THE EFFECTS OF SPATIAL CONTEXT AND SOCIAL EXPERIENCE ON THE TERRITORIAL AGGRESSION OF MALE THREESPINE STICKLEBACK

by

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Summary

1. Territorial male threespine stickleback, \textit{Gasterosteus aculeatus}, attacked their neighbors more and escaped from their neighbors less when at their own nests than when at their neighbors' nests. In this study, attacks decreased gradually and escape attempts increased gradually as males moved from the center to the edge of their territories.

2. As predicted by the conflict hypothesis, males performed head-down threat displays most at their territory boundaries. The conflict hypothesis holds that aggression and fear are two opposing tendencies that regulate the expression of attack and escape behavior.

3. This experiment did not find evidence for a threshold effect of aggression or fear in which new behaviors would abruptly replace attack or escape once a certain level of motivation had been activated.

4. Males in our study that had social experience outside of their territories, either fighting with a rival or spending time with a sympatric heterospecific, made fewer attacks overall in later encounters with their neighbors in their and their neighbors' territories than did males without social experience. This finding demonstrates that the influence of encounter site on territorial aggression is modified by a male's past experience.

5. Males with winning and losing experiences outside of their territories did not respond differently to their neighbors in later encounters.

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Introduction

We investigated how the agonistic behavior of neighboring territorial male threespine stickleback (*Gasterosteus aculeatus*) varies with encounter location and past experience to address questions arising from three different conceptual perspectives. First, how does an animal with a discrete resource in its territory modulate its aggressive response to intruders encountered from the center to the edge of the territory? We analyzed the center-edge effect, in which animals exhibit higher aggression towards intruders at the territory's center than at its edge (Falls, 1982), of male stickleback to illustrate a possible strategy used by animals with central-resource territories (Giraldeau & Ydenberg, 1987). Second, can the conflict hypothesis, a model of underlying motivational tendencies of animals, accurately predict the behavior of territorial male stickleback? We evaluated the conflict hypothesis' ability to predict the behavior of neighboring males at the territory boundary. Third, what experiences of males shape how they respond to their neighbors during territorial intrusions? We varied the specific experiences of males to determine how these influence a male's aggressive behavior towards its neighbor.

Encounter location

Agonistic encounters between neighboring territorial conspecifics may occur at various locations within an animal's territory. The location of the encounter may influence the degree to which each animal exhibits aggressive or fearful behavior (Bolyard & Rowland, 1996). Tinbergen (1953) recognized the effect of encounter location on agonistic behavior and considered it to be an important factor in the limitation of intraspecific fighting. By limiting intraspecific fighting to its territory, a male would thus have time to perform other important activities such as courtship.

Despite the significance of particular fight-evoking stimuli possessed by a rival, Tinbergen (1953) hypothesized that certain features of a male's territory result in the restriction of fighting to that area. In support of this hypothesis, Tinbergen reported that a territorial male threespine stickleback will attack its neighbor only when in its own territory. Tinbergen took two males from an aquarium in which each had a territory and put each into a glass tube. He then placed the tubes first into one male's territory and then into the other male's territory. When in its own territory, each male attacked, and when in the rival's territory, each male fled. Unfortunately, no
quantitative data were provided, and the pattern of the change in attacks as males moved from one territory to another was not reported. Thus, we were left with Tinbergen's suggestion that the effect of territory on aggression by neighboring males is all-or-none: all attack when in their own territories, none attack when in a rival's territory.

Van Iersel (1958) investigated the relationship between intraspecific aggression and distance from a male's nest by presenting a rival in a glass tube to territorial males at various locations within a male's territory. Males directed the most bites towards the intruder when it was the shortest distance from the male's nest, and the fewest bites towards the intruder when it was the greatest distance from the male's nest. Van Iersel (1958) concluded that the nest serves as the functional center of the male's territory. Symons (1966) also found that proximity to the nest influences aggression; males in his study directed more bites to intruders presented at 10 cm from the nest than at 100 or 280 cm. The same pattern exists in interspecific aggression; males attack interspecific intruders more when they are close to the nest and less when they are far from the nest (Rowland, 1983a, b). Male stickleback, however, do tend to attack conspecifics at a higher rate than heterospecifics when presented at the same distances from the nest (Huntingford, 1977; Rowland, 1983a, b), and may be considered to have a series of concentric territory boundaries around the nest, with different boundaries for different intruders, as described for three-spot damselfish, *Eupomacentrus planifrons* (Myrberg & Thresher, 1974).

