STIMULUS-SPECIFIC AND RESPONSE-SPECIFIC HABITUATION IN COURTING STICKLEBACK: DEVELOPMENTAL AND FUNCTIONAL CONSIDERATIONS

by

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Summary

Male threespine stickleback, *Gasterosteus aculeatus*, from a marine population on Long Island, New York were presented simultaneously with two dummies, one simulating a normally distended ('thinner') gravid female and the other a highly distended ('fatter') one. Males initially courted the dummies much as they do real females, but showed stimulus-specific and response-specific habituation to the dummies. Males initially courted the fatter dummy slightly more than the thinner one but showed clear signs of habituation toward the thinner dummy after about 4 min while courtship to the fatter one continued throughout the 1 hr presentation period. Thus, within 12 min males were directing a much greater proportion of courtship to the fatter dummy, and this difference increased over time. Males also attacked both dummies and, in contrast to their courtship response, divided biting equally between the two dummies. Moreover, bite rates to the thinner and the fatter dummy doubled within the first 12 min and then fluctuated around that level for the remainder of the trial. The stimulus-specific and response-specific nature of habituation may be adaptive for male mating success because it leads the male to focus courtship on the preferred female and to direct attack against the fish presenting a greater threat to the nest but a lower potential reproductive payoff.

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Introduction

In the spring of the year, threespine stickleback, *Gasterosteus aculeatus*, form breeding aggregations in which males nest in close proximity to rivals (e.g. Black & Wootton, 1970; Wootton, 1972; Kynard, 1978; FitzGerald, 1983; Mori, 1985; Goldschmidt & Bakker, 1990; Goldschmidt et al., 1992). Here the territory owners may simultaneously encounter several receptive females (e.g. Wunder, 1934; Kynard, 1978; Li & Owings, 1978; Borg, 1985) as well as unreceptive females and other males (e.g. Wootton, 1971, 1972; Kynard, 1978; Li & Owings, 1978; FitzGerald, 1983; Borg, 1985; Whorisky et al., 1985; Ward & FitzGerald, 1987, 1988; Foster, 1990; Goldschmidt & Bakker, 1990). To fully understand the factors affecting the behaviour and mating success of males, it is crucial to study the dynamics of their response during these interactions.

Most studies of stickleback courtship have focused on interactions between one male and one female (e.g. ter Pelkijk & Tinbergen, 1937) but interactions involving additional individuals become more complicated as members of like sex interfere with each other and disrupt or prolong courtship (Wunder, 1934; van den Assem, 1967; Li & Owings, 1978). Females are also known to devour the spawn of conspecifics (e.g. Foster 1990; Whorisky & FitzGerald, 1985, 1994). Thus, males should adjust their response to individual females, depending on how the interaction proceeds.

Previous studies reveal that males presented with a choice of two prospective mates or dummies initially court both but court the larger or fatter one more (Rowland, 1982, 1989; Sargent et al., 1986; Kraak & Bakker, 1998). Even males presented with 4 dummies simultaneously tend to distribute courtship to each in proportion to its abdominal distention (Rowland, 1994). Preference for the fatter female is assumed to benefit males because abdominal distention reflects the female's sexual receptivity and the number of ova she carries (Rowland, 1989).

In the present study we investigated how reproductive male stickleback respond over time when they can choose between two gravid females (dummies) that differ in abdominal distention. We chose this paradigm because it allows us to control for certain factors that could unduly complicate our investigation and because it reflects a situation that males sometimes face in nature (Kynard, 1978; Borg, 1985). By employing this simple and well-tested technique over longer time periods than used in previous studies (e.g.
Rowland, 1989; Jenkins & Rowland, 1997), we hoped to better understand how the male's internal state may change over time and affect his response to prospective mates under natural conditions. This could help us understand how adaptive mate choice develops in males and how habituation and other experiential effects influence this process.

**Materials and methods**

*Subjects*

Adults from a marine population of threespine stickleback were seined from salt marsh pools (30-32 ppt salinity) on eastern Long Island, NY shortly after their arrival in March 1996. Most males showed a trace of greenish blue coloration on the iris and red on the inside corners of their mouth and females showed slight distention of the abdomen, indicating that both sexes were just attaining reproductive condition.

