

Changes in agonistic, courtship and parental displays of the male brook stickleback, *Culaea inconstans*, across the breeding cycle

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Summary

Although the individual elements of stickleback behaviour are well known, to date a robust investigation of the temporal cycling of aggressive, courtship and parental behaviour during the breeding cycle has been undertaken in only one species, the threespine stickleback, *Gasterosteus aculeatus*. In order to understand the evolution of behaviour in this fascinating group of fishes, comparative studies conducted upon other members of the Gasterosteidae are necessary. We thus documented the cyclic changes and structure of sixteen agonistic, parental and courtship displays across the breeding cycle for a population of brook sticklebacks, *Culaea inconstans*, from Nebraska and compared it to the temporal cycling of reproductive behaviour in the threespine. Our results indicate that in the brook stickleback: (1) aggressive behaviour varies temporally in response to a male, but not a female, intruder. (2) During those phases wherein the young are nest bound, aggressive and parental elements are negatively correlated. (3) The location of the focal fish within the tank during the trial is an accurate indicator of his primary motivation and (4), a sex specific differential response to intruding fish is apparent only during the courtship interval.

The temporal structure of reproductive behaviour in the brook stickleback mirrors that of the threespine stickleback in three respects: (1) aggressive motivation across the breeding cycle exhibits a U-shaped distribution, with peak levels of aggression occurring in the nest-building stage and final days of parental care. (2) During the courtship phase sexual motivation supercedes aggression in response to the presence of a nuptially coloured female and (3), the pattern of parental fanning shows an inverted U-shaped distribution with peak frequencies coinciding with the hatching of the eggs.

Keywords: aggression, courtship, parental care, temporal change, Gasterosteidae

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The threespine stickleback, *Gasterosteus aculeatus*, is a model system for a wide range of evolutionary studies from the genetic and physiological control of behaviour (Kingsley et al., 2004; Mayer et al., 2004) to the involvement of behaviour in rapid bursts of ecological speciation (McPhail, 1994; Cresco & Baker, 1996; Schluter, 1998). The species, or rather species complex, is so well studied that entire books are now devoted to its biology. Other members of the Gasterosteidae, the family containing the threespine and its relatives, have not been investigated in nearly the same detail. This is unfortunate because a complete evolutionary explanation ultimately requires information from both microevolutionary and macroevolutionary levels of analysis. Without comparative studies, it is impossible to determine when a particular behaviour originated, the sequence of character origin and diversification, and how often a particular adaptive response has appeared under similar selective regimes. Without models and experimental manipulations of individual organisms, it is impossible to test hypotheses concerning the processes underlying the patterns of origin, maintenance and diversification revealed by comparative analysis. In this way, the two research programmes reinforce one another in the search for answers concerning the production of biological diversity (Brooks & McLennan, 1991, 2002, and references therein).

Of all the experiments conducted with *G. aculeatus*, some of the most elegant are still some of the oldest and involved asking questions about how a male's behaviour changes across his breeding cycle. Ter Pelkwijk & Tinbergen (1937) pioneered the use of captive presentations to document the motivation of a territorial male during the courtship phase. This protocol was quickly extended to examine the responses of that territorial male at different stages of his breeding cycle. The existence of such a cycle, in turn, generated questions about the physiological processes underlying and controlling those cycles (e.g. Wootton, 1970; Bakker, 1986; Smith & Wootton, 1999; Pall et al., 2004; Mayer et al., 2004). To this day, those ground-breaking experiments have only been conducted with the threespine. The question thus remains, is the cycling of behaviour documented for the threespine unique to that species or is it a reflection of an older (plesiomorphic) pattern?

In this paper, we examine this question using the brook stickleback, *Culaea inconstans*. Elements of the brook stickleback's behavioural repertoire have been described for different stages of the breeding cycle (Hall, 1956; Winn, 1960; Reisman & Cade, 1967; McKenzie, 1969a, b, 1974; Foster,

1973), however, no one has examined changes in those behaviours across a complete cycle for individual males.

Materials and methods

Adult brook stickleback were collected in minnow traps from Sutherland Creek, Nebraska (41°08'13"N, 101°07'28"W) as they moved from deeper over-wintering waters into the shallow breeding areas in May 2004. Sutherland Creek is shallow and steep banked, runs between farmland and is characterized by clear water flowing gently over a moderately vegetated, soft silt and pebble substrate. Once caught, fish were placed in large, covered, plastic day packers and immediately transported back to the University of Toronto in chilled, continually aerated water.

Fish were maintained under winter conditions (10–12°C; 12 h: 12 h light-dark cycle) and high population density (Reisman, 1961; Reisman & Cade, 1967) in a 200 liter opaque tank with continual flow-through of dechlorinated water. Reproductive behaviour was further discouraged by a lack of suitable nest-building materials and substrate, although large sticks and stones were provided for shelter. Individuals were removed as needed from this holding tank and placed in 58-liter community tanks lined with 2.5 cm of fine gravel, fitted with a charcoal filter and aeration system, and covered on three sides with light green paper. These tanks were maintained under a 16 h light: 8 h dark regime, a constant temperature of 16°C, and low density (three fish per tank), all of which promote breeding (Winn, 1960; Reisman & Cade, 1967). Each tank was supplied with nesting material consisting of soft moss and algae and one or more aquatic plants. Under these conditions, fish usually became reproductively active within three days.

