needed to 58-l and 120-l community tanks, covered on three sides with light green paper, lined with 2.5 cm of fine gravel, and supplied with nesting material consisting of soft moss, algae, submerged grass roots and bunches of *Hygrophila*. These tanks were maintained under a 16h:8h light-dark regime, a constant temperature of 16 C and low density, all of which promote breeding (Winn, 1960; Reisman and Cade, 1967). Fish usually became reproductively active within 3 d.

Once a male began nest building in a community tank he was moved to a test tank lined with 2.5 cm of fine gravel and covered on three sides with light green paper and a photograph of aquatic vegetation. Each tank was supplied with *Hygrophila* rooted in a peat-filled 10 cm diam plastic dish and an abundance of soft algae, grasses and twigs collected from a nearby marsh. All males began nest building in the *Hygrophila* within 72 h. Males that failed to build a complete nest within five days were replaced. Trials were conducted between May and Aug. in 1992, 1993, 2004 and 2006. For all years, the experimental protocol remained the same with the exception that in 2006, males were observed in 40-l test aquaria, whereas in all other years they were viewed in 58-l tanks. Mann-Whitney U tests, Bonferroni corrected for multiple comparisons, revealed that there was no difference in the behavior of males from the same population examined in the two tank sizes, so they were pooled in subsequent analyses.

COURTSHIP AND SPAWNING

Each male was presented with a nuptially coloured, free-swimming gravid female from his respective population with whom he was permitted to spawn. If a female lost her nuptial colour during the interaction, or did not enter the nest within 15 min, she was removed and the male allowed to rest for three hours before being presented with another female. All females were used only once. All interactions were videotaped with a Sony Handycam video camera from behind a green fabric blind. Twenty-one behavioral variables (Table 1) were scored from the tapes at a later date.

RESULTS

We observed 51 males successfully court and spawn with a gravid female from their respective populations (Nebraska $n = 22$; Tooley Creek $n = 14$; New York $n = 8$; Algonquin $n = 7$). The nest placement of one Nebraskan male made it impossible to record the duration of behavioral elements performed while the female was in the nest so only frequency data for this breeding pair are included in the analysis of spawning activity. Mann-Whitney U tests, adjusted for multiple comparisons via sequential Bonferroni correction (Peres-Neto, 1999), indicated that the behavior of Nebraskan fish collected in 2004 and 2006, and the behavior of Tooley Creek fish collected in 1992 and 2006, did not differ significantly between experimental years so the data were pooled in subsequent analyses.

COURTSHIP BEHAVIOR

Male brook sticklebacks from Nebraska and Tooley Creek performed all courtship and agonistic displays. Algonquin Park males did not perform headdown threats and New York males did not perform broadside/tailbeating or acts of nest boring (Table 2; Fig. 1). None of the 51 males exhibited any novel aggressive or sexual behaviors.

For all of the populations except New York, which did not display broadside/tailbeat, our observations agreed with McKenzie's (1969a) ethogram indicating that tailbeating is

TABLE 1.—Behavioral variables measured during courtship and spawning in 51 male brook stickleback *Culaea inconstans* from four allopatric populations. f = frequency; d = bout duration

Behavior	Description
<i>Courtship - Agonistic displays</i>	
Headdown Threat (f)	Male assumes a 45–90° head-down position relative to the substrate (McKenzie, 1969a)
Broadside Threat (f)	Male assumes a horizontal, concave posture, displaying laterally to the female. The dorsal and pelvic spines are often flared and the pectoral fins erected (McKenzie, 1969a)
Tailbeating (f)	Male beats his tail laterally in a slow (approximately 4–10 beats per second), exaggerated manner (McKenzie, 1969a)
Bite (f)	Male snaps his jaws at the female with all spines flared
<i>Courtship - Sexual displays</i>	
Pummeling (f)	Hovering slightly above, and approximately 10–15 cm away from the female (McKenzie, 1969b), the male dashes downward, striking the female on her head and flanks with his snout (Hall, 1956)
Nest lead (f, d)	Male turns away from female and swims towards the nest. Leads were divided into two categories, complete (male arrives at nest) and partial (male terminates the lead prior to reaching the nest). Duration of nest leading included both partial and complete leads
Nest showing (f, d)	Positioned facing into the nest entrance, the male presses his mouth onto the upper rim of the nest while moving his pectoral fins rapidly back and forth, with emphasis on the forward stroke. Position is maintained by countering the forward pectoral stroke with powerful lateral beats of the caudal fin. Nest showing may assist the female in locating the nest entrance (Wootton, 1976 and references therein)
Boring (f)	Male repeatedly thrusts his head into the nest entrance, widening the nest entrance and cavity
Tailflagging (f)	Male raises his caudal fin, such that the caudal peduncle and fin curve upward (Reisman and Cade, 1967)
Wagging (f, d)	Male assumes a distinctive mode of swimming that is characterized by exaggerated lateral tail movement and an oscillating torso (Hall, 1956). Although usually observed during a nest leading episode, males may occasionally waggle during a pre-lead courtship interaction
Total courtship time	Time from the male's first approach to the female's entry into his nest
<i>Spawning</i>	
Male quivering (f, d)	Male pushes his snout along the flank of the female from the tip of the caudal peduncle to approximately the anal spine while vibrating his pectoral fins and body
Female shaking (f, d)	Female shudders and struggles slightly forward in the nest
Total spawning time	Time from the female's entry into the nest to her exit
Time to 1 st quiver	Time from the female's nest entry to the first male quiver
Time to 1 st shake	Time from the female's nest entry to her first shake

preceded by broadside display the majority of the time (London, Ontario population). Kruskal-Wallis H tests, followed by Mann-Whitney U tests adjusted for multiple comparisons, indicated that males from Algonquin Park performed a greater total number of broadside displays ($Z = -3.87$, $P < 0.004$) and more displays per second ($Z = -3.89$, $P = 0.004$), than Nebraskan fish and New York males performed more headdown threats than their counterparts in Tooley Creek ($Z = -2.99$, $P = 0.006$). There was no difference