While these data show that the stickleback's nest is more aggressively defended than the territory edge, they do not describe how a male's aggression changes from the center to the edge of its territory. The center-edge effect (Falls, 1982) can result from either the distance between the two contestants varying as a function of encounter site (an intruder might be more likely to be close to a territory owner when at the territory's center) or from the center of the territory having a higher value than the periphery (Giraldeau & Ydenberg, 1987). One hypothesis put forward by Giraldeau & Ydenberg (1987) to explain a higher value of the territory center is the central-resource hypothesis. The central-resource hypothesis holds that a discrete resource present in the territory center gives that location a higher value than other areas within the territory and leads to a high intensity aggressive response by territory holders to intruders encountered there. Based on a war of attrition between two contestants, the evolutionarily stable strategies depend on the
ratio \((V/K)\) of the value of winning \((V)\) to the rate of costs expenditure \((K)\); Parker, 1984). The decrease in the intensity of aggression from the center to the edge of a territory could decrease gradually or abruptly based on how the value of winning and the rate of costs expenditure change (Giraldeau & Ydenberg, 1987). We measured how the aggressive responses of one species of central-resource territory holders, male stickleback, changed as intruders were encountered at different locations within their territories. In addition, we staged the encounters between adjacently territorial males which allowed us to reevaluate Tinbergen's findings (1953) quantitatively.

\textit{Conflict hypothesis}

The conflict hypothesis (Tinbergen, 1952; Hinde, 1970) explains the variation in territory defense of male threespine stickleback as a result of the interaction of two causal factors: aggression and fear. According to the conflict hypothesis, aggression and fear are opposing motivational states, and attack and escape behaviors result from the varying levels of each. When the aggressive tendencies exceed the fearful tendencies, as happens at a male's territory center, he attacks (Archer, 1988). When the fearful tendencies exceed the aggressive tendencies, as happens outside the male's territory, he flees. The response tendencies correspond to the probabilities of attack and escape and can vary according to individual differences in genetics, hormonal state, past fighting experience, stimuli eliciting the defensive response, and the context of the encounter (Archer, 1988). According to the conflict hypothesis, threat displays result from a particular balance of these two incompatible tendencies (Hinde, 1981) and thus should occur at the territory boundary, where aggression and fear are likely to be equally activated.

\textit{Experience}

To understand more about how the aggression of adjacently territorial males develops, we extended Tinbergen's original experiments by examining how attack, escape, and threat behaviors vary with respect to both the encounter site and past experience of males. The experiences we tested were fighting with a neighbor, fighting with a non-neighboring rival, spending time in a neighbor's territory, spending time with a heterospecific fish, and spending time alone. We expected that a male would learn the extent of its own
territory through the physical exploration of its neighbor's territory while males with only visual experience of the neighbor's territory would not. We also expected that among males with fighting experience, previous winners would behave differently than previous losers.

*Our predictions*

In accordance with the conflict hypothesis, we predicted that as a male approaches its nest, attacks would increase as aggression exceeds fear. As it approaches its neighbor's nest, however, fear would be increasingly activated and would suppress attacks. At the territory boundary where attack and escape tendencies are balanced, threat displays should be maximally expressed. We predicted that how aggression decreased from a male's own nest to the territory boundary, known as the center-edge effect, and also to its neighbor's nest would depend on a male's past experience.

We predicted that males with experience in their neighbors' territories would decrease attacks abruptly at the boundary because they would have learned where their territories stop and their neighbors' territories start. In other words, exploring a neighbor's territory might impose a threshold effect on the expression of aggression at the territory boundary. We also expected that losing a fight would be a more salient experience for males than winning, and would lead to fewer attacks towards neighbors in the neighbors' territory. Several studies have shown that fish that lose a fight tend to lose again (e.g. Frey & Miller, 1972; Bakker & Sevenster, 1983; Francis, 1983; Bakker et al., 1989; Chase et al., 1994; Hsu & Wolf, 1999). Thus, we hypothesized that the encounter location of two adjacent territorially males would have different implications for each male, depending on whether he won or lost a previous fight.