Individual male brook sticklebacks were introduced directly from the community tanks into 58-liter aquaria lined with 2.5 cm of fine gravel and fitted with a charcoal filter and aeration system in one corner. Two sides of each tank were covered with light green paper and a third with a photographic backdrop of aquatic vegetation. To minimize disturbance, a green curtain was hung in front of each tank and removed during filming. Each tank was supplied with *Hygrophila* rooted in a peat-filled 10 cm diameter plastic dish, and an abundance of soft algae, grasses, and twigs collected from a nearby marsh. All males who successfully completed a breeding cycle began nest-building in the *Hygrophila* within 72 hours. Males that failed to build a nest within five days were replaced.

The reproductive cycle

Elaborating on protocols developed for the threespine stickleback (ter Pelkewijk & Tinbergen, 1937), males were presented with captive male and female intruders at six stages of the breeding cycle (McLennan, 1993):

i) Nest-building (Day 2).

ii) Courtship (Day 3): defined by the presence of a complete nest, which is usually marked by the appearance of nest fanning. Males were allowed to spawn following filming.

iii) Egg guarding (Day 6): embryos are approximately halfway to hatching. The male spends most of his time fanning (aerating) the eggs and removing those that are damaged or diseased (McKenzie, 1974).

iv) Fry hatch (Day 10): newly hatched fry are immobile within the nest. The parental male begins to tear apart the nest and construct a loosely tangled algal nursery.

v) Post-nursery construction (Day 12): nursery is complete and fry have been transferred to the new structure.

vi) Free-swimming fry (Day 17): fry begin attempting to leave the nest but the male actively retrieves them in his mouth and returns them to the nest.

For each presentation, a nuptially coloured male or female stimulus fish was placed in a 12 × 5.5 cm glass jar, capped with fine mesh to permit the exchange of chemical cues (McLennan, 2004, 2005). The size of the jar restricted excess movement, minimizing the behavioural response of the intruder. Presentation order of the male and female intruder was randomized and each male was permitted a three hour rest between trials. All presentations were videotaped for five minutes using a SONY digital handycam and later analyzed using a SONY Digital8 Video Cassette Recorder.

Behavioural analysis

Temporal cycling in the behaviour of male brook sticklebacks was investigated by recording the frequency and duration of sixteen aggressive, courtship and parental displays from video playback of the interactions between the focal male and each captive intruder. The sixteen displays were operationally defined as follows:

Frontal approach: focal male approaches the glass jar in a straight path.

Head-down threat: male assumes a 45-90° head-down position relative to the substrate (McKenzie, 1969b).

Broadside threat: male assumes a horizontal, concave posture, displaying laterally to the opposing fish. The dorsal and pelvic spines are often flared and the pectoral fins erected (McKenzie, 1969b).

Circle-threat: male circles the stimulus fish while engaged in the lateral broadside posture described above (Morris, 1958; McKenzie, 1969b).

S-maneuver: male curves his body into an S-shaped, sigmoid posture, usually while directly facing the stimulus fish (McKenzie, 1969b).

Tail-beating: male beats his tail laterally in a slow (approximately 4-10 beats per second), exaggerated manner (McKenzie, 1969b).

Pummeling: from a position slightly above, and approximately 10-15 cm away from the female (McKenzie, 1969a), the male dashes downward, repeatedly striking the female on her head and flanks with the anterior jaw (Hall, 1956).

C-body: male brings his tail forward until the caudal fin is in line with his head. The tail is then whipped back into line with the body, accompanied by simultaneous forward lunging. Likely a propulsion mechanism, this behaviour has not previously been described for the brook stickleback, although a description by Burks et al., (1985) of a display observed in a population from Wisconsin and termed 'sigmoid Atkinson' may be comparable.

Complete leads to the nest: male attempts to lead the stimulus fish to his nest, typically terminating the attempt in a bout of fanning/nest-showing.

Partial leads to the nest: male terminates the lead prior to reaching the nest.

Fanning: positioned facing into the nest entrance, the male rapidly vibrates his pectoral fins, forcing fresh water over the clutch. Most commonly an element of parental care (Winn, 1960; Reisman, 1961; Reisman & Cade, 1967, McKenzie, 1974; see also Wootton, 1976 and references therein), males may fan an empty nest during courtship, particularly following a lead attempt. The latter display, termed 'displacement fanning' or 'nest-showing', differs from parental fanning behaviour in that the male presses his mouth onto the upper rim of the nest while simultaneously vibrating his pectoral fins. For the purposes of discrimination, we shall call the former display 'parental fanning' and the latter 'nest showing'.

Boring: male repeatedly thrusts his head into the nest entrance, widening the nest cavity.

Tail-flagging: male raises his caudal fin, such that the caudal peduncle and fin curve upward (Reisman & Cade, 1967).

Waggle-dance: males assume 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-dance during pre-lead courtship interaction.

Bite: male attempts to bite the captive fish through the glass jar.

Dorsal threat: male modifies a broadside threat display by tilting his body on a sagittal 45° angle and erecting his dorsal spines (McKenzie, 1969b; McLennan 1993).