The fish were transported to the laboratory in insulated chests of chilled seawater (6 ± 1°C), then acclimated to the simulated spring conditions of the laboratory (16:8 h light:dark cycle and 18°C). Soon after their arrival in the laboratory, males were placed into individual territory tanks (60 × 50 × 20 cm) where they were maintained throughout the study period. Each territory tank contained brackish water (15-20 g/l salinity), a sand-filled nesting dish (11 × 11 × 4 cm), and tufts of filamentous marine algae (*Urospora* spp.) scattered over the bottom to provide nesting material and cover. Opaque screens were placed between each tank to visually isolate males from their neighbors. All fish were fed frozen adult *Artemia* daily throughout the study period.

Within 1-3 days after introduction into the territory tanks, all males developed full nuptial coloration (blue irises and red undersides) and built a nest in their nesting dish. Testing trials were begun for each male once it had completed its nest, as indicated by the presence of a complete tunnel through the nest (van Iersel, 1953). A total of 10 males were tested, each in its own territory tank, from 22-24 May 1996.

*Dummies*

We presented male subjects with a simultaneous choice of two dummies that differed only in the extent of abdominal distention. The less distended or ‘thinner’ dummy (dummy D2; Rowland, 1989) consisted of an epoxy casting molded from a dead, normally gravid female (SL = 48 mm) from which all fins were removed to facilitate the molding process (Rowland, 1979). The more distended or ‘fatter’ dummy (dummy D4; Rowland, 1989) consisted of an otherwise similar casting whose abdomen was enlarged by adding epoxy putty to its ventral surface and sculpting it appropriately. Both castings were painted shiny silver (Plasti-kote # CC3729 lacquer), and black pupils were drawn in with waterproof markers to simulate eyes. A thin green wire was inserted into the back of each dummy so that they both assumed a horizontal posture when suspended into the tank. The two dummies were therefore virtually identical except for the degree of their abdominal distention.
Testing procedure

Both dummies were suspended into each subject's tank from a rack that rested on the top edge of the tank. The dummies were positioned 22 cm apart from each other and 5 cm from the bottom, each dummy 45 cm from and facing the subject's nest. The side on which a given dummy was presented was alternated from subject to subject to control for possible side biases.

Once a subject approached a dummy, the trial began and continued for 1 hr. The entire trial for each subject was recorded with a video camera-recorder positioned 1 m in front of the tank. All trials were conducted in the absence of observers, to minimize disturbance to the subjects as they responded to the dummies. Videotape recordings of the trials were observed later in real time while the observer scored each subject's courtship and aggression (zigzags and bites, respectively; Sevenster, 1961) to each dummy.

Trials from two males were recorded on each standard (2 hr) videotape, and slight variation in the length of videotapes and the recording procedure led to incomplete recording of the final 4-min time block for several subjects. Hence, only the first fourteen 4-min time blocks (56 min) of each subject were plotted and analyzed in this study.

Statistical analysis

All statistical tests were conducted using the programs Statistix® (version 4.1) and SigmaStat® (version 2.03). Standard parametric tests were applied to the untransformed frequency data and, in cases where these data were found to violate the assumptions of normality, a corresponding nonparametric test was also applied to the data. All probabilities presented herein are two-tailed.

Results

General response

The behaviour that males directed toward the dummies (Fig. 1) varied in intensity but closely resembled that which they direct to real females. That is, when the dummies were introduced into the tank, the male, sometimes after brief hesitation, rapidly approached and directed a quick series of zigzags and a few bites to one or both of them. Female-directed behaviour was interrupted at more or less regular intervals as the male returned to his nest to conduct various nest-directed activities such as nosing, gluing and fanning, and then rushed back to the dummy to continue responding to it (e.g. Sevenster, 1968; Rowland, 1994). At some point, usually early on, males crept through their nest and then continued courting, typically with increased vigour.
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Fig. 1. Mean ± SE of total number of zigzags (filled circles) and bites (open circles) males performed during each 4-min block.

Specific effects of stimuli

Overall, males in this study courted persistently during the trial. A two-way ANOVA reveals individual differences: males differed in total zigzags (i.e. the sum of zigzags, irrespective of the dummy to which they are directed) per block ($F_{9,117} = 10.539, p < 0.001$ for male effect), as some males zigzagged more than others ($\bar{x} = 33.4 \pm 4.7$ SD, range = 7.9-55.5, averaged over the 14 blocks). The overall pattern of total zigzags per block (Fig. 1) did not, however, change significantly over the trial ($F_{13,117} = 0.966, p = 0.489$ for block effect).