We then considered the implications of these predictions on the interaction of aggression and fear, the two motivational tendencies of the conflict hypothesis. If attack behaviors gradually decline, while escape attempts gradually increase, as a defender encounters its neighbor farther from its own nest, then this would imply that aggressive tendencies are increasingly inhibited by fearful tendencies. Alternatively if attack behaviors drop off precipitously and escape attempts increase sharply, at the territory boundary, then this would imply that the aggressive tendency may decrease abruptly at the boundary. These scenarios would also indicate different changes in
V/K, and possibly the existence of multiple evolutionarily stable strategies, for males with different experiences. The evolutionary implications of our study will be considered elsewhere.

Methods

Subjects

We collected threespine stickleback from tidal pools on Long Island, NY in late March 1996 and 1997. We transported all subjects to the laboratory in Bloomington, IN. Subjects were housed in community tanks (122 × 40 × 41 cm) for a few days until males began to develop nuptial coloration and we could reliably sex individuals. Males were then transferred to individual tanks (60 × 50 × 20 cm or 51 × 26 × 16 cm) where they were allowed to nest and held until tested. All subjects were maintained under spring conditions (brackish water, 12-15 ppt salinity; 18°C; 16L:8D) and fed frozen brine shrimp, *Artemia* spp., daily. All subjects were cared for in accordance with the guidelines established by the Indiana University Animal Care and Use Committee.

Test tank

The test tank (120 × 32 × 42 cm; Fig. 1) was divided in half across its length by a plastic mesh divider. Two males were placed into the tank, one on each side of the divider. The divider ensured that each male had the same territory size and permitted visual, chemical, and auditory communication between the two males. Each tank half was 60 cm in length, which is greater than the approximate minimum territory size required by a male, 30 cm in length, according to van Iersel (1958). Each tank half also contained a sand-filled nesting dish (11 × 11 × 3.5 cm) in the far back corner; the location of the nesting dish served to hold the inter-nest distance constant between pairs of males and served as the functional center of a male's territory. Filamentous algae were provided to males for nest construction. The nest of each male was removed after testing, the bottom of the tank and the nesting dish were cleaned, and fresh algae were placed in the tank for each new subject. One artificial plant was fixed approximately 10 cm from the divider on each side of the tank. The plant provided males with a fixed boundary marker when the divider was removed during staged encounters between them.

Once males were placed into the test tank, they were given three days to build a nest in the nesting dish. If a male did not build a nest within this time, it was not used and another male was put into the test tank. Neighbor males were selected from different initial laboratory community tanks to minimize any effects of previous experience between any two males. We were not able to insure that males had been collected from different tidal pools, however, and so males may have had experience with each other before collection.

Since males weighing 15% or more than their opponents tend to dominate contests (Rowland, 1989), neighbor males were matched for weight and differed by 15% or less, except one pair of males in 1997 which differed by 17%. After both males had built a nest, they were given a particular experience treatment lasting 30 min (see Experiences I and II and Table 1). Four to five hours after the experience treatment, encounters were staged between the neighboring males in the test tank (see Testing procedure). Previous work (Bakker et al., 1989) has shown that the effects of losing an encounter last at least six hours.
Fig. 1. (a) Front and (b) top views of the experimental test tank including the test box and floating cup (present only between test trials). A plastic mesh divider separated the tank into two equal halves, and each half contained an artificial plant fixed 10 cm from the divider and a nesting dish with algae in the far corner. A-G represent test locations. Note that at test locations A and G, the center of the test box could not be aligned with the test location.

<table>
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<tr>
<th>Experience group</th>
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<tr>
<td>View neighbor's territory</td>
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<tr>
<td>Fight unfamiliar rival</td>
<td>14</td>
<td>1997</td>
</tr>
<tr>
<td>Time with pupfish</td>
<td>14</td>
<td>1997</td>
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Males were tested only once; hence each group of males is different from every other group.
Experience I

To investigate the effects of experience in a neighbor's territory versus experience with a neighbor, we gave subject males one of four types of experience (April-May 1996), each lasting 30 min: (1) time with the neighbor in a neutral tank (‘fight neighbor’, \( N = 16 \)), (2) time in the neighbor's territory while the neighbor was absent (‘explore neighbor's territory’, \( N = 8 \)), (3) time viewing the neighbor's territory while the neighbor was absent (‘view neighbor's territory’, \( N = 8 \)), and (4) time alone in a neutral tank (‘solitary’, \( N = 16 \)). The ‘view neighbor's territory’ experience controlled for any potential effects of the neighbor's absence on the males in the ‘explore neighbor's territory’ experience. The ‘solitary’ experience controlled for any effects of the transfer to and time spent in the neutral tank on males in the ‘fight neighbor’ experience.