TABLE 2.—Mean frequencies of behaviors (\pm SE) recorded during courtship and spawning in male brook sticklebacks *Culaea inconstans* from four allopatric populations

Frequency of behavior	Sutherland, nebraska (n = 22)	Two mile creek, New York (n = 8)	Algonquin park, Ontario (n = 7)	Tooley creek, Ontario (n = 14)
<i>Courtship - Aggressive</i>				
Headdown threat	2.25 (0.96)	3.13 (2.15)	...	0.29 (0.16)
Broadside threat	0.05 (0.05)	...	2.14 (1.03)	1.79 (1.29)
Tailbeat threat	0.05 (0.05)	...	0.71 (0.42)	0.43 (0.43)
Bite	0.70 (0.49)	0.38 (0.26)	0.71 (0.57)	0.36 (0.20)
<i>Courtship - Sexual</i>				
Pummel	6.25 (1.88)	8.75 (3.13)	9.14 (2.43)	4.36 (0.62)
Complete Lead	2.10 (0.33)	2.13 (0.44)	2.00 (0.44)	2.07 (0.32)
Partial Lead	0.40 (0.21)	0.63 (0.26)	1.57 (0.48)	0.29 (0.13)
Nest Showing	2.20 (0.41)	1.75 (0.31)	2.43 (0.69)	2.14 (0.39)
Boring	0.25 (0.20)	...	0.43 (0.20)	0.29 (0.19)
Tailflag	0.25 (0.16)	0.38 (0.26)	0.29 (0.49)	0.36 (0.17)
Wagging	2.0 (0.33)	3.00 (0.80)	4.00 (0.76)	2.21 (0.89)
<i>Spawning</i>				
Male Quiver	120.35 (15.98)	97.13 (30.29)	155.71 (32.89)	99.50 (26.19)
Female Shake	12.15 (1.46)	12.00 (2.18)	11.29 (2.23)	7.86 (1.14)

between any of the populations in the total frequency of threat displays performed (broadside + headdown + tailbeat: Kruskal-Wallis test: $\chi^2 = 2.50$, $P = 0.43$).

Pummeling was the dominant behavior signaling sexual motivation in all four populations (Table 2). There was no difference in the total amount of time spent engaged in courtship (Kruskal-Wallis test: $\chi^2 = 5.80$, $P = 0.12$) nor in the total frequency of sexual behaviors performed (pummel + nest leads + nest show + boring + tailflag + waggle dance: Kruskal-Wallis test: $\chi^2 = 5.04$, $P = 0.17$). Nebraskan males performed wagging bouts that were significantly shorter than the more moderate bouts performed by Tooley Creek males ($Z = -2.73$, $P = 0.006$) and the long bouts performed by Algonquin Park males ($Z = -3.16$, $P = 0.001$) (Table 3). Algonquin Park males spent more time wagging than males from either Nebraska ($Z = -3.05$, $P = 0.001$) or Tooley Creek ($Z = -2.46$, $P = 0.01$), but did not differ significantly from New York males ($Z = -0.926$, $P = 0.397$).

SPAWNING BEHAVIOR

Males from Nebraska and Algonquin Park performed more quivering bouts than males from New York (Nebraska vs. New York: $Z = -2.68$, $P = 0.006$; Algonquin Park: $Z = -2.79$, $P = 0.004$; Fig. 2a) and each bout was significantly shorter (Nebraska vs. New York: $Z = -4.02$, $P < 0.001$; Algonquin Park: $Z = -3.24$, $P < 0.001$; Fig. 2b). The duration of a quivering bout was also shorter in the Algonquin, versus the Nebraskan, males ($Z = -2.60$, $P = 0.008$). Because Tooley Creek males performed quivering bouts of moderate length and moderate intensity, they were not significantly different from males of any other population. New York males lagged behind Algonquin Park fish in the time to the first quivering bout (34.5 s vs. 3.67 s.; $Z = -2.78$, $P = 0.001$). There was no difference amongst females of any two populations in the amount of time spent depositing eggs in the nest (Kruskal-Wallis test: $\chi^2 = 1.26$, $P = 0.76$).

Correlation between male and female behavior and the spawning duration.—Spearman rank correlations indicated that the total amount of time that the male spent quivering was positively correlated with the amount of time that the female spent in the nest in fish from Nebraska ($R =$