In addition to recording behavioural frequencies for all of the preceding characters, the total amount of time each male spent fanning, engaging in bouts of biting, waggle-dancing, boring at the nest and tail-beating was recorded, as well as the total amount of time spent by each male within the plants, or within one fish length of the jar containing the captive individual.

Results

Of the initial twenty-three males selected for breeding trials, seven failed to build nests and five spawned with a gravid female but did not successfully raise the eggs to term, thus the results are based on the remaining eleven males who successfully completed the breeding cycle ($N = 11$). Unless indicated otherwise, all analysis of the temporal cycling of behaviour was undertaken via Bonferroni corrected t -tests.

Temporal cycling in aggressive behaviour

There was no change in the frequency and duration of aggressive displays directed towards a female intruder across the breeding cycle. The response to a male intruder, however, varied temporally for five behaviours: the frequency of head-down (HD), broadside (BR), tail-beating (TB) and C-body (CB) displays, and the frequency of bites (BT). Temporal cycling of aggressive behaviour in this population follows a U-shaped distribution, peaking during nest-building, decreasing to a plateau during egg-guarding and fry-hatching, then increasing after nursery movement to a second peak when fry are free swimming (Figure 1). In general, aggression at the two peak stages is significantly greater than during the egg-guarding / fry-hatching plateau (Table 1).

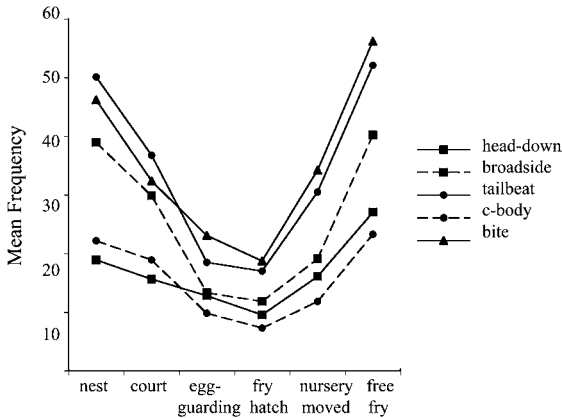


Figure 1. Mean frequencies of five behavioural components of aggression across the breeding cycle in response to a male captive intruder ($N = 11$).

Table 1. Results of Bonferroni corrected t -tests of differences in the frequencies of head-down display, broadside threat, tailbeats, C-body display and bites in response to a male captive intruder ($N = 11$; $df = 10$). Although all combinations of breeding stages were tested, only those comparisons that exhibit significant differences in the frequency of behaviours are presented.

		Egg-guarding									
		Headdown		Broadside		Tailbeat		C-Body		Bite	
		t	p	t	p	t	p	t	p	t	p
Nest	Free fry	NS	NS	4.66	0.001	4.39	0.002	4.76	0.001	4.47	0.002
Free fry	Nest	-4.89	0.001	-5.08	0.001	-4.77	0.001	NS	NS	-3.97	0.002
		Fry hatch									
Nest	Free fry	NS	NS	5.15	0.001	4.53	0.001	3.82	0.003	NS	NS
Free fry	Nest	-5.73	0.001	-5.61	0.001	-6.22	0.001	-5.09	0.001	-6.37	0.001

The amount of time spent engaged in bouts of biting and tail-beating shows a similar U-shaped distribution (Figure 2). Territorial male sticklebacks spend significantly more time attempting to bite the captive male intruder during nest-building and when their fry are free swimming, than they do during the egg-guarding / fry-hatching plateau [nest/egg-guarding ($t = 4.59$, $df = 9$, $p = 0.001$); nest/fry hatch ($t = 4.24$, $df = 10$, $p = 0.002$);

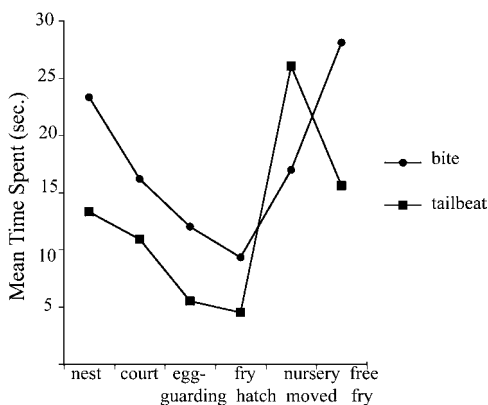


Figure 2. Mean duration of time spent engaging in bouts of biting or tail-beating across the breeding cycle in response to a male captive intruder ($N = 11$).

free-swimming fry / egg-guarding ($t = -3.94$, $df = 9$, $p = 0.003$); free-swimming fry / fry hatch ($t = -6.19$, $df = 10$, $p < 0.001$]. The total amount of time spent tail-beating is significantly greater when fry are free swimming than it is during the egg-guarding and fry hatch phases [free-swimming fry / egg-guarding ($t = -4.76$, $df = 9$, $p = 0.001$); free-swimming fry / fry hatch ($t = -7.14$, $df = 10$, $p < 0.001$)]. The significantly greater number of bouts of tail-beating performed during the nest-building stage than during the first two stages of parental care, combined with the statistically constant total activity duration across this period, suggests that in the early stages of the breeding cycle, tail-beating bouts are short and increase in duration when the male acquires a clutch.