Before the experience treatment, each male from the ‘fight neighbor’ experience was marked for individual recognition with a small triangular (2 × 2 × 2 mm) piece of tan or black plastic (a flag) glued onto the middle dorsal spine. Males were given a few minutes to recover from the marking procedure before they were released into the neutral tank. The flag did not interfere with normal swimming and had no apparent effect on the males' behavior other than to restrict the downward movement of the spine. The flag and glue were removed immediately after the experience treatment and all males quickly regained full control of the spine.

Males in the ‘fight neighbor’ and ‘solitary’ experiences were netted and transferred to the neutral tank (50 × 25.5 × 31 cm) for their experience treatments. The neutral tank was devoid of any landmarks and nesting materials so that males would not attempt to build a second nest and establish a new territory after fighting, and it was not in view of other test tanks in the laboratory. Males were placed into clear floating cups (12 cm diameter) in the neutral tank, with neighbor males being placed into the cups within 1 min of each other. Immediately after both males were in the cups (or after 30-60 s for the solitary males), the cups were turned over and removed from the tank, thereby releasing the males simultaneously into the neutral tank and beginning the experience treatment. Although the neutral tank was not cleaned in between subjects and experience treatments, no more than one pair of males was placed into the neutral tank per day; hence, any short-term chemical cues from one pair of subjects (or one solitary male) would not be present in the tank when the next subjects were placed into the tank.

During the ‘explore neighbor's territory' and ‘view neighbor's territory' trials, the test tank was divided visually, but not physically, into 12 equal sections by placing a section of lined paper behind the test tank. For the ‘explore neighbor's territory' experience, the neighbor and the divider were removed from the test tank. The subject male was netted and released over its neighbor's nest to insure that it visited the neighbor's territory at least once. For the ‘view neighbor's territory' experience, the neighbor was removed but the divider was not. The subject male was netted and placed back into its own territory to control for any effects on the ‘explore neighbor's territory' males of being netted and removed from the water. In each case the neighbor male received the ‘solitary' experience for 30 minutes in the neutral tank.

All except the solitary experience trials were videotaped (Nikon VN750, Hi-8mm video camera recorder). We analyzed the videotapes of the treatments to verify that males had obtained the intended experience.
Experience II

To investigate whether the effects of previous fighting experiences on male-male aggression between neighbors were a result of fighting with a known rival or were a general effect which would result from any fighting experience, we gave males fighting experience with males that were not their neighbors (April-May 1997). We let each neighbor male from the test tank spend time individually with an unfamiliar rival in the neutral tank ('fight unfamiliar rival', \( N = 14 \)). Two neutral tanks were used to allow neighbor males to have their experience treatments simultaneously. The general procedure for the ‘fight unfamiliar rival’ experience treatment was the same as that for the ‘fight neighbor’. In the ‘fight neighbor’ experience, each male of the pair in the neutral tank came from the same test tank (they had adjacent territories in the test tank). In the ‘fight unfamiliar rival’ experience, one of the two males came from the test tank (the subject male), while the other male (the unfamiliar rival) came from a community tank in the lab of which the subject male had not been a part.

To control for any unknown social effects of the fighting experiences and to isolate the effect of fighting per se (versus spending time with another fish), we allowed another group of males to spend time with pupfish, *Cyprinodon variegatus*, in the neutral tank ('time with pupfish', \( N = 14 \)). Pupfish occur sympatrically with stickleback in the tidal pools on Long Island, NY and represent a species that male stickleback would encounter in the natural habitat. Each subject male was netted and placed into a floating cup in the neutral tank where a pupfish waited in another floating cup. Both fish were then poured into the tank at the same time and allowed to interact. Neighbor males had simultaneous experience treatments in separate neutral tanks.

As in the Experience I treatments, the experience treatments lasted 30 min, were videotaped, and analyzed later for evaluation of the intended experience. All stickleback were marked individually as before to control for any effects of flag placement on behavior.