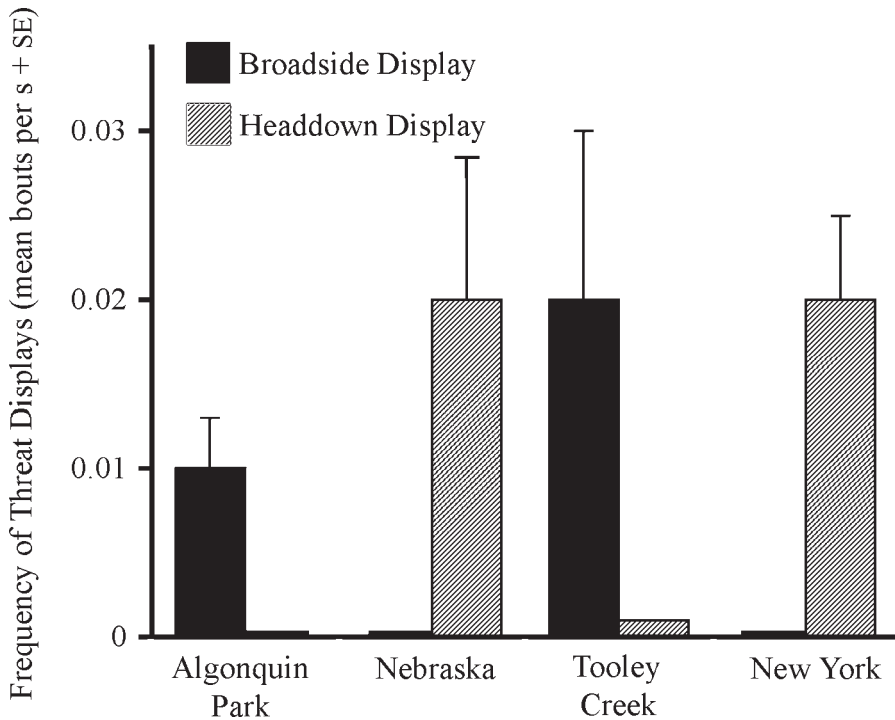


FIG. 1.—Interpopulation differences in the relative frequencies of broadside threat displays and headdown threat displays performed during courtship (# per s: mean \pm SE) by male *Culaea inconstans* from Tooley Creek, Ontario (n = 14), Algonquin Park, Ontario (n = 7), Two Mile Creek, New York (n = 8) and Sutherland Creek, Nebraska (n = 22)

0.88, $P < 0.001$), New York ($R = 0.86$, $P = 0.007$) and Tooley Creek ($R = 0.787$, $P = 0.001$), whereas the frequency of quivering bouts was positively correlated with total spawning time in the Nebraskan ($R = 0.89$, $P < 0.001$) and Tooley Creek ($R = 0.87$, $P < 0.001$) populations. The total amount of time that a female spent engaged in shaking behavior was positively correlated with the total amount of time spent in the nest in the Nebraskan population ($R = 0.623$, $P = 0.003$), as was the total number of shakes a female performs ($R = 0.662$, $P = 0.001$). Conversely, the total number of shakes performed by females from New York was negatively correlated with the amount of time she spent in the nest ($R = -0.74$, $P = 0.04$).

Correlation between male and female behavior during spawning.—The total number of shakes that a female performed was positively correlated with several aspects of male quivering behavior in the Nebraskan population, including the total number of quivering bouts ($R = 0.790$, $P < 0.001$), the intensity of quivering ($R = 0.54$, $P = 0.01$) and the total amount of time spent quivering ($R = 0.75$, $P < 0.001$). The total number of shakes performed by a New York female was negatively correlated with the mean length of a male quivering bout ($R = -0.790$, $P = 0.02$).

DISCUSSION

We detected several differences in the courtship and spawning repertoires of brook sticklebacks from four geographically disjunct populations. While we did not observe any

TABLE 3.—Mean bout durations ($s \pm SE$) and total durations of behaviors recorded during courtship and spawning in male brook sticklebacks *Culaea inconstans* from four allopatric populations

	Sutherland, Nebraska (n = 21)	Two mile creek, New York (n = 8)	Algonquin, Ontario (n = 7)	Tooley creek, Ontario (n = 14)
<i>Bout duration (s)</i>				
Nest Showing	5.59 (0.45)	5.26 (0.98)	5.54 (0.84)	6.38 (0.71)
Quivering	1.39 (0.04)	2.14 (0.12)	1.06 (0.01)	1.69 (0.21)
Shaking	1.56 (0.07)	1.40 (0.28)	1.03 (0.16)	1.31 (0.15)
Waggle dance/lead	2.21 (0.19)	3.19 (0.43)	4.45 (0.66)	3.50 (0.37)
Time to 1st Quiver	21.55 (12.75)	34.5 (11.52)	3.67 (1.94)	49.16 (36.33)
Time to 1st Shake	128.95 (24.36)	254.38 (65.30)	112.64 (12.64)	170.33 (41.19)
<i>Total time (s)</i>				
Courtship	122.60 (38.92)	131.50 (50.76)	163.77 (46.23)	152.33 (48.14)
Spawning	540.05 (58.45)	609.00 (103.33)	468.75 (48.02)	542.26 (99.40)
Nest Showing	12.48 (2.46)	10.36 (3.63)	12.83 (3.76)	14.14 (3.37)
Quivering	163.78 (21.16)	203.11 (59.25)	144.34 (32.65)	155.97 (37.22)
Shaking	18.11 (2.16)	15.82 (7.15)	10.14 (1.28)	10.59 (1.99)
Waggle dance/lead	4.84 (1.05)	13.36 (4.35)	16.17 (3.02)	8.21 (1.94)

behavior to be unique to a single population (for similar results see the territorial, courtship and spawning behaviors of *Salaria fluviatilis*: Neat *et al.*, 2003 and the courtship behaviors of threespine populations in Japan: Nagata *et al.*, 1988; Ishikawa and Mori, 2000), in several instances we found inter-group differences manifested as variability in the performance of a trait (frequency and duration). It is possible that we may have missed some additional differences in the Algonquin Park and New York populations due to small sample sizes. As we discuss below, this is particularly relevant to the problem of missing versus rare characters.

COURTSHIP BEHAVIOR

Aggressive displays, co-opted from male-male communication (Berglund *et al.*, 1996), are often included in the courtship repertoire of groups with complex pre-mating dialogues (McInerney, 1969; Mateos and Carranza, 1999; Borgia and Coleman, 2000). In fishes such as sticklebacks that nest close to one another and/or exhibit extensive paternal care, these behaviors may deter neighboring males from interfering during courtship (van den Assem, 1967; Sargent and Gebler, 1980; Rowland, 1988; Candolin, 2000) and/or may communicate information about a male's ability to defend his clutch to potential mates. Levels of courtship aggression are often positively correlated with future parental care behaviors such as egg aeration and/or survival (Cacho *et al.*, 2006; Gozlan *et al.*, 2003) and females often prefer more aggressive males (Rogers and Barlow, 1991; Gozlan *et al.*, 2003; Rosenfield and Kodric Brown, 2003; Cacho *et al.*, 2006). The direct relationship between courtship aggression and egg survival has never been investigated in sticklebacks, but a positive correlation between levels of aggression (frequency of biting) during courtship and both the parental (Huntingford, 1976) and territorial (Bakker, 1986) phases of the breeding cycle suggest that a male's level of aggression in one context is a reliable indicator of his aggression in another (Huntingford, 1976).

