Consistent individual differences in fathering in threespined stickleback *Gasterosteus aculeatus*

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Abstract   There is growing evidence that individual animals show consistent differences in behavior. For example, individual threespined stickleback fish differ in how they react to predators and how aggressive they are during social interactions with conspecifics. A relatively unexplored but potentially important axis of variation is parental behavior. In sticklebacks, fathers provide all of the parental care that is necessary for offspring survival; therefore paternal care is directly tied to fitness. In this study, we assessed whether individual male sticklebacks differ consistently from each other in parental behavior. We recorded visits to nest, total time fanning, and activity levels of 11 individual males every day throughout one clutch, and then allowed the males to breed again. Half of the males were exposed to predation risk while parenting during the first clutch, and the other half of the males experienced predation risk during the second clutch. We detected dramatic temporal changes in parental behaviors over the course of the clutch: for example, total time fanning increased six-fold prior to eggs hatching, then decreased to approximately zero. Despite these temporal changes, males retained their individually-distinctive parenting styles within a clutch that could not be explained by differences in body size or egg mass. Moreover, individual differences in parenting were maintained when males reproduced for a second time. Males that were exposed to simulated predation risk briefly decreased fanning and increased activity levels. Altogether, these results show that individual sticklebacks consistently differ from each other in how they behave as parents [*Current Zoology* 58 (1): 45–52, 2012].

Keywords   Paternal care, Personality, Behavioral syndrome, Fathers, Temperament, Antipredator behavior

Consistent individual differences in behavior ("personality") have been well-documented (Bell et al., 2009) and may have important fitness consequences (Dingemanse and Reale, 2005). Studies of animal personality have primarily focused on behavioral variation in traits such as aggressiveness, boldness and exploratory behavior (Reale et al., 2007). Fewer studies have examined whether how an individual behaves as a parent is part of an individual's personality (but see Budaev et al., 1999). While there is evidence that variation in parental care is an important axis of inter-individual variation in a range of taxa, including mammals (Maestripieri, 1998; Meaney, 2001) and birds (Nakagawa et al., 2007), we know less about variation in parental care in species where males are the sole providers of parental care.

Threespined sticklebacks *Gasterosteus aculeatus* have become an important model system in the study of individual variation in behavior. Individual sticklebacks consistently differ in how they behave in ecologically-relevant contexts such as in the presence of a predator (Huntingford, 1976), during competition and other social interactions (Pike et al., 2008; Harcourt et al., 2009) and in a novel environment (Bell and Stamps, 2004). Another important context with a direct link to fitness in sticklebacks is parenting: in this species, parental care is necessary for offspring survival (Wootton, 1984) and is provided solely by the male. During the breeding season, male sticklebacks establish territories, build nests, and court females. After a female spawns, she leaves the territory and the male provides all of the parental care while defending the territory and nest from predators and intruders. During the incubation period (approximately 3–6 days), the male "fans" the eggs with his pectoral fins, providing oxygen and clearing carbon dioxide (Wootton, 1984) and removes rotten eggs and debris. After the eggs hatch, the male tends the fry for approximately 4–7 days until the fry are free-swimming, fanning the young immediately after hatching and retrieving individuals that stray from the nest. In addition to physically protecting and meeting physiological requirements of his offspring, Tulley and Huntingford (1987) suggested that behavioral interactions between fathers and fry at the nest

might also influence the development of his offspring's antipredator behavior.

While directly related to male's reproductive success, parenting in sticklebacks is costly: it is energetically expensive (Chellappa et al., 1989; Smith and Wootton, 2005) and exposes males to predation risk (Candolin, 1998). Moreover, while simultaneously rearing his offspring, male sticklebacks must also defend them and maintain a territory. Therefore we might not expect male sticklebacks to show within-individual consistency in parental behavior over time because they must be behaviorally flexible in order to manage several competing demands; this flexibility has been empirically demonstrated in other fishes (Hale et al., 2003; Lissaker and Kvarnemo, 2006). Another reason why we might not expect to see strong behavioral consistency is because male stickleback behavior changes dramatically over the course of the nesting cycle – rates of fanning, for example, change with the age of the eggs and fry (van Iersel, 1953).