Testing procedure

Encounters between neighboring males were staged by placing each neighbor into one side of a transparent test box, removing the divider in the middle of the test tank, and moving the test box to seven different locations within the test tank. By placing both males into a confined container (the test box), rather than just the intruder, we were able to maintain a constant distance between the two males; hence, changes in aggression by a male as the encounter location changed could not be due to changes in its proximity to its neighbor.

The test box (12 \( \times \) 12.7 \( \times \) 21 cm; Fig. 1) consisted of clear plexiglass and was divided into two adjacent chambers. Males could view the entire test tank and each other but could not physically interact. Each half of the box had holes in one outside wall and in the wall between the males to allow chemical cues from the rest of the tank to flow freely into the test box and to allow chemical and auditory information to flow between males. Neighboring males were netted and placed into clear floating cups in their territories. The cups were then emptied into the test box simultaneously; each male was released into the side of the test box closest to its own nest.

The test locations were equally spaced (20 cm) from one another (Fig. 1). We calculated the distance a male was from his nest at each test location by measuring longitudinally from the middle wall of the test box at each test location to the closest edge of a male's nesting dish. Thus, the actual distances from a male's nest to each test location were not equidistant from
one another (0, 9, 29, 49, 69, 89 and 109 cm). The order of the test locations was randomized for each pair of males.

We videotaped the behavior of the males in the test box at each test location for two min. The experimenter sat at least one meter away from the tank and waited until males just began to resume normal locomotion before starting the video camera; this always occurred within two minutes after the experimenter sat down. We allowed the males 10-20 min of rest away from their neighbors between test locations while minimizing the stress associated with repeated net captures by removing each male from the test box and putting it back into its floating cup.

Data collection and analysis

For analysis of the experience treatments, we confirmed that ‘explore neighbor's territory’ males visited their neighbors' nests and that ‘fight neighbor' and ‘fight unfamiliar rival' males fought. We also assessed the degree of locomotion and aggression during two randomly chosen 5 minute segments of each 30 minute experience treatment (except the solitary experience). We measured the overall amount of movement by males in the ‘explore neighbor's territory' and ‘view neighbor's territory' groups by counting how many times they crossed one of the lines that demarcated the test tank into equal sections. For males in the ‘fight neighbor', ‘fight unfamiliar rival', and ‘time with pupfish' groups, we measured the time male sticklebacks spent swimming, the number of turns they made, and the number of times each male made mouth contact with the other fish. We used SigmaStat 2.0® to analyze data from the experience treatments using non-parametric tests where the assumptions for parametric tests were not met, except for the binomial tests, which were calculated by hand and used critical values from Table D in Siegel & Castellan (1988).

To analyze the staged encounters between neighbors, we scored the number of times each male made either open or closed mouth contact with the test box. Mouth contact with the test box in the direction of the neighbor was recorded as an attack, and contact in the direction of the male's own nest was scored as an escape attempt. Contacts made with the halves of the front and rear walls of the test box closest to the neighbor were scored as attacks, and contacts made with the halves of the front and rear walls of the test box closest to the male's own nest were scored as escape attempts. We also counted the number of times each male made mouth contact with the bottom of the test box. Such ground picking is an extreme form of the head-down threat display, which is thought to have evolved via ritualization of sand digging used in nest construction (Tinbergen, 1952).

In consultation with the Indiana University Statistics Consortium and using SPSS 8.0®, we applied an analysis of variance (ANOVA) on the log transformed frequency data with distance from the nest and treatment type as fixed factors and fish as a random factor nested in treatment, which accounted for the repeated measures of the fish at the different distances. The test data of males that had experience with another fish were also analyzed with an analysis of variance (ANOVA) on the log transformed data with win, lose, or draw as a fixed factor in place of treatment type. We made post-hoc comparisons with the Sheffé test.
Results

Experience I

All ‘explore neighbor's territory' males visited the neighbor's nest at least once in addition to the initial placement over the neighbor's nest. ‘Explore neighbor's territory' and ‘view neighbor's territory' males did not differ in their overall locomotion, as measured by the number of times a male moved from one section to another (t-test, two-tailed: \( t = -1.723, \text{df} = 14, p = 0.107 \)) and the males spent equal amounts of time in the sections of the tank containing their nests (t-test, two-tailed: \( t = 1.837, \text{df} = 14, p = 0.088 \)).