A male who attacks a potential mate, however, risks driving her away (Ward and FitzGerald, 1987) and in our study, the frequencies of bites directed towards the female were substantially lower than the levels recorded during male-male interactions during another experiment (*e.g.*, Algonquin Park males: 0.71 bites towards females, results this study; 16.38

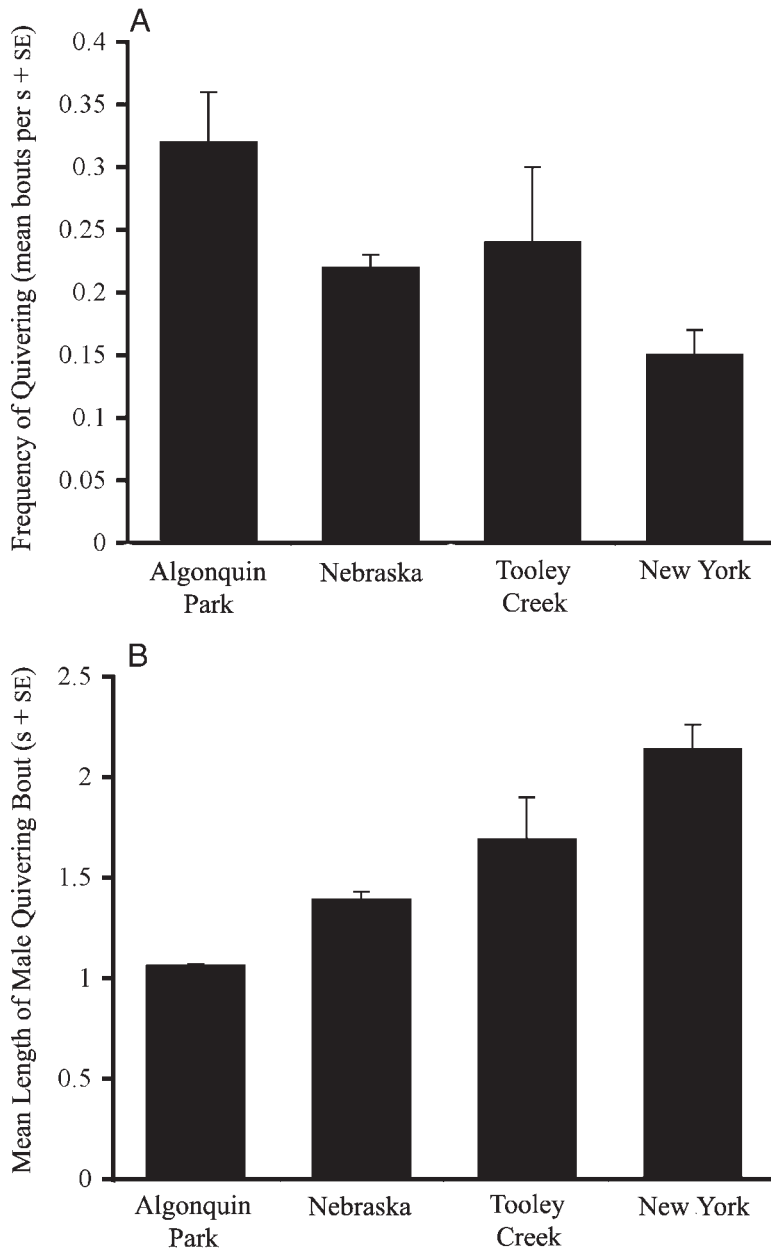


FIG. 2.—Interpopulation differences in (A) the relative frequency of quivering bouts performed during spawning ($\#$ per s: mean \pm SE) by male *Culaea inconstans* from Tooley Creek, Ontario ($n = 14$), Algonquin Park, Ontario ($n = 7$), Two Mile Creek, New York ($n = 8$) and Sutherland Creek, Nebraska ($n = 22$) and (B), the mean length of a single quivering bout ($s \pm SE$)

bites towards males; [McLennan and Ward, *in press*]). Threat displays, on the other hand, might be capable of transmitting information concerning a given male's clutch-defensive abilities without harming the female or driving her away. The type of threat display used most often differed between the four populations; broadside/tail beat dominated the agonistic repertoire of Tooley Creek males (88.4%: *cf.*, 79.5% in male-male interactions, McLennan and Ward, *in press*) and Nebraskan males generally used headdown threats (73.8%: *cf.*, 73.5% in male-male interactions; McLennan and Ward, *in press*). New York and Algonquin males displayed only one of the two threat behaviors, headdown and broadside/tailbeat respectively. Data from previous studies of male-male interactions indicates that Algonquin males are capable of displaying head down threats, however the frequency with which they do so is extremely low (mean frequency = 0.1 displays per 5 min: McLennan and Ward, *in press*). We have also, on occasion, observed New York males performing broadside displays in the lab. We therefore suggest that the absence of one threat display in the courtship repertoire of these two populations reflects a decrease in its frequency, rather than the actual loss of a behavioral component. Presumably we would have detected these rare displays if our sample sizes for New York and Algonquin Park had been larger.