Temporal cycling in parental fanning

The frequency of fanning behaviour increases with deposition of eggs within the nest, and peaks when the fry are newly hatched. Following hatching, parental males gradually decrease the number of fanning bouts performed, returning to minimum levels with the emergence of fry from the nest (Figure 3). In response to a male intruder, parental males perform significantly more bouts of fanning when the fry are newly hatched than they do during nest-building ($t = -5.17$, $df = 10$, $p < 0.001$), courtship ($t = -5.17$, $df = 10$, $p < 0.001$) or when the fry are free swimming ($t = 5.17$, $df = 10$, $p = 0.001$). In response to a female intruder, males perform more bouts of fanning during the fry-hatching and post-nursery construction stages than

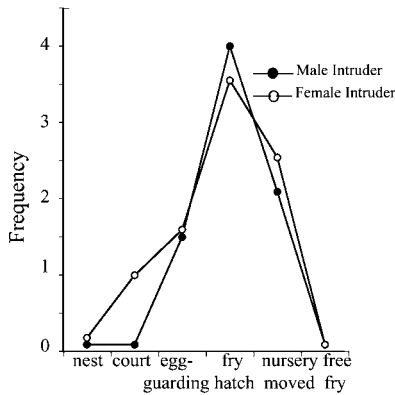


Figure 3. Mean frequency of fanning behaviour across the breeding cycle in response to either a male (●) or female (○) captive intruder ($N = 11$).

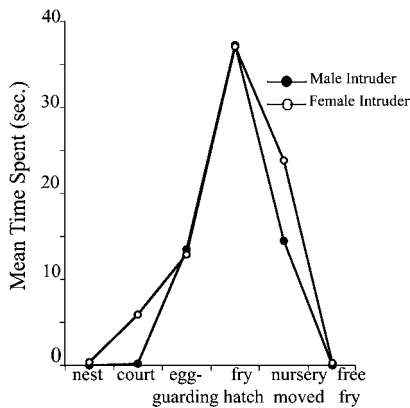


Figure 4. Mean duration of time spent engaging in bouts of fanning across the breeding cycle in response to either a male (●) or female (○) captive intruder ($N = 11$).

they do during the nest-building phase (fry hatch: $t = -6.18$, $df = -6.18$, $p < 0.001$; nursery moved $t = -4.10$, $df = 10$, $p = 0.002$) or in the final stage of parental care when the fry are free-swimming (fry hatch: $t = 6.33$, $df = 10$, $p < 0.001$; nursery moved $t = 4.50$, $df = 10$, $p = 0.001$).

The above results are mirrored in the amount of time that parental males spend engaged in fanning behaviour (Figure 4). Males spend more time fanning at fry-hatching versus all other stages except egg-guarding when the intruder is male [nest-building ($t = -4.77$, $df = 10$, $p = 0.001$); courtship ($t = -4.74$, $df = 10$, $p = 0.001$); nursery movement ($t = 3.92$, $df = 10$,

$p = 0.003$); free-swimming fry ($t = 4.77$, $df = 10$, $p = 0.001$), and more time fanning at fry-hatching versus nest-building ($t = -5.42$, $df = 10$, $p = 0.001$) or free swimming fry ($t = 5.43$, $df = 10$, $p < 0.001$) when the intruder is female.

Differential response towards a male vs female intruder

A comparison of the mean behavioural frequencies at each stage of the breeding cycle reveals that the behaviour of the focal male changes with respect to the sex of the intruding fish only during the courtship phase (Figure 5). During this phase, courting males direct significantly more bites towards toward intruding males ($t = 3.98$, $df = 10$, $p = 0.003$), and spend more time engaged in biting bouts ($t = 3.95$, $df = 10$, $p = 0.003$). Conversely, courting males direct more pummels towards females ($t = -5.05$, $df = 10$, $p = 0.007$) and spend more time attempting to lead her to the nest ($t = -2.65$, $df = 10$, $p = 0.02$).

In response to a female intruder, pummeling behaviour is the most commonly performed display during both the courtship and egg-guarding stages (mean: 15.18 bouts during courtship and 10.27 bouts during egg-guarding). Across the breeding cycle, the frequency of pummeling behaviour shows an inverted U-shaped distribution, sharply increasing with the completion of the nest to peak frequency during the courtship stage and decreasing equally as sharply to minimal levels once the fry have hatched. In contrast, territorial males did not perform pummeling behaviour towards a male intruder at any stage of the breeding cycle.

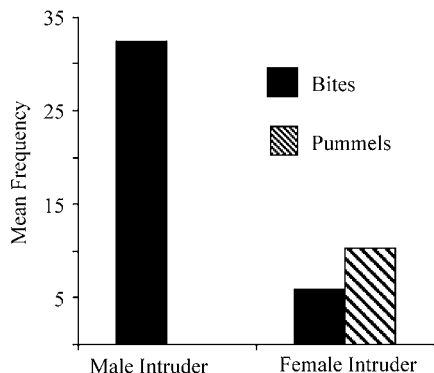


Figure 5. Mean frequency of bites and pummels directed towards a male and female captive intruder during the courtship stage of the breeding cycle ($N = 11$).