All ‘fight neighbor' males did fight with their neighbors. In all contests, one male came to dominate the neutral tank, swimming around freely, while the other male hovered at the top of the water column to avoid the dominant male. Whether a male was wearing a tan or black flag did not affect which fish won the fight (6/8 winners had tan flags; Binomial test, two-tailed: \( k = 2, p = 0.29 \)). Initiating the fight did not significantly affect winning; males that initiated the encounter subsequently won the fight in 6/8 cases (Binomial test, two-tailed: \( k = 2, p = 0.29 \)). There also was no significant effect of weight on winning; the heavier fish won the fight in 6/8 cases (Binomial test, two-tailed: \( k = 2, p = 0.29 \)).

Experience II

All males placed in a neutral tank with an unfamiliar conspecific fought with that male. The fights resulted in one male dominating the other in all cases but one. In that pairing, there was no clear dominant male at the end of the 30 min and the contest was scored as a draw. As before, the color of the flag marking individuals did not influence the outcome of the fight (6/14 winners had tan flags; Binomial test, two-tailed: \( k = 6, p = 0.79 \)). Initiating the fight did not significantly affect which fish won the fight. In 10/14 cases, the fish that initiated the encounter won the encounter (Binomial test, two-tailed: \( k = 4, p = 0.18 \)). The test fish initiated 8/14 (Binomial test, two-tailed: \( k = 6, p = 0.79 \)) and won 8/14 encounters with the unfamiliar rival (Binomial test, two-tailed: \( k = 6, p = 0.79 \)). Of the eight encounters initiated by the different test fish, they won six, lost one, and ended one in a draw. Of the remaining six encounters, the test fish lost four and won two. There was no
significant effect of weight; of the thirteen fights with a clear winner, the heavier fish won 10 (Binomial test, two-tailed: \( k = 3, p = 0.058 \)).

Of the 14 males that spent time with pupfish, the stickleback attacked the pupfish in five cases and can be said to have had winning experiences since the pupfish never retaliated and always fled from the stickleback. The other 9/14 cases were scored as draws because neither fish attacked or dominated the other.

**Experiences I and II**

Males with different social experiences in the neutral tank (‘fight neighbor’, ‘fight unfamiliar rival’, and ‘time with pupfish’ males) did not spend different amounts of time moving or turning, nor did they deliver significantly different numbers of bites to their social partners (Kruskal-Wallis one way ANOVA on Ranks: time spent moving, \( H_2 = 4.971, p = 0.083 \); number of turns, \( H_2 = 1.514, p = 0.469 \); number of bites given, \( H_2 = 4.290, p = 0.117 \)).

**Effect of experiences I and II on encounters with neighbors**

There was a significant effect of distance from the nest on all three behaviors measured (ANOVA: attacks, \( F_{6,420} = 11.082, p < 0.001 \); escape attempts, \( F_{6,420} = 5.498, p < 0.001 \); ground picks, \( F_{6,420} = 8.015, p < 0.001 \); Figs 2, 3 and 4). Males in each experience group attacked their neighbors most at their own nest and least at their neighbors' nest (Sheffé test: 0 vs 29 cm, \( p = 0.034 \); 0 vs 49, 69, 89 and 109 cm, \( p < 0.001 \); 9 vs 89 cm, \( p = 0.005 \); 9 vs 109 cm, \( p < 0.001 \); 29 vs 109 cm, \( p = 0.011 \)).

Males in each experience group made more escape attempts from their neighbors when at their neighbors' nests than when at their own nests and the statistical reliability of this effect increased with increasing distance from the nest (Sheffé test: 0 vs 49 cm, \( p = 0.020 \); 0 vs 69 cm, \( p = 0.001 \); 0 vs 89 cm, \( p = 0.001 \); 0 vs 109 cm, \( p < 0.001 \)). Males in each experience group performed the most ground picks at the territory boundary (Sheffé test: 49 cm vs all other test locations, \( p < 0.001 \)).

There was a significant effect of experience on the number of attacks males directed towards their neighbors (ANOVA: \( F_{5,70} = 2.952, p = 0.018 \); Fig. 5). Males that had fighting experience with their neighbors directed fewer attacks towards their neighbors than did males lacking that experience
Fig. 2. Mean number of attacks (± 1 SE) males of each experience treatment directed towards their neighbors at various distances (0, 9, 29, 49, 69, 89 and 109 cm) from their nests during the two min encounters.