On the other hand, studies in biparental systems have suggested that males behave consistently as parents. Indeed, some studies on birds have shown that males behave more consistently than their female mates, who adjust more readily to the needs of their offspring (Schwagmeyer and Mock, 2003; Nakagawa et al., 2007). A plausible cause of consistent individual differences in paternal behavior is a hormonal constraint; in other vertebrates, including some teleost fishes, tradeoffs between territory defense and parenting in males are mediated by androgens, where high levels of androgens increase defense behaviors and decrease parenting (Wingfield et al., 1990; Oliveira et al., 2002; McGlothlin and Ketterson, 2008; but see Rodgers et al., 2006; Dey et al., 2010). Given the well-documented variation in aggressive behavior during territory defense in threespined sticklebacks (Huntingford, 1976), it is possible that males differ in how they resolve the tradeoff, with some males prioritizing territory defense while others prioritize paternal care.

Another important factor potentially influencing behavioral consistency in parenting is predation risk. Pressures imposed by predation might favor behavioral flexibility in parenting behaviors of prey if prey adaptively decrease nest-directed parental care such as incubation and nestling provisioning (Martin et al., 2000; Ghalambor and Martin, 2002) and increase nest defense (Lissaker and Kvarnemo, 2006; Cooke et al., 2008) under predation risk. However, if predation risk is constant and predictable, then we might expect animals to adopt

a fixed behavioral strategy, if a fixed behavioral strategy minimizes risk of depredation (Tarwater and Brawn, 2008). Indeed, high levels of predation risk have been implicated in increasing behavioral consistency outside of the parental care context in threespined stickleback (Bell, 2005; Bell and Sih, 2007; Dingemanse et al., 2007).

Several authors have already suggested that individual stickleback males consistently differ in how they behave as fathers (van Iersel, 1953; Feuth-De Bruijn and Sevenster, 1982), in nest site preference (Moodie, 1971), and in size and composition of nests (Rushbrook et al., 2008). However, consistent variation in parental behaviors has not been explicitly examined. In this study, we assess whether wild-caught stickleback males differ in parental care, and whether they behave consistently across clutches in controlled conditions in the lab. We used a within-subject experimental design wherein males were bred twice (two "clutches"). During the first clutch, male parental behaviors were observed every day for approximately 10 days, the average length of a nesting cycle from spawning through fry dispersal. After completing the first clutch (clutch one), males were transferred to a new tank for the second clutch (clutch two) and behavior was observed as in clutch one. Males were randomly assigned to the control or experimental treatment in clutch one, and experienced the other treatment in clutch two. The experimental treatment consisted of the introduction of a model nest predator (sculpin) to the tank. As theory predicts parental response to predation should increase as eggs approach hatching and fry develop (Cooke et al., 2008), we presented the model predator to experimental males on two occasions: once when eggs were near hatching, and again after the eggs hatched into fry. The experimental treatment was designed to simulate the presence of predation risk in the environment. We predicted that males exposed to the predator model would be more "cautious" in their parental behaviors after exposure to predation risk, reducing fanning behaviors and spending less time at the nest.

1   Materials and Methods

1.1   Study population

Adult sticklebacks were collected from Putah Creek in April 2010. Putah Creek is a dammed, regulated freshwater stream in the Central Valley of California. While native fish predators are not common in this creek, prickly sculpin *Cottus asper* have been observed there and are a natural nest predator of the threespined

stickleback (Pressley, 1981). Sticklebacks were shipped to the University of Illinois at Urbana-Champaign and kept in 83L (107 × 33 × 24 cm) mixed sex aquaria until the beginning of the experiment (May 2010) at 20 degrees Celsius on a summer (16L: 8D) photoperiod. Water was cleaned via a recirculating flow-through system that consists of a series of particulate, biological, and UV filters (Aquaneering, San Diego, USA). 10% of the water volume in the tanks was replaced each day. Fish were fed a mixed diet consisting of frozen bloodworm, frozen brine shrimp, and frozen Mysis shrimp *ad lib* each day.

1.2   Experimental set-up

Before being introduced into separate 9.5L (36 × 21 × 18 cm) tanks, 12 males were weighed and measured for standard length. Each tank had four equal-sized squares (9 × 9 cm) drawn on the front in sequence. Each tank contained a refuge (plastic "plant"), an open plastic box (13 × 13 × 3 cm) filled with fine sand, and filamentous algae for nest building. The floor of the tank was covered in coarse gravel. Dividers were placed between tanks to prevent visual interactions between neighboring males. Twenty females were housed across two 38L tanks (53 × 33 × 24 cm), each with at least one refuge. To induce gravidity, females were provided with extra bloodworm in addition to regular feeding.