Although not statistically significant, males are also more likely to engage in bouts of nest-showing when courting a female, then when presented with a male intruder (mean: 1.00 bout(s) of nest-showing fanning in response to a female versus 0.09 in response to a male). The failure to detect a significant difference, in this case, may be a statistical artifact due to a reduced sample size.

Correlation between location and behavioural motivation

When considering each stage of the breeding cycle separately, a sex-specific differential response is not detected in the relative amounts of time the fish spends within the plants or within one fish length of the jar, so the responses to males and females were pooled in graphical analysis (Figure 6). General trends indicate that the relative amount of time that the territorial male spends in the plants when presented with a captive intruder corresponds to the temporal pattern of fanning behaviour, and conversely, the amount of time he spends within one fish length of the conspecific exhibits a U-shaped distribution reminiscent of the temporal cycling of aggressive elements.

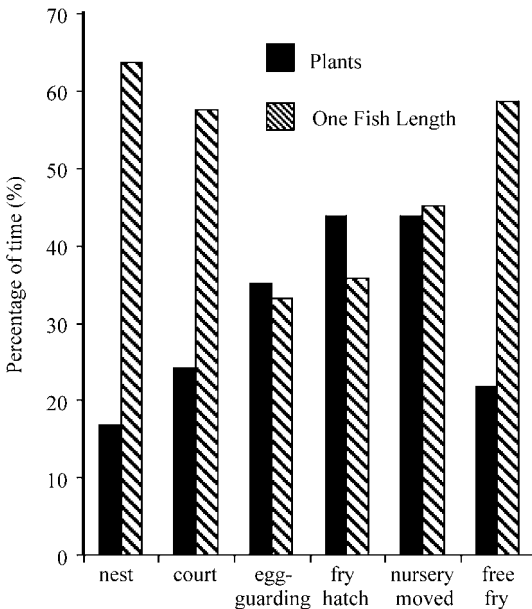


Figure 6. Mean percentage of time territorial male brook sticklebacks spend engaging in interaction with a territorial intruder or in nest activity at each stage of the breeding cycle ($N = 11$).

Across the breeding cycle however, subtle differences in the location of the male with respect to sex of the intruding individual are detectable. Territorial male sticklebacks spend significantly more time engaged in close encounters with a captive male intruder during the courtship ($t = 4.21$, $df = 9$, $p = 0.002$) and free-swimming fry ($t = -4.03$, $df = 9$, $p = 0.003$) phases than they do during the egg-guarding phase. Additionally, territorial males spend less time engaged in female encounters during the fry hatch stage than at any other phase of the breeding cycle [nest-building ($t = 5.67$, $df = 10$, $p < 0.001$); courtship ($t = 5.25$, $df = 10$, $p < 0.001$); egg-guarding ($t = 4.64$, $df = 10$, $p = 0.001$); nursery moved ($t = -5.26$, $df = 10$, $p < 0.001$); free-swimming fry ($t = -5.94$, $df = 10$, $p < 0.001$)].

Discussion

The pattern of behavioural cycling from nest-building to free-swimming fry shown by brook stickleback males mirrors the pattern reported for *Gasterosteus aculeatus* in three respects. First, the aggressive response of a territorial male towards a conspecific male intruder is U-shaped, with maximal levels of aggression characterizing the first (nest-building) and last (free-swimming fry) stages of the breeding cycle and minimal levels corresponding to the care of nest-bound young. Studies using the threespine stickleback have also documented either the complete U-shaped pattern of aggressive cycling (Segaar, 1961; Wootton, 1971; Black, 1971) or components of this pattern, including high levels of aggression during the territory acquisition stage (van Iersel, 1958; Sevenster, 1961; Sevenster-Bol, 1962; McInerney, 1969; Wilz, 1972; Wootton, 1970) and increasing levels of aggression with progression of the parental phase (Huntingford, 1976; Kynard, 1978; McLennan & McPhail 1989; 1990; McLennan 1993; Bakker, 1994).

At the onset of the breeding cycle, male brook sticklebacks are equally aggressive towards both male and female conspecifics and will readily threaten or attack territorial intruders (Reisman & Cade, 1967; results, this study). Obtaining a suitable territory is of paramount importance and, if the system in *C. inconstans* is similar to that of *G. aculeatus*, a male's nest placement will have a profound effect on his reproductive success. Male brook sticklebacks preferentially nest in heavily weeded areas (Reisman & Cade, 1967; and references therein) where they are (presumably) less vulnerable

to predators (Degraeve, 1970; Reist, 1980, 1983), are likely to face fewer courtship/parental care intrusions (van Iersel, 1953; van den Assem, 1967; Wootton, 1971, 1972; Li & Owings, 1978; Rowland, 1988) and/or raids by cannibalistic neighbors (McKenzie, 1974; Sargent & Gebler, 1980; FitzGerald, 1991). The attributes of a male's territory may also have a considerable effect on his chances of enticing a female to mate. In threespines, reproductive success is positively correlated with increasing territory size (van den Assem, 1967; Black, 1971; Li & Owings, 1978; Goldschmidt & Bakker, 1990), nest concealment (Sargent & Gebler, 1980; Sargent, 1982; Kraak et al., 1999, 2000) and/or shelter (Moodie, 1972; Kynard, 1978; Mori, 1990). Laboratory (Morris, 1958; van den Assem, 1967; McInerney, 1969; Black, 1971; Sargent & Gebler, 1980), and field studies have found that when space is limited, males actively compete with each other for suitable territories (Black & Wootton, 1970; Wootton, 1972; Kynard, 1978; Borg, 1985; Whorisky & FitzGerald, 1987). Our results are thus congruent with the hypothesis that during the territory acquisition/nest-building stage, the importance of a well-chosen nest site means that agonistic intraspecific interactions are likely to be frequent and aggressive motivation correspondingly high.