Although the composition of the threat repertoire varied across populations, we did not detect a difference in the total number of threat displays performed by males in different populations, indicating that a decline in the performance of one threat display is compensated for by an increase in the other (Fig. 1). Interestingly, this suggests that it is the message of aggressive motivation transmitted by the displays (Hinde, 1981; Hurd and Enquist, 2001 and references therein), rather than the structure of the display itself, that a female might use during mate assessment. Overall, the relative percentage of the courtship repertoire that is dedicated to biting and threatening did not vary significantly across the four populations (range of 15.4%–19.7%: Kruskal Wallis test $\chi^2 = 1.36$, $P = 0.72$), which implies that there might be an upper limit to aggression during courtship; possibly because aggressive and sexual motivation are mutually inhibitory in sticklebacks (Sevenster, 1961; Wilz, 1972; Bakker, 1986).

The composition of the sexual component of the courtship repertoire was less variable than the aggressive component; all seven displays were present in the four populations with the exception of nest boring, a rare display missing in the New York fish. Again, small sample size coupled with the rarity of the behavior may have been responsible for this omission rather than the actual loss of nest boring itself. Courtship behaviors generally have a number of different functions, such as stimulating ovulation in females (Degani, 1993; Perkins and FitzGerald, 1994; Junca and Rodrigues, 2006), synchronizing the mating dialogue between partners (Fleming *et al.*, 1996; de Gaudemar and Beall, 1998) and allowing females to recognize and discriminate among potential suitors (*e.g.*, Kotiaho, 2002; Rantala and Kortet, 2003; Galeotti *et al.*, 2005), so it is possible that a complex hierarchy of functions requires the presence of all traits. In this hierarchy, one display is not "the same" as another, as we have hypothesized for threat behaviors.

Pummeling was the dominant sexual display in all males and no differences were detected in its frequency across populations. Traits used for species recognition are expected to vary little throughout a species' geographical range (Paterson, 1985; Ferreira and Ferguson, 2002), suggesting that pummeling might convey such information in this system. There was, however, a substantial difference in the rate of pummeling among males within populations (Algonquin Park: 2 to 22 pummels/male; Tooley Creek: 2–9 pummels/male; New York: 2–29 pummels/male; Nebraska 2–36 pummels/male). It is possible that pummeling contributes to the female's assessment of general male attractiveness by transmitting

information about both species identification (consistent across populations) and mate quality (variable within populations). Mate recognition and mate discrimination might thus emerge from a common perceptual process in this species in which different dimensions of one signal provide overlapping information (Phelps *et al.*, 2006).

SPAWNING BEHAVIOR

In both sticklebacks and salmon the mechanical stimulation of quivering is required before a female will release her eggs (Tinbergen, 1951 and references therein; Hall, 1956); gently stroking the female's caudal peduncle area with a glass rod achieves the same effect (ter Pelkwijk and Tinbergen, 1937). The use of vibrational signals is common in fishes for which synchronized gamete release is essential to successful fertilization (Keenleyside, 1979; Halliday, 1983). For example, male quivering increases in frequency as spawning approaches in *Oncorhynchus keta*, *O. mykiss* (Tautz and Groot, 1975; Schroder, 1981), *O. kisutch* (Berejikian *et al.*, 1997) and *O. nerka* (Satou *et al.*, 1991, 1994), signaling male spawning readiness to the female.

Culaea inconstans males from all populations responded immediately to a bout of female shaking by moving to the side they had not been quivering, then vibrating in either many, very short, intense bouts (Algonquin Park, Nebraska), or fewer, less intense, longer bouts (Tooley Creek, New York). In the present case, the total duration of male quivering was positively correlated with spawning time in all populations but Algonquin Park (although there was a positive trend: $R = 0.71$, $P < 0.08$) and comparing spawning times across populations reveals a continuum of responses, with Algonquin Park males at one end (quickest to begin quivering, many short intense bouts, females spend the least amount of time in the nest [mean = 468 s]) and New York males at the other (slowest to begin, few low intensity bouts, longest time in the nest [mean = 609 s]).

The precise function of female shaking in brook sticklebacks is currently unknown, although a similar display elicits courtship and sperm release from males in *Oncorhynchus nerka* (Satou *et al.*, 1991, 1994) and courtship from females in sex-role reversed pipefish (Fiedler, 1954). In brook sticklebacks, shaking may communicate a female's spawning progress to her mate. All females shake as they leave the nest, thus shaking is ultimately a reliable signal of female departure. Not all shakes, however, end with the female exiting the nest (mean number of shakes during spawning ranges from 7.86 to 12.15: Table 2). Sneak fertilizations by neighboring males have been reported in every other member of the Gasterosteidae (threespine: Goldschmidt *et al.*, 1992; Jamieson and Colgan, 1992; fourspine: Willmott and Foster, 1995; fiftenspines: Jones *et al.*, 1998; ninespine: Morris, 1952). Thus, being able to predict when his mate was about to leave the nest would be advantageous to a male because he would be in position to pass quickly through the nest after her, fertilizing her clutch and ensuring greater paternity. From the female's perspective, shaking behavior may serve two functions. First, it may aid in her struggle to move forward in the small constricting nest until she finds the right place for egg deposition. Second, shaking may prolong a male's sexual motivation; all males increased their quivering rates after a female shake (Hall, 1956; pers. obs. this study). This latter point is particularly important because brook stickleback females spend approximately 9.14 min (mean of 51 spawnings) in the nest; far longer than any of the other gasterosteids [*Gasterosteus aculeatus*: <2 min (Wootton, 1976, and references therein); *G. wheatlandi*: 1 min (McInerney, 1969); *Apeltes quadracus*: 1 min (Rowland, 1974); *Spinachia spinachia*: 45 s–2 min (Leiner, 1934); *Pungitius pungitius*: <2 min (Hall, 1956)].

In summary, despite their separation in time and space, most of the differences in courtship and spawning behaviors that we observed among four populations of *Culaea*

inconstans have occurred in terms of performance parameters (frequency and duration), similar to the extent of diversification in agonistic displays used during male-male interactions (McLennan and Ward, *in press*). Whether or not this moderate level of behavioral variation, coupled with interpopulation differences in the structure of the nuptial colour signal (Ward and McLennan, 2006), would produce assortative mating is unknown. Explorations into the interaction between genetic divergence and the intensity of both pre and postmating isolation between the allopatric brook stickleback lineages are currently being undertaken in our lab.