Nest completion was determined when a visible opening in the nest was present. After nest completion, a gravid female was selected at random, patted dry, weighed, and placed with the male. If spawning did not occur within half an hour, the female was removed and a new gravid female was introduced after 24 hrs. Immediately after spawning, the female was removed, patted dry, and weighed again. The difference in female mass pre- and post-spawning provided a measure of total egg mass. Each male was presented with a unique female, and each female spawned only once.

1.3   Behavioral observations

Behavioral observations began the day after spawning. Between 10:00 and 13:00 every day, an observer (LS) recorded the following parental behaviors for five minutes: *Visits to nest*, the number of times the male was within half a body length from his nest while oriented toward the nest; *Total time fanning*, the total amount of time a male spent fanning within an observation period; and *Total squares moved*, the total number of squares a male moved during the observation period. Observations were made once per day.

1.4   Experimental treatment

At the start of the first breeding attempt (clutch 1),

six males were randomly assigned to the experimental treatment; the other six served as controls. Three days after spawning, experimental males were chased with a 4-inch rubber sculpin model (Jewel Bait Company) for two minutes to simulate a nest predation attempt. The model "nosed" the nest, circled the perimeter of the tank, and approached the nest again before being removed. This process was repeated again three days after the eggs hatched. For control clutches, we removed the top of the tank and gently splashed the water when the eggs were three days old, and again when the fry were three days old to simulate the water disturbance caused when the sculpin model entered the tank. The simulated predation threat occurred after the daily behavior observation.

Observations ceased five days after the eggs hatched, and males were removed from their tank and placed in new individual tanks with nesting material and a refuge. Males were allowed to construct second nests and the entire process, including daily behavioral observations, was repeated (clutch 2); however, for the second clutch males previously exposed to the experimental treatment were now controls and vice versa. After males were removed from their fry a second time, they were placed in new individual tanks.

1.5   Data analysis

To quantify individual differences in behavior, we tested for the effect of treatment, individual male ID, and day in the nesting cycle ('nesting stage') on each behavior throughout the nesting cycle using ANOVA. We used the mean squares from these ANOVA to estimate repeatability within a clutch; this allowed us to control for differences between nesting stages when evaluating individual differences (Lessels and Boag, 1987), with standard errors estimated as in Becker (1992). Treatment was never significant in the analyses of behavior over the entire nesting cycle (see Results), but the males were only exposed to predation risk during certain days rather than throughout the entire nesting cycle. Therefore we had a closer look at the effect of treatment on behavior by comparing the control and experimental group just on the days immediately following exposure to predation risk. To examine consistency in behavior across clutches, we computed average behavior during each clutch, and tested for correlations between the averaged values. We examined the effect of clutch (1st or 2nd breeding attempt), male standard length and egg mass on the averaged values. All statistics were performed using PASW 18 (SPSS Inc.).

2   Results

Eleven males completed one clutch, eight males completed two clutches. Only one of the original twelve males aborted his clutch via cannibalization; these data were excluded from analysis. In the first clutch, five males were exposed to a predator model and six were controls. Of the eight males that completed two clutches, four were exposed to a predator model and four were controls during their second clutch. Males that did not complete two clutches either failed to build new nests or failed to spawn. There were no obvious differences between males that had one or two clutches, i.e. no significant differences in male length, egg mass, visits to nest, or total time fanning (independent samples t-test, $P > 0.5$ for all), except that males that had one clutch visited the nest significantly more often (mean 5.36 ± 0.94 visits) than males that had two clutches (mean 2.98 ± 0.88 visits; independent samples t-test, $t_9 = 3.93$, $P = 0.025$).

For the analyses of individual differences in parental behaviors within a clutch, we focus on the data from the first clutch only ($n = 11$). During the five-minute observation periods, males engaged in multiple parental behaviors, including fanning and visiting the nest. On average, males spent one of the five minutes fanning the nest during the behavioral observation. Males were also active during the observation period, moving between areas of the tank on average approximately seven times in five minutes. Some of these behaviors, however, showed a distinctive pattern of temporal change across days throughout the nesting cycle (Fig. 1, Table 1). For example, the *Visits to nest* as well as *Total time fanning* increased as the eggs approached hatching, and then rapidly decreased. Similar temporal changes were observed in clutch 2, e.g. increase in fanning before hatching, etc (data not shown).