Once the territory has been obtained and the male has built his nest he is ready to court receptive females. In the Nebraskan population of brook sticklebacks, courtship is the only stage of the breeding cycle during which the territorial male exhibits a differential response towards male versus female intruders; directing significantly more bites towards the male and more pummels and leads towards the female. The interaction between sex and aggression manifests itself subtly in sticklebacks. When a male threespine courts a female in the presence of a rival, he generally experiences reduced spawning success, in part because the rival occasionally swims through the nest and fertilizes the eggs before the territory holder and in part because the male spends less time courting the female (Rowland, 1988). This second component occurs because the male must spend more time trying to drive his rival away and, more critically, because aggression and sexual behaviour appear to be mutually inhibitory motivational states in sticklebacks. The territorial male must therefore switch between agonistic and sexual behaviour, and this increases the overall time required for a successful courtship, which in turn increases the chance that the female will break off the interaction (Sevenster, 1961, 1973; Wilz, 1972). In our experiment we found that

a courting male attempts to bite an intruding male with a frequency comparable to that observed during territory acquisition. When he encounters a nuptially coloured female however, he switches from biting to pummeling and nest leading (Hall, 1956; Foster, 1973; Winn 1960; Reisman & Cade, 1967; McLennan, 1995). These results provide additional support for the hypothesized inhibitory action of sexual motivation upon aggressive tendencies; the discrepancy in the number of biting bouts directed towards a male versus a female intruder stems from a sharp decrease in aggression towards the female as aggression is superseded by sexual motivation, rather than an increase in aggression towards males.

Finally, the third way in which the pattern of behaviour cycling is similar between brook sticklebacks and other gasterosteid species involves parental fanning; in all species studied to date the time spent fanning is inversely related to the time spent involved in aggressive interactions (*Gasterosteus aculeatus*: van Iersel, 1953; Wootton, 1971; Black, 1971; Kynard, 1978; Páll et al., 2002a, b, 2005; *Gasterosteus wheatlandi*: McInerney, 1969; *Spinachia*: Sevenster, 1951; *Pungitius*: McKenzie & Keenleyside, 1970; *Apeltes*: Hall, 1956; Rowland, 1974; Reisman 1963; *Culaea inconstans*: McKenzie, 1974; results this study). Following fertilization, fanning in the brook stickleback gradually increases, reaching peak levels nine or ten days after egg deposition, corresponding with fry hatching, while intraspecific aggression falls to its minimal level. During the initial parental care stages the male is restricted to the immediate vicinity of the nest, aerating the eggs via fanning and removing those that are diseased or dead (Winn, 1960; Reisman & Cade, 1967; McKenzie 1974). Two pieces of evidence suggest that the decrease in aggressive motivation during this interval is correlated with the parental status of the male: (1) a decrease in the amount of time engaging in close interactions with the captive intruder, and a corresponding increase in the amount of time spent in the nest plant, suggesting that parental males often ignore the presence of a conspecific during the egg-guarding and first stages of fry care. In threespine sticklebacks, egg-guarding males were also less likely to attack intruders and their territories contracted somewhat as attention was focused on the eggs and nest (van den Assem, 1967); and (2), as mentioned above, the frequency of the aggressive response of the parental male is inversely related to the fanning frequency. In response to a male intruder, parental fanning and aggression are decoupled during the egg-guarding [spearman rank correlation: headdown ($r = -0.74$, $p = 0.01$), bite ($r = -0.74$, $p = 0.01$)]

and fry hatch [headdown ($r = -0.75$, $p = 0.01$), broadside ($r = -0.61$, $p = 0.05$), tailbeat ($r = -0.72$, $p = 0.01$), bite ($r = -0.62$, $p = 0.04$)] stages and during the fry hatch [frontal approach ($r = -0.65$, $p = 0.03$), headdown ($r = -0.65$, $p = 0.03$), tailbeat ($r = -0.75$, $p = 0.01$)] stage when the intruder is a captive female. These two variables are also negatively correlated in the threespine (Sevenster, 1961).