Fig. 3. Mean number of escape attempts (± 1 SE) males of each experience treatment directed away from their neighbors at various distances (0, 9, 29, 49, 69, 89 and 109 cm) from their nests during the two min encounters.
Fig. 4. Mean number of ground picks (± 1 SE) males of each experience treatment performed at various distances (0, 9, 29, 49, 69, 89 and 109 cm) from their nests during the two min encounters.

Fig. 5. Mean number of attacks (± 1 SE) by males of each experience treatment averaged across distance from the nest.

(Sheffé test: vs ‘explore neighbor's territory' males, \( p = 0.026 \); vs ‘view neighbor's territory' males, \( p = 0.031 \); vs ‘solitary' males, \( p < 0.001 \)). Males that had fighting experience with an unfamiliar rival attacked their neighbors less than males with solitary experience (Sheffé test: \( p = 0.012 \)).
Males that spent time with a pupfish attacked their neighbor less than did males who had non-social experiences or had fought unfamiliar rivals (Sheffé test: vs ‘explore neighbor’s territory’ males, $p < 0.001$; vs ‘view neighbor’s territory’ males, $p < 0.001$; vs ‘solitary’ males, $p < 0.001$; vs ‘fight unfamiliar rival’ males, $p = 0.001$).

Experience did not affect the number of escape attempts males made or the number of ground picks males performed (ANOVA: escape attempts, $F_{5,70} = 1.735, p = 0.138$; ground picks, $F_{5,70} = 1.110, p = 0.363$). There was no interaction between distance and experience for any of the behaviors (ANOVA: attacks, $F_{30,420} = 1.161, p = 0.259$; escape attempts, $F_{30,420} = 0.814, p = 0.748$; ground picks, $F_{30,420} = 0.990, p = 0.485$).

Whether a male ended the encounter with his neighbor, unfamiliar rival, or pupfish during the experience treatment by winning, losing, or drawing did not affect his behavior during the encounter with his neighbor (ANOVA: attacks, $F_{2,41} = 1.592, p = 0.216$; escape attempts, $F_{2,41} = 1.184, p = 0.316$; ground picks, $F_{2,41} = 0.358, p = 0.701$).

**Discussion**

**Encounter location**

Tinbergen (1953) reported a strong influence of the location of an agonistic interaction on threespine stickleback aggression: whether a male will fight ‘depends entirely on where it is’ (p. 63). Our research confirms the importance of encounter site on attack behavior by territorial males but shows that their aggressive response is not all-or-none.

In this study, we found that male threespine stickleback decrease their attacks and increase their escape attempts gradually as they move from their nest to their neighbor’s nest regardless of their experience in that territory or with that neighbor. Experience in a neighbor's territory did not result in a precipitous drop in attacks and increase in escape attempts at the boundary as predicted. Males of every experience type gradually decreased their attacks and increased their escape attempts as distance from their nests increased.

Our description of the center-edge effect in male stickleback suggests that the central-resource hypothesis about the high value of a territory center does not differ from the central-place foraging hypothesis (Giraldeau & Ydenberg, 1987). The central-place foraging hypothesis is another game theoretic
model of contest behavior based on a war of attrition in which a contestant's fighting time is determined by the value of winning over the rate of cost expenditure. In that hypothesis, the rate of cost expenditure is set by the individual's resource holding potential (RHP), and is not likely to change as an individual moves from the territory center to the territory edge (Giraldeau & Ydenberg, 1987). The value of winning a contest, however, would be higher at the territory center because it contains an important food resource, and as distance from the food resource increases, there is a 'decelerating decline in V' (Giraldeau & Ydenberg, 1987, p. 537). Such changes in the value of winning a contest should change an individual's persistence time in a contest, which would lead to gradual changes in the intensity of an individual's aggressive response (Giraldeau & Ydenberg, 1987). We may conclude, then, that the expected changes in V/K that produce a gradual center-edge effect for central-place foragers may also produce a gradual center-edge effect in at least one central-resource territory holder, threespine stickleback.

Conflict hypothesis

Males of every experience performed the most ground picking at the boundaries between the two territories, where aggression and fear were predicted to be equally activated. Ground picking is an extreme form of the head-down threat display, which male stickleback perform towards rivals. Ground picking represents conflict behavior because the balanced activation of aggression and fear prevents a male from performing either attack or escape behavior.