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LITERATURE CITED

- BAKKER, T. C. M. 1986. Aggressiveness in sticklebacks (*Gasterosteus aculeatus* L.): a behavior genetic study. *Behavior*, **98**:1–144.
- BEREJKIAN, B. A., E. P. TEZAK, S. L. SCHRODER, C. M. KNUDSEN AND J. J. HARD. 1997. Reproductive behavioral interactions between spawning wild and captive reared coho salmon (*Oncorhynchus kisutch*). *ICES J. Mar. Sci.*, **54**:1040–1050.
- BERGLUND, A., A. BISAZZA AND A. PILASTRO. 1996. Armaments and ornaments: an evolutionary explanation of traits of dual utility. *Biol. J. Linn. Soc.*, **58**:385–389.
- BORGIA, G. AND S. W. COLEMAN. 2000. Co-option of male courtship signals from aggressive display in bowerbirds. *Proc. R. Soc. Lond. B.*, **267**:1735–1740.
- BURKS, D. J., D. A. SCANTLAND, R. S. SINCLAIR AND J. H. WOOLPY. 1985. Geographic variation in agonistic responses of territorial male brook sticklebacks, *Culaea inconstans*. *Ohio J. Sci.*, **85**:23–29.
- CACHO, M. D. R. F., S. CHELLAPPA AND M. E. YAMAMOTO. 2006. Reproductive success and female preference in the Amazonian cichlid angel fish, *Pterophyllum scalare* (Lichtenstein, 1823). *Neotrop. Ichthyol.*, **4**:87–91.
- CANDOLIN, U. 2000. Male-male competition facilitates female choice in sticklebacks. *Proc. R. Soc. Lond. B.*, **266**:785–789.
- DEGANI, G. 1993. Effect of sexual behavior on oocyte development and steroid changes in *Trichogaster trichopterus* (Pallas). *Copeia*, **4**:1091–1096.
- DE GAUDEMAR, B. AND E. BEALL. 1998. Reproductive behavioral sequences of single pairs of Atlantic salmon in an experimental stream. *Anim. Behav.*, **57**:1207–1217.
- FERREIRA, M. AND J. W. H. FERGUSON. 2002. Geographic variation in the calling song of the field cricket *Gryllus bimaaculatus* (Orthoptera: gryllidae) and its relevance to mate recognition and mate choice. *J. Zool.*, **257**:163–170.
- FIEDLER, K. 1954. Vergleichende Verhaltensstudien an Seenadeln und Seepferdchen. *Z. Tierpsychol.*, **11**:358–416.
- FLEMING, I. A., B. JONSSON, M. R. GROSS AND A. LAMBERG. 1996. An experimental study of the reproductive behavior and success of farmed and wild Atlantic salmon (*Salmo salar*). *J. Appl. Ecol.*, **33**:893–905.
- FOSTER, J. 1973. Some aspects of the social systems of the brook stickleback, *Culaea inconstans*. M.Sc Thesis, University of Toronto, Toronto.
- FOSTER, S. A. 1988. Diversionary displays of paternal stickleback: defenses against cannibalistic groups. *Behav. Ecol. Sociobiol.*, **22**:335–340.
- . 1994a. Evolution of the reproductive behavior of threespine stickleback, p. 381–397. In: M. A. Bell and S. A. Foster (eds.). *The Evolutionary Biology of the Threespine Stickleback*. Oxford University Press, New York.