Classical parental investment theory (PI) stresses that the cost of reproductive effort will be selected to maximize future reproductive success and is independent of past cumulative effort or investment (Trivers, 1972). Increases in the cost of parental care are therefore expected as the young age and increase their reproductive value (Fisher, 1930; Pianka & Parker, 1975; Pressley, 1976, 1981). Research investigating a positive correlation between the age of the eggs and parental investment in sticklebacks have so far been equivocal (Huntingford, 1976; Kynard, 1978; Pressley, 1981; FitzGerald & von Havre, 1985; Ukegbu & Huntingford, 1988; Lachance & FitzGerald, 1992). Most of these studies however, have used risk taking and/or increased aggressive defense during the egg-guarding stage as the primary criterion of PI. Our results suggest that, in the brook stickleback at least, aggressive behaviour is a less reliable measure of PI during these stages than parental fanning, as levels of aggressive defense are minimal throughout the duration of the parental interval when fry are confined to the nest. Fanning, on the other hand, increases as embryo development progresses. Smith & Wootton (1999) estimated that the energy expenditure of a threespine male increased from $3.9 \text{ J g}^{-1}\text{h}^{-1}$ during courtship to $12.3 \text{ J g}^{-1}\text{h}^{-1}$ during paternal care. Since a large part of paternal care in these fishes involves driving oxygenated water over the developing eggs via vigorous movements of the pectoral fins, it appears that fanning is an alternative energetically costly activity that fits the criterion of parental investment.

Using aggressive defense as a measure of parental investment may be more reliable during the later stages of parental care when the parental male begins to actively defend his territory once more, guarding the fry and returning any errant individuals to the nest (Wootton, 1971; Black, 1971; Huntingford, 1976; Kynard, 1978; McLennan & McPhail, 1989, 1990; McLennan, 1993). During this time the young are particularly vulnerable to predation (McPhail, 1969; Moodie, 1972; Wootton, 1970, 1972; Semler, 1971; McKenzie & Keenleyside, 1970; McKenzie, 1974; FitzGerald, 1991; Foster, 1994). The high energetic costs of parental care (Chellappa et al., 1989;

Chellappa & Huntingford, 1989; Wootton, 1994; Smith & Wootton, 1999) and the short breeding season (Winn, 1960; Reisman & Cade, 1967) means that, for any given parental male, the young that emerge from the nest will probably represent his entire reproductive output. Because of these costs, the energy available for a second reproductive cycle decreases as the male's first clutch ages. Indeed most males only complete one breeding cycle in the wild. The value of that clutch to the male should thus increase over time, reaching a peak somewhere between the time when the fry emerge from the nest and swim-up (fry with a physoclistous gas bladder must fill that bladder with a one time only gulp of air at the surface), after which it becomes impossible for the parental male to control their movements any further. Aggressive defense of offspring should reach maximal levels when fry are free swimming, which is exactly what we saw.

If we consider the two breeding phases characterized by the highest levels of aggression, that of nest-building and the final stage of parental care, we may ask whether there are any qualitative differences in the aggressive response of males at the onset, versus the final days, of the breeding cycle. In general, Nebraskan males appear more aggressive during the latter phase, although the scoring method used here did not permit quantification of behavioural intensity. Thus, while a given aggressive element (e.g. *broadside*) may have occurred with equal frequency in the territory acquisition and final days of parental care, it often appeared more 'intense' at the latter stage (e.g. more sharply curved body).

The greatest disparity between the two breeding phases in terms of aggressive elements lies in the relative frequencies of head-down and bite displays; these displays are often observed during intense aggression and fighting and are more frequent in the final stage of parental care. A biting attack, in particular, is clearly intensely agonistic. The broadside, tail-beating and C-body threat behaviours, however, do not differ noticeably between the two phases. McKenzie (1969b) noted that both broadside threat and tail-beating were equally as likely to be followed by fleeing as by an attack, and suggested that the lateral structure of the broadside threat (neither facing towards, nor away from the opposing fish) reflected the balance of indecision between the two extremes of attacking and fleeing. This suggests that although overall levels of aggression are statistically similar between the two phases, while the male is building his nest, relevant agonistic interactions contain more of a threat element. Once fry have begun to leave the nest however, the male

has reached near maximal levels of reproductive investment, aggressive motivation is more intense and the tendency to attack an intruder more likely.

Conclusion

The striking similarities between our results and those available for other members of the stickleback family suggest that the temporal cycling of aggressive, sexual and parental care behaviours are old, highly conserved patterns within the family (McLennan et al., 1988; McLennan, 1991, 1996). Cycling of male behaviour in the threespine is controlled, in part, by changes in circulating hormones. The plasma level of 11-ketotestosterone (11kT) increases during nest-building, peaks during courtship when it is correlated with an increase in zigzag dancing, then drops rapidly as the male enters the parental phase (Páll et al., 2002a, b and references therein). During egg- and fry-guarding, the appearance of parental fanning and the suppression of courtship is modulated by increasing levels of prolactin (PRL) (Slijkhuis et al., 1984; Páll et al., 2004). The involvement of 11kT and PRL in courtship and parental behaviour, respectively, has been demonstrated for numerous teleost species (reviewed in Mayer et al., 2004), so it would appear that at least part of the physiological control of the behavioural cycle is also older than the Gasterosteidae (McLennan, 1993). It is important at this point to note that the conservative nature of the temporal cycle does not imply that there is no behavioural variability in the clade. It simply implies that we should search for such variability in performance factors like frequency and intensity and differences in the structure or presence of traits comprising the entire breeding repertoire. Overall, we would expect this variability to be expressed within the context of the plesiomorphic behavioural cycle.