Total bite rate varied between males ($F_{9,117} = 16.488, p < 0.001$) ($\bar{x} = 79.82 \pm 27.93$, range = 35.5-118.56) but the average bite rate for each male was uncorrelated with its zigzag rate ($r = 0.35, p = 0.329$). There was also a significant block effect on bite rate ($F_{13,117} = 2.637, p = 0.003$) which was confirmed by Friedman's repeated measures ANOVA ($\chi^2 = 28.53, N = 10, k = 14, p < 0.008$). This block effect was due to a difference between total bite rate in the first block and those attained later in the trial (Dunnett's multiple comparisons procedure, $p < 0.05$); thus males averaged about 40 bites per block in the first 4 min, doubled that rate by 12 min, then fluctuated around that level for the rest of the trial (Fig. 1).

The behaviour that males directed to each dummy clearly differed. First, zigzag rate (Fig. 2a) to the fatter dummy exceeded that to the thinner one (two-way repeated measures ANOVA: $F_{1,9} = 44.01, p < 0.001$).
Males directed 24.75 zigzags per block to the fatter dummy and 8.63 to the thinner one, averaged over the trial. Although total zigzag rate did not change over the trial, as noted above, there was a dummy × block interaction ($F_{13,117} = 2.09, p = 0.0192$) indicating that males modulated zigzags differently to the two dummies. Page's test for ordered alternatives revealed that zigzags to the thinner dummy decreased over time blocks ($L = 8553.5, Z_L = 3.4, p < 0.001$) but this was not the case for zigzags to the fatter dummy ($L = 7841.5, Z_L = -0.16, NS$) (Fig. 2a). When the percentage of total zigzags directed to the fatter dummy is plotted across blocks (Fig. 3), it
becomes clear that males did indeed focus courtship more onto that dummy as the trial progressed ($r = 0.39, p < 0.01$).

Bite rate to each dummy (Fig. 2b) increased steeply early in the trial ($F_{13,117} = 3.79, p < 0.001$ for block effect), and males bit the two dummies at a similar rate ($F_{1,9} = 0.01, p = 0.9447$ for dummy effect), averaging 39.42 and 40.19 bites per block over the trial to the fatter and thinner dummies, respectively. A plot of the percentage of total bites that males directed to the fatter dummy over blocks (Fig. 3) shows that this behaviour was distributed equitably between the two dummies throughout the trial ($r = 0.119; \text{NS}$).

**Discussion**

This study confirms previous reports that male stickleback court real or dummy females in proportion to the relative size or abdominal distention of the females (Rowland, 1982, 1989; Sargent et al., 1986; Kraak & Bakker, 1998), at least initially. But an unexpected finding of our study is that males with longer access to females (dummies) courted them more persistently than we predicted, based on other recent findings. For example, Hampton (1984) reported that copulatory attempts by male mosquitofish, *Gambusia affinis*, habituate to about half of their initial rate in the first 3 min with a live female then drop to 20% or less of the initial rate after 15 min. Peeke &
Figler (1997) found similarly that when male threespine stickleback from a freshwater population in California were presented with just the fatter dummy (D4), zigzagging habituated about 50% within 10 min of continuous exposure. These fish initially zigzagged much more than Long Island fish did, and when presented with the dummy for 10 min longer, the California fish habituated less during the second half of their 20-min exposure period. Although the contrasting results may reflect in part some methodological differences between the two studies, they suggest that habituation, like other forms of learning in this species (Huntingford & Wright, 1992; Mackney & Hughes, 1995; Girvin & Braithwaite, 1998) may differ among populations.

The stimulus-specific nature of the habituation we observed to the two dummies is especially interesting. Zigzagging to the thinner and weaker of the two sexual stimuli produced a typical habituation curve, but zigzagging to the fatter dummy did not. In relative terms, sexual preference for the fatter dummy increased because males continued to court it while their courtship to the thinner dummy habituated.

Another noteworthy finding is that the habituation is response-specific. While zigzagging to the thinner dummy decreased, biting to it (and to the fatter dummy) initially increased and then held steady. Because zigzagging and biting reflect sexual and aggressive tendencies, respectively (van Iersel, 1953; Sevenster, 1961; Sevenster-Bol, 1962), we conclude that males were becoming less sexual and more aggressive to the thinner dummy as the trial progressed, much as they may do to a live female held in a glass tube (e.g. 't Hart, 1978). Even though the males in our study became more aggressive toward the fatter dummy too, they still continued to court it after 1 hr of continuous exposure. Indeed, males continued to court a dummy female in lordosis (receptive) posture during 2 hr of continuous exposure, even after their courtship to a similar dummy in horizontal (neutral) posture had dropped to baseline levels (Rowland, in press).