- . 1994b. Inference of evolutionary pattern: diversionary displays of threespine sticklebacks. *Behav. Ecol.*, **5**:114–121.
- . 1995. Understanding the evolution of behavior in threespine stickleback: the value of geographic variation. *Behavior*, **132**:1107–1129.
- . 1999. The geography of behavior: an evolutionary perspective. *Trends Ecol. Evol.*, **14**:190–195.
- AND J. A. ENDLER. 1999. Thoughts on geographic variation in behavior, p. 287–307. *In*: S. A. Foster and J. A. Endler (eds.). *Geographic variation in behavior: perspectives on evolutionary mechanisms*. Oxford University Press, Oxford.
- GACH, M. H. 1996. Geographic variation in mitochondrial DNA and biogeography of *Culaea inconstans*. *Copeia*, **1996**:563–575.
- GALEOTTI, P., R. SACCI, D. P. ROSA AND M. FASOLA. 2005. Female preference for fast-rate, high-pitched calls in Hermann's tortoises. *Testudo hermanni*. *Behav. Ecol.*, **16**:301–308.
- GOLDSCHMIDT, T., S. A. FOSTER AND P. SEVENSTER. 1992. Inter-nest distance and sneaking in the three-spined stickleback. *Anim. Behav.*, **44**:793–795.
- GOZLAN, R. E., C. J. FLOWER AND A. C. PINDER. 2003. Reproductive success in male sunbleak, a recent invasive fish species in the U.K. *J. Fish Biol. Suppl. A.*, **63**:131–143.
- HALL, M. F. 1956. A comparative study of the reproductive behavior of the sticklebacks (Gasterosteidae). Ph.D. Thesis, Oxford University.
- HALLIDAY, T. 1983. Information and communication, p. 43–81. *In*: T. R. Halliday and P. J. B. Slater (eds.). *Animal behavior vol 2: communication*. Blackwell, Oxford.
- HINDE, R. A. 1981. Animal signals: ethological and games-theory approaches are not incompatible. *Anim. Behav.*, **29**:535–542.
- HUNTINGFORD, F. A. 1976. The relationship between inter- and intra-specific aggression. *Anim. Behav.*, **24**:485–497.
- . 1982. Do inter- and intraspecific aggression vary in relation to predation pressure in sticklebacks? *Anim. Behav.*, **30**:900–916.
- AND S. COYLE. 2006. Anti-predator defenses in sticklebacks: tradeoffs, risk sensitivity and behavioral syndromes, p. 127–156. *In*: S. Ostlund-Nilsson, I. Mayer and F. A. Huntingford (eds.). *Biology of the three-spined stickleback*. CRC Press, New York.
- HURD, P. L. AND M. ENQUIST. 2001. Threat display in birds. *Can. J. Zool.*, **79**:931–942.
- ISHIKAWA, I. AND S. MORI. 2000. Mating success and male courtship behaviors in three populations of the threespine stickleback. *Behavior*, **137**:1065–1080.
- JAMIESON, I. G. AND P. W. COLGAN. 1992. Sneak spawning and egg stealing by male threespine sticklebacks. *Can. J. Zool.*, **70**:963–967.
- JONES, A. G., S. OSTLUND-NILSSON AND J. C. AVISE. 1998. A microsatellite assessment of sneaked fertilizations and egg thievery in the fifteen-spine stickleback. *Evolution*, **52**:848–858.
- JUNCA, F. A. AND M. T. RODRIGUES. 2006. Morphological changes in the female reproductive organs during mating in *Colostethus stephensi* and associated behavior. *Amphibia-Reptilia*, **27**:303–308.
- KEENLENSIDE, M. H. A. 1979. *Diversity and adaptation in fish behavior*. Springer, Berlin.
- KIRTLAND, J. P. 1840. Descriptions of four new species of fishes. *Boston J. Nat. Hist.*, **3**:273.
- KOTIAHO, J. S. 2002. Sexual selection and condition dependence of courtship display in three species of horned dung beetles. *Behav. Ecol.*, **13**:791–799.
- LAHTI, K., A. LAURILA, K. ENBERG AND J. PIIRONEN. 2001. Variation in aggressive behavior and growth rate between populations and migratory forms in the brown trout, *Salmo trutta*. *Anim. Behav.*, **62**:935–944.
- LEINER, M. 1934. Die drei europäischen Stichlinge (*Gasterosteus aculeatus* L., *Gasterosteus pungitius* L. and *Gasterosteus spinachia* L.) und ihrer Kreuzungsprodukte. Vergleichende Betrachtung ihrer Laichzeit, ihrer Korperformen und ihrer Brutpfl egetätigkeit. *Z. Morph. Okol. Tiere, Berlin.*, **28**:107–154.
- MATEOS, C. AND J. CARRANZA. 1999. Effects of male dominance and courtship display on female choice in the ring-necked pheasant. *Behav. Ecol and Sociobiol.*, **45**:235–244.
- MATTERN, M. Y. M. 2006a. The phylogeny of the Gasterosteidae with emphasis on the relationships within *Culaea inconstans*. Ph.D. Thesis, University of Toronto.

- . 2006b. Phylogeny, systematics and taxonomy of sticklebacks, p. 1–40. *In*: S. Østlund-Nilsson, I. Mayer and F. A. Huntingford (eds.). *Biology of the three-spined stickleback*. CRC Press, New York.
- MCINERNEY, J. E. 1969. Reproductive behavior of the blackspotted stickleback, *Gasterosteus wheatlandi*. *J. Fish. Res. Bd. Can.*, **26**:2061–2075.
- McKENZIE, J. A. 1969a. A descriptive analysis of the aggressive behavior of the male brook stickleback, *Culaea inconstans*. *Can. J. Zool.*, **47**:1275–1279.
- . 1969b. The courtship behavior of the male brook stickleback, *Culaea inconstans* (Kirtland). *Can. J. Zool.*, **47**:1281–1286.
- McLENNAN, D. A. 2006. The Umwelt of the three-spined stickleback, p. 179–224. *In*: S. Østlund-Nilsson, I. Mayer and F. A. Huntingford (eds.). *Biology of the three-spined stickleback*. CRC Press, New York.
- AND J. L. WARD. Conservation and variation in the agonistic repertoire of the brook stickleback, *Culaea inconstans* (Kirtland). *Environ. Biol. Fish.*, in press.
- MORRIS, D. 1952. Homosexuality in the ten-spined stickleback (*Pygosteus pungitius* L.). *Behavior*, **4**:233–261.
- NAGATA, Y., T. YOSHIMURA AND S. MORI. 1988. Courtship behavior of the three-spined stickleback, *Gasterosteus aculeatus* (leirus form) in Japan. *Memoirs of Osaka Kyouiku University Ser. Osaka, Japan*, **37**:29–36.
- NEAT, F. C., W. LENGKEEK, E. P. WESTERBEEK, B. LARHOVEN AND J. J. VIDELER. 2003. Behavioral and morphological differences between lake and river populations of *Salaria fluviatilis*. *J. Fish Biol.*, **63**:374–387.
- NELSON, J. S. 1969. Geographic variation in brook stickleback, *Culaea inconstans*, and notes on nomenclature and distribution. *J. Fish. Res. Board Can.*, **26**:2431–2447.
- PARZEFALL, J., U. GAGELMANN AND M. SCHARTL. 1997. Aggressive behavior and optomotor response in different populations of *Poecilia mexicana* (Pisces, Poeciliidae). *Mem. Biospeol.*, **24**:63–69.
- PATERSON, H. E. H. 1985. The recognition concept of species, p. 21–29. *In*: E. S. Vrba (ed.). *Species and speciation*. Transvaal Museum Monograph No. 4, Transvaal Museum Pretoria.
- PERES-NETO, P. R. 1999. How many statistical tests are too many? The problem of conducting multiple ecological inferences revisited. *Marine Ecology Progress Series*, **176**:303–306.
- PERKINS, A. AND J. A. FITZGERALD. 1994. The behavioral component of the ram effect: the influence of ram sexual behavior on the induction of estrus in anovulatory ewes. *J. Anim. Sci.*, **72**:51–55.
- PHELPS, S. M., A. S. RAND AND M. J. RYAN. 2006. A cognitive framework for mate choice and species recognition. *Amer. Nat.*, **167**:28–42.
- RANTALA, M. J. AND R. KORTET. 2003. Song and immune function in the field cricket *Gryllus bimaculatus*. *Biol. J. Linn. Soc.*, **79**:503–510.
- REISMAN, H. AND T. CADE. 1967. Physiological and behavioral aspects of reproduction in the brook stickleback, *Culaea inconstans*. *Am. Midl. Nat.*, **77**:1245–1252.
- RIDGWAY, M. S. AND J. D. MCPHAIL. 1987. Rival male effects on courtship behavior in the Enos Lake species pair of threespine sticklebacks (*Gasterosteus*). *Can. J. Zool.*, **65**:1951–1955.
- ROGERS, W. AND G. W. BARLOW. 1991. Sex differences in mate choice in a monogamous biparental fish, the midas cichlid (*Cichlasoma citrinellum*). *Ethology*, **87**:249–261.
- ROSENFELD, J. A. AND A. KODRIC-BROWN. 2003. Sexual selection promotes hybridization between Pecos pupfish, *Cyprinodon pecosensis* and sheepshead minnow, *C. variegates*. *J. Evol. Biol.*, **16**:595–606.
- ROWLAND, W. J. 1974. Reproductive behavior of the fourspine stickleback, *Apeltes quadracus*. *Copeia*, **1974**:183–194.
- . 1988. Aggression versus courtship in threespine sticklebacks and the role of habituation to neighbors. *Anim. Behav.*, **36**:348–357.
- RYAN, M. J., A. S. RAND AND L. A. WEIGT. 1996. Allozyme and advertisement call variation in the Túngara frog, *Physalaemus pustulosus*. *Evolution*, **50**:2435–2453.
- SARGENT, R. C. AND J. GEBLER. 1980. Effects of nest site concealment on hatching success, reproductive success, and paternal behavior of the threespine stickleback, *Gasterosteus aculeatus*. *Behav. Ecol. Sociobiol.*, **7**:137–142.