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Appendix 1. Mean frequencies and standard errors (brackets) of sixteen behaviours performed by male brook sticklebacks in response to a male or female intruder, across the breeding cycle ($N = 11$).

Behaviour	Mean response to male intruder					
	Nest	Court	Egg-guarding	Fry hatch	Nursery moved	Free fry
Frontal approach	3.45 (0.56)	5.18 (1.03)	5.90 (1.23)	5.18 (0.74)	7.09 (0.85)	5.36 (1.06)
Headdown	18.91 (2.96)	15.63 (2.36)	12.80 (3.38)	9.55 (3.19)	16.09 (4.14)	27.00 (3.23)
Broadside	38.91 (6.78)	29.82 (7.79)	13.30 (4.18)	11.82 (3.79)	19.09 (5.92)	40.18 (7.11)
Circle threat	9.18 (3.31)	7.00 (3.09)	2.20 (1.02)	2.55 (1.11)	4.45 (1.68)	7.09 (2.22)
S-maneuver	2.55 (1.19)	1.55 (0.56)	1.0 (0.63)	0.18 (0.12)	0.64 (0.28)	1.09 (0.37)
Tailbeat	50.09 (11.25)	36.73 (11.39)	18.50 (5.94)	17.00 (6.52)	30.45 (8.45)	52.09 (8.70)
Pummel	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
C-body	22.18 (4.55)	18.91 (5.20)	9.80 (3.22)	7.27 (2.33)	11.82 (3.26)	23.27 (3.81)
Complete lead	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Partial lead	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Fanning	0.09 (0.09)	0.09 (0.09)	1.50 (0.79)	4.00 (0.79)	2.09 (0.61)	0.09 (0.09)
Boring	0.18 (0.18)	0.09 (0.09)	0.20 (0.13)	0.09 (0.09)	0.00 (0.00)	0.00 (0.00)
Tailflag	7.27 (1.99)	6.00 (1.29)	5.50 (2.42)	5.00 (1.58)	6.09 (1.53)	8.18 (2.38)
Waggle dance	0.00 (0.00)	0.36 (0.28)	0.70 (0.70)	0.09 (0.09)	0.00 (0.00)	0.00 (0.00)
Bite	46.18 (7.97)	32.36 (7.03)	23.1 (6.13)	18.73 (5.89)	34.18 (8.71)	56.18 (7.96)
Dorsal threat	2.36 (1.56)	1.09 (0.58)	0.90 (0.50)	0.73 (0.49)	0.64 (0.36)	0.36 (0.15)

Appendix 1. (Continued).

Behaviour	Mean response to female intruder					
	Nest	Court	Egg-guarding	Fry hatch	Nursery moved	Free fry
Frontal approach	4.00 (0.82)	5.45 (0.84)	6.91 (1.06)	4.91 (0.53)	6.55 (0.80)	5.00 (1.00)
Headdown	18.82 (3.49)	12.73 (3.07)	12.09 (2.21)	8.36 (3.05)	9.00 (2.39)	21.00 (5.03)
Broadside	29.73 (7.18)	11.55 (3.37)	10.64 (2.75)	9.27 (4.61)	15.45 (5.65)	36.55 (9.30)
Circle threat	4.73 (2.64)	1.91 (1.07)	1.64 (0.56)	2.27 (1.21)	1.36 (1.00)	8.35 (3.21)
S-maneuver	2.09 (0.48)	0.18 (0.12)	0.45 (0.31)	0.45 (0.25)	0.73 (0.33)	0.91 (0.41)
Tailbeat	33.09 (11.76)	14.91 (5.08)	13.64 (3.46)	13.09 (5.94)	17.64 (6.00)	44.18 (11.32)
Pummel	2.82 (2.11)	15.18 (4.55)	10.27 (3.45)	0.36 (0.28)	0.00 (0.00)	0.45 (0.25)
C-body	16.45 (5.45)	12.91 (3.51)	6.73 (1.96)	5.64 (2.08)	8.27 (1.92)	21.00 (5.12)
Complete lead	0.00 (0.00)	0.91 (0.55)	0.45 (0.21)	0.09 (0.09)	0.00 (0.00)	0.00 (0.00)
Partial lead	0.36 (0.36)	1.63 (0.66)	1.82 (0.58)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Fanning	0.18 (0.18)	1.00 (0.63)	1.55 (0.59)	3.55 (0.56)	2.55 (0.53)	0.09 (0.09)
Boring	0.18 (0.18)	0.09 (0.09)	0.18 (0.12)	0.09 (0.09)	0.09 (0.09)	0.00 (0.00)
Tailflag	5.09 (1.23)	7.75 (3.12)	6.73 (2.00)	5.09 (1.91)	4.09 (0.83)	5.27 (2.03)
Waggle dance	0.81 (0.58)	2.45 (1.08)	1.82 (0.54)	0.09 (0.09)	0.00 (0.00)	0.00 (0.00)
Bite	30.73 (8.88)	5.91 (2.24)	12.55 (3.22)	13.82 (5.62)	22.00 (6.89)	44.82 (10.60)
Dorsal threat	1.73 (1.35)	0.55 (0.28)	0.27 (0.14)	0.64 (0.45)	0.00 (0.00)	0.36 (0.36)