Development of stimulus-response specificity

The innate releasing mechanism concept originally maintained that animals recognize and respond innately to the salient features of a sign-stimulus (e.g. Tinbergen, 1951; Ewert, 1980; Drickamer et al., 1996). Because our dummies ‘behaved’ identically, they could not provide differential external feedback to the male. The different responses males developed to them
probably resulted from an interaction between a mechanism for shape recognition that was already functional before males were exposed to the dummies and a habituation process that occurred as males responded to this cue. We suggest that males are predisposed to perceive the fatter dummy as a stronger sexual stimulus than the thinner one, and that this predisposition facilitates the differential habituation by which males come to respond even more differently to them.

While reexamining Lorenz's (1939) and Tinbergen's (1948) experiment on the response of ducklings to a goose/hawk silhouette flown overhead, Canty & Gould (1995) confirmed that ducklings fear the hawk configuration (short neck and long tail) more than the goose configuration (long neck and short tail) as a result of habituation. But Canty & Gould also found that ducklings habituate to the goose pattern faster than to the hawk even before the ducklings have had the opportunity to encounter geese more often than hawks, as they would in nature. The present study and those by Canty & Gould (1995) and Rowland (in press) reveal how 'innate' predisposition and experience may interact to produce stimulus-response specificity in reaction to sign stimuli.

The resistance of males to habituate to the fatter dummy raises the question why they continue to vigorously court this seemingly unresponsive 'fish'. A proximate explanation is that a female just remaining in place (i.e. not fleeing from the courting male) is perceived by the male as responding positively, and so stimulates him to court. Moreover, male stickleback obtain positive feedback by courting (e.g. Sevenster-Bol, 1962), and the effect may be enhanced when a stronger stimulus is presented, much as increased palatability of food increases its stimulatory effect on eating (Mcfarland, 1970; Lemagnen, 1985). Because male stickleback perceive the gravid shape as a sexual stimulus (Tinbergen, 1951; Rowland, 1982, 1989), they would receive positive feedback as they approach and court it. This would initiate a process of self-stimulation in which courting the female — the fatter one in particular — would further arouse the male sexually and to a lesser extent, aggressively. Males would therefore continue to court and bite the preferred female increased over time.

**Functional aspects of stimulus-response specificity**

The specific response males direct to the female depends on the feedback they receive from her. If she faces the male, holds her position, or assumes a
head-up posture, he will continue to perform the initial activities of courtship (zigzagging and circling). If she begins to follow the courting male he may proceed to the next stage of courtship and lead her to the nest. If she tries to flee or is otherwise unresponsive, he will become increasingly aggressive and chase her from his territory.

The differential habituation and resulting shift in courtship allocation to each dummy could be highly adaptive for the male. By initially courting both females, the male retains the opportunity to mate with either of them. Because the two stationary dummies behave similarly, the male first courts them relative to their distention, a response that may simply reflect the design properties of his visual system (Rowland, 1989; Ryan, 1990; Guilford & Dawkins, 1991). Because distention is correlated with receptivity and fecundity in females, this preference should improve male mating efficiency and therefore be maintained or enhanced by selection (Rowland, 1989).

As the male gains more information while courting he should adjust his response and use other cues to direct it. The stimulus-specific nature of habituation accomplishes this by helping the male direct courtship toward the fatter female and away from the thinner one. Moreover, habituation to the thinner dummy is also response-specific: only courtship declined, whereas attack increased. Males maintained attention to both dummies but the ratio of courtship to aggression changed such that males would attack and chase off the thinner female and so improve their chance for uninterrupted spawning with the fatter one. By keeping other females away from the nest area the male also reduces the risk of nest raids and egg canibalism during courtship (e.g. Whoriskey & FitzGerald, 1985, 1994; Foster, 1990).

Would males benefit by courting so persistently in nature? Hinde (1970) pointed out that the rate at which responses habituate is determined by natural selection. The single, short breeding season of males from our study population and the sexual competition they face may select against courtship habituation, particularly to highly distended (fecund) females. If the cost of losing matings by reduced courting time exceeds the benefit of lower energy output and risk of predation, males should court persistently. This would be especially likely if males compete strongly for mates and if energy and predation costs of courtship are relatively low. Field data on the length of time that females remain in a male's territory could provide insight into the extent to which courtship habituation may be adaptive for male stickleback in nature.
References