While there were dramatic temporal changes in behavior within the breeding cycle, individuals showed distinctive parenting types. There was a significant effect of 'individual' on all of the behaviors measured, with repeatability within a clutch ranging from 0.22 ± 0.11 to 0.33 ± 0.12 (Table 1). There was no indication that a male's parental behavior was influenced by the mass of the clutch within his nest (*Visits to nest: r = 0.309, P = 0.355; Total time fanning: r = 0.255, P = 0.450; Total squares moved: r = 0.418, P = 0.201*) or his body size (*Visits to nest: r = −0.068, P = 0.841; Total time fanning: r = −0.416, P = 0.204; Total squares moved: r = −0.123, P = 0.718*). When the males
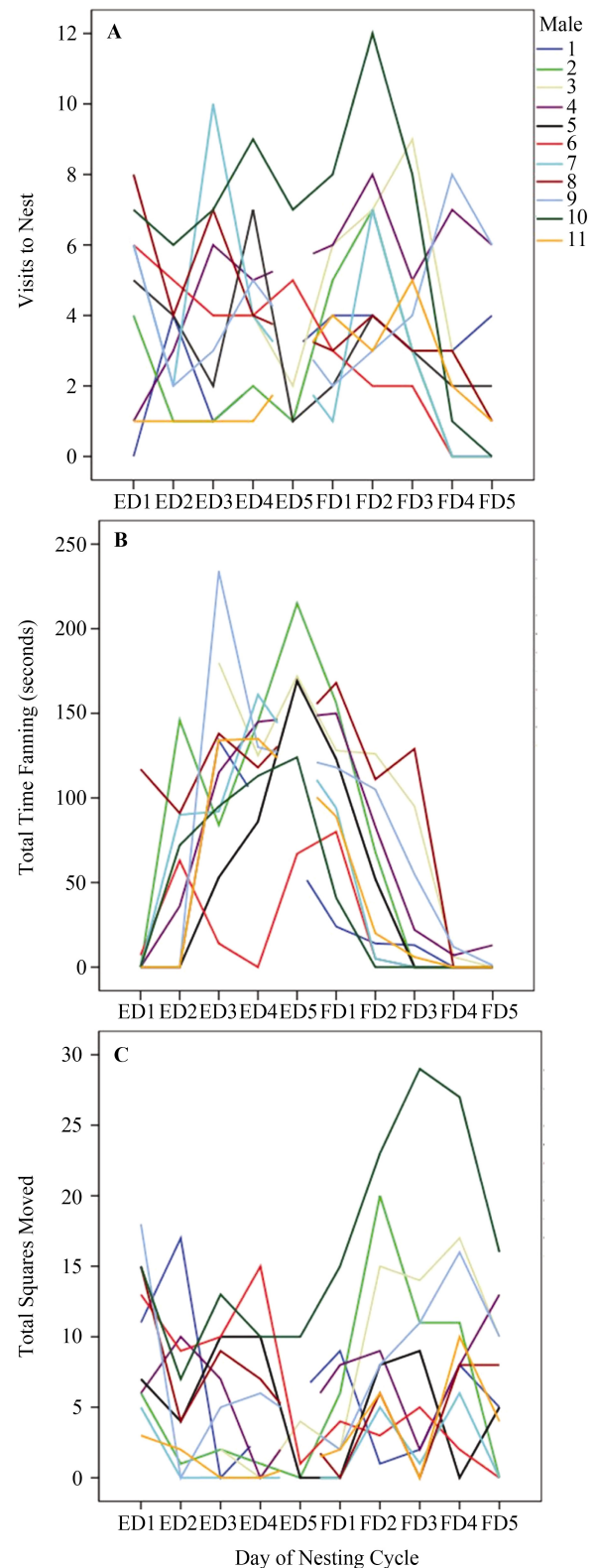

Fig. 1   Individual differences in parental behaviors across days within a nesting cycle (clutch 1)

A. Visits to nest. **B**. Total time fanning. **C**. Total squares moved. ED1 = Eggs day #1 after fertilization; ED2 = Eggs day #2 after fertilization, etc. FD1 = Fry day #1 after hatch, etc. Each individual male is represented by a different color, with separate panels for each behavior; $n = 11$.