These results support the conflict hypothesis. Attack, escape, and threat behavior seem to be controlled by two opposing tendencies, aggression and fear. The high number of threat displays at the territory boundary suggests that at this location, aggression and fear are equally activated and in balance (Fig. 6). It is this motivational conflict that results in the behavior intermediate between attack and escape, i.e. threat. The data do not suggest a threshold effect for either aggression or fear, which would instead result in rapid and abrupt shifts from one behavior to another.

Past experience

In our study, encounter site was not the only critical factor in determining a male's territorial aggression. Social experience with a rival or another fish
modified the expression of aggressive behavior both within and outside a male's territory. Males that had social experience made fewer attacks overall during the test period than males with solitary experience. In addition, males that had fought their neighbors or spent time with pupfish, but not males who had fought unfamiliar rivals, made fewer attacks overall than did males that explored their neighbors' territories or spent time alone in their own territories while their neighbors were absent. Although this finding is interesting, it may not be biologically relevant to compare males that spent time in the neutral tank (with or without another fish) to males that spent time in the test tank. In other words, males that spent time in their territories may not be appropriate control groups for males that had social experience outside of their territories. Because males with solitary experience did not differ in behavior during the staged encounters with neighbors from males with explore neighbor's territory or view neighbor's territory experiences, further discussion will be limited to males with social and solitary experience away from their nests and territories.

Males with social experience gradually decreased their attacks as they moved from their own nests to their neighbors' nests, but overall they attacked less than males with solitary experience. Thus, the probability of attack, but not escape, was modified in males with social experience. Archer (1988) states that it is difficult to distinguish how the relationship between the aggression and fear tendencies changes with variables such as past experience. We found no overall increase or decrease in escape attempts by
males with social experience *versus* males with solitary experience, but there was an overall decrease in the attacks by males with social experience. This finding supports the idea that aggression, but not fear, has been modified by social experience (Fig. 6, A2).

The different attack rate by males with social experience may also mean that these males have different strategies for territorial defense based on different estimates of their own RHP, resource values, or both. The ramifications of this result for the central-resource hypothesis and its underlying game theory model are worthy of further investigation.

In light of the findings that individuals with previous losing experiences tend to lose future fights (*e.g.* Bakker *et al.*, 1989), we expected males that had lost fights in our experiment to be less aggressive than winners. Interestingly, whether a male's fighting experience ended in a win, lose, or draw had no effect on his subsequent behavior in staged encounters with his neighbor. This finding contrasts with Bakker *et al.*'s (1989) report on successive encounters between unfamiliar rivals. They found that a male stickleback was more likely to win a second encounter with an unfamiliar rival immediately after a winning experience but not if it occurred three hours later. Prior losers never won second encounters when they occurred immediately or three hours after the losing experience, and they won less than 25% of second encounters if these occurred six hours after the losing experience. In our experiments, we tested males against their neighbors 4-5 hr after their experience treatments, so losers should have exhibited less aggression than winners if losing results in a decrease in aggressive acts. Our experiment, however, was not designed specifically to test the effects of winning and losing on future contests, and there may be good methodological reasons why we did not find any (*e.g.* individuals did not physically interact during the testing part of the experiment).

Other research has also shown that past experience influences male-male aggression. For example, Jenni *et al.* (1969) found that stickleback males held in complete isolation attacked intruders less than did males held in tanks with views of fish in other tanks. Males with solitary experience in our experiment were more aggressive than males with social experience, but the solitary experience lasted only 30 min, compared to four days or more in Jenni *et al.*'s (1969) experiment. Males with solitary experience in Jenni *et al.*’s experiment may have been in a different physiological state than were males in our experiment.
Conclusion

The attack, escape, and threat display behavior of male threespine stickleback appears to be a function of the location of an encounter and the past social experience of males. Specifically, we have shown that: (1) males decrease their attacks towards and increase their escape attempts from their neighbors gradually when moved from their own nests to their neighbors' nests, even if they have not been in their neighbors' territories or fought with their neighbors; (2) males perform head-down displays most at the boundaries between two territories; and (3) males with social experience outside their territories attack their neighbors less than males without this experience. These results support the conflict hypothesis as an explanatory model of agonistic behavior by male threespine stickleback. The results also suggest that males with different past experiences may have different strategies with respect to how they defend their territories. Thus, male threespine stickleback appear to have great flexibility in their expression of aggression and fear based on their past fighting experience and perceived changes in the context of an agonistic encounter.

References