- SATOU, M., A. SHIRAIISHI, T. MATSUSHIMA AND N. OKUMOTO. 1991. Vibrational communication during spawning behavior in the Hime salmon (landlocked red salmon, *Oncorhynchus nerka*). *J. Comp. Physiol.*, **168**:417–428.
- , H. A. TAKEUCHI, K. TAKEI, T. HASEGAWA, T. MATSUSHIMA AND N. OKUMOTO. 1994. Characterization of vibrational and visual signals which elicit spawning behavior in the male hime salmon (landlocked red salmon, *Oncorhynchus nerka*). *J. Comp. Physiol. A-Sen. Neur. Behav. Physiol.*, **174**:527–537.
- SCHRODER, S. L. 1981. The role of sexual selection in determining the overall mating patterns and mate choice in chum salmon. Ph.D. Dissertation, University of Washington, Seattle.
- SCOTT, M. L. AND S. A. FOSTER. 2007. Phenotypic plasticity and the ecotypic differentiation of aggressive behavior in threespine stickleback. *Ethology*, **113**:190–198.
- SEVENSTER, P. 1961. A causal analysis of a displacement activity. *Behavior Suppl.*, **9**:1–170.
- SIH, A., A. BELL AND J. CHADWICK JOHNSON. 2004a. Behavioral syndromes: an ecological and evolutionary overview. *Trends Ecol. Evol.*, **19**:372–378.
- , ———, AND R. ZIEMBA. 2004b. Behavioral syndromes: an integrative overview. *Quart. Rev. Biol.*, **79**:241–305.
- TAUTZ, A. F. AND C. GROOT. 1975. Spawning behavior of chum salmon (*Oncorhynchus keta*) and rainbow trout (*Oncorhynchus tshawytscha*). *Can. J. Fish. Aqua. Sci.*, **56**:1172–1181.
- TER PELKWIJK, J. J. AND N. TINBERGEN. 1937. Eine reizbiologische analyse einiger verhaltenweisen von *Gasterosteus aculeatus* L. Z. *Tierpsychol.*, **1**:103–200.
- TINBERGEN, N. 1951. *The Study of Instinct*. Oxford University Press, Oxford.
- UTTER, F. 2004. Population genetics, conservation and evolution in salmonids and other widely cultured fishes: some perspectives over six decades. *Reviews in Fish Biology and Fisheries*, **14**:125–144.
- VAN DEN ASSEM, J. 1967. Territory in the three-spined stickleback (*Gasterosteus aculeatus* L.), an experimental study in intraspecific competition. *Behavior, Suppl.*, **16**.
- WARD, G. AND J. FITZGERALD. 1987. Male aggression and female mate choice in the threespine stickleback, *Gasterosteus aculeatus* L. *J. Fish Biol.*, **30**:679–690.
- WARD, J. L. AND D. A. McLENNAN. 2006. The relative influences of sexual and natural selection upon the evolution of male and female nuptial colouration in the brook stickleback, *Culaea inconstans*. *Behavior*, **143**:483–510.
- WILLMOTT, H. E. AND S. A. FOSTER. 1995. The effects of rival male interaction on courtship and parental care in the fourspine stickleback, *Apeltes quadracus*. *Behavior*, **132**:997–1010.
- WILZ, K. 1972. Causal relationships between aggression and the sexual and nest behaviors in the threespined stickleback. *Anim. Behav.*, **20**:335–340.
- WINN, H. 1960. Biology of the brook stickleback, *Eucalia inconstans* (Kirtland). *Amer. Midl. Nat.*, **63**:424–438.
- WOOTTON, R. J. 1976. *The Biology of the Stickleback*. Academic Press, New York.

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