Table 1 **ANOVAs testing for male ID, nesting stage, and treatment on A) visits to nest; B) total time fanning; C) total squares moved on behavior during clutch 1**

A) Visits to nest					B) Total time fanning					C) Total squares moved				
Source	*df*	MS	*F*-value	*P*-value	Source	*df*	MS	*F*-value	*P*-value	Source	*df*	MS	*F*-value	*P*-value
Male ID	10	17.74	3.61	0.001	Male ID	10	5819.77	4.33	< 0.0001	Male ID	10	135.2	5.6	< 0.0001
Nesting Stage	11	9.07	1.84	0.06	Nesting Stage	11	22953.1	17.07	< 0.0001	Nesting Stage	11	67.92	2.81	0.004
Treatment	1	12.18	2.47	0.12	Treatment	1	36.67	0.027	0.869	Treatment	1	0.062	0.003	0.96
Error	81	4.92			Error	81	1345.06			Error	81	24.163		
R ± *SE*: 0.22 ± 0.11					R ± *SE*: 0.27 ± 0.11					R ± *SE*: 0.33 ± 0.12				

n = 11. Repeatabilities were estimated from these ANOVA.

reproduced for a second time, they retained their individually distinctive parenting type (Fig. 2). For example, male 10, which had a high number of *Visits to nest* during clutch 1, also visited the nest often during clutch 2. Fig. 2 shows the correlation between the average *Visits to nest* in clutch 1 and clutch 2. We did not detect any differences in average behavior between the first and the second clutch ($P > 0.05$), but note that only eight males successfully spawned twice, so our power to detect differences was limited.
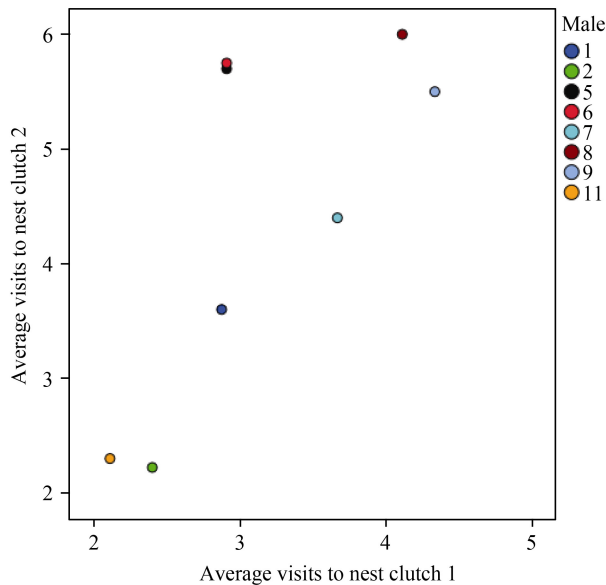

Fig. 2   Individual differences in parental behaviors across two nesting cycles (clutches)
Each point represents the average *Number of visits to nest* of a male within a clutch ($r = 0.717$, $P = 0.045$, $n = 8$).

When the sculpin was introduced into a male's tank, males reacted by hiding, orienting to it and/or attacking the model. There were subtle, transient effects of exposure to predation risk on parental behaviors on the days immediately following exposure to predation risk (analysis restricted to first clutch only). For example, after being exposed to the sculpin with eggs in the nest, males spent less *Total time fanning* compared to control males (One day after exposure: 82.7 ± 26 s, $n = 6$ vs 132 ± 13 s, $n = 5$; Two days after exposure: 63.7 ± 36 s, $n = 3$ vs 185.3 ± 15 s, $n = 3$). The sample size is lower two days after exposure because some clutches hatched 4−5 days after fertilization. Another intriguing pattern was that individual variation among males exposed to predation risk was higher compared to control males. For example, the *Visits to nest* was not repeatable among males in the control group, but the repeatability of *Visits to nest* was 0.54 when males were exposed to predation

risk (Table 2). The greater repeatability in the experimental group was due to an increase in between-individual variation (MS between vs MS within, Table 2). We detected the same pattern (repeatability of experimental males higher than repeatability of control males) for *Total time fanning*, and *Total squares moved*. In each case, the increase in repeatability within a clutch was due to greater variation among males when they experienced the experimental treatment, rather than smaller within-individual variation (Table 2).

Table 2   Repeatability of behavior was greater for experimental males

Behavior	Treatment	MS_{among}	MS_{within}	$R \pm SE$
Visits to nest	C	9.24	5.66	0.10 ± 0.15
Visits to nest	E	27.62	3.4	0.54 ± 0.20
Total time fanning	C	5011.4	1333.3	0.33 ± 0.1
Total time fanning	E	10356.4	1768.6	0.45 ± 0.2
Total squares moved	C	31.5	27.6	0.025 ± 0.13
Total squares moved	E	182	31.5	0.44 ± 0.21

Repeatabilities calculated from MS from ANOVA with male ID and nesting stage as fixed effects (first clutch only). Control (C): $n = 6$; Experimental (E): $n = 5$.

3   Discussion

Despite dramatic temporal changes in behavior over the course of the nesting cycle, fathers exhibited consistent individual differences in their parental behaviors both within and across breeding episodes (clutches). Parental behaviors varied substantially among males, ranging from males that spent a large fraction of their time near the nest and fanning to males that were less 'attentive', and those differences were maintained even when males were transferred to a new environment (tank) and reproduced for a second time, with a different female. Moreover, these individual differences in parental behavior were not related to a male's body size or the mass of the eggs in his nest, suggesting that the individual differences do not reflect transient differences in 'state'. Altogether these results show that wild-caught threespined stickleback males show an individually distinct and consistent parenting style within and across clutches, even though their behavior changed dramatically over time.

The failure to find an influence of egg mass on parenting behaviors is unusual. Larger clutch size is typi-

cally associated with increased parental behaviors in many fish with paternal care (Sargent, 1988; Ridgway, 1989; Karino and Arai, 2006). Egg mass was highly variable during clutch 1 (range 0.1 g – 0.7 g) and so this result cannot be attributed to low variance. However, it is important to note that we used egg mass as a measure of clutch size; to minimize disturbance to the male we did not count the eggs in each nest. It has been suggested that parental investment in fish is driven primarily by total number of eggs rather than egg size (Kolm et al., 2006), and so if egg mass is not a reliable indicator of egg number we would not necessarily expect to see a strong effect of egg mass on parental behaviors.

It is likely that the temporal changes in behavior reflect a male's response to the changing needs of eggs or fry at different stages of development (van Iersel, 1953). For example, rates of fanning increased just prior to hatching, perhaps due to increased energetic needs of the eggs (Sevenster, 1961) Importantly, the differences among males in behaviors such as fanning do not reflect differences in territory quality or oxygen availability because males were all housed in identical tanks, and in a flow-through system with standardized conditions across tanks.

Males that were exposed to predation risk by a model sculpin altered their behavior on the days immediately following the threat by decreasing their amount of time fanning and increasing activity (*Total squares moved*). These results are consistent with the hypothesis that a threat to a male's territory causes him to reallocate activity toward territory defense at the expense of parental care. However, unlike previous studies in birds and other teleost fishes where exposure to predation risk resulted in long-term changes in parenting both within and across clutches (Eggers et al., 2005; Cooke et al., 2008; Chalfoun and Martin, 2010), the effect of predation risk on behavior in this experiment was relatively transient. This suggests that although males respond immediately to predation risk, they relatively quickly return to their behavioral type following a disruption. The other intriguing consequence of exposure to predation risk that we observed in this experiment is that males exposed to predation risk showed higher variation compared to control males. The increased between-individual variation under predation risk could reflect individual differences in strategies for coping with risk in the environment. This result is consistent with previous studies showing that exposure to predation risk increases behavioral consistency across contexts (Bell, 2005; Bell and Sih, 2007).

In a wide range of taxa, there are strong inter-individual differences in parental behavior, both among fathers and among mothers (Feuth-De Bruijn and Sevenster, 1982; Budaev et al., 1999; Champagne et al., 2001; Maestripieri, 2001; Meaney, 2001; Schwagmeyer and Mock, 2003; Nakagawa et al., 2007). Other studies have shown that there are consequences of fathering for offspring behavior in sticklebacks: in high-predation populations, offspring raised by their fathers show greater antipredator behavior than orphans (Tulley and Huntingford, 1987). Therefore, consistent individual differences in parental behavior in sticklebacks may influence the strength and direction of parental effects in this species.

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