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Nest sites of the threespine stickleback: Can site characters alone protect the nest against egg predators and are nest sites a limiting resource?

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In a field population of *Gasterosteus aculeatus*, analysis of the patterns of nest destruction following removal of males indicated that nest site variables *per se* offered little protection against egg predation in the absence of paternal defense. These experiments also indicated that male breeding densities were not limited by a lack of space for territories. We suggest that site quality and male quality, which is determined in part by the quality of parental defense, interact to determine the number of eggs a male can hatch.

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Dans une population naturelle de *Gasterosteus aculeatus*, les patrons de destruction des nids à la suite du prélèvement des mâles résidents ont indiqué que les caractéristiques du nid offrent peu de protection contre la prédation des oeufs en l'absence de soins parentaux. Ces expériences ont montré aussi que l'espace nécessaire à l'établissement d'un territoire n'est pas une ressource limitante à cet endroit. La qualité d'un site et la qualité d'un mâle, celle-ci déterminée en partie par la qualité des soins parentaux, agissent de concert pour déterminer le nombre d'oeufs qu'un mâle peut faire éclore.

Introduction

Territorial behavior presumably permits animals to control one or a number of resources that can increase their fitness (Wilson 1975). Such resources may include food (Dill *et al.* 1981; Hixon 1980; Ebersole 1980; Brown 1964), shelter (Larson 1980), space for sexual display (Fitzpatrick and Wellington 1983; Loiselle and Barlow 1978; Wilson 1975), nest sites (Krebs 1971; Brown 1969), or all of the above (Myrberg and Thresher 1974).

Male threespine sticklebacks (*Gasterosteus aculeatus* L.) aggressively defend breeding territories (Wootton 1976). After establishing a territory and building a nest, they court one or several females, and then provide parental care to their eggs and young. While in some populations of this species males steal fertilizations from other males (Wootton 1976), we have never observed such behavior at our study site and to our knowledge there is no evidence to suggest that fertilization-stealing is an evolutionarily stable strategy for the species (see Gross 1985, 1984, 1982). Thus a male's best strategy for reproductive success is probably to establish a nest site.

Certain nest site characters (i.e., site "quality") may be important determinants of male reproductive success in *G. aculeatus*. In a classic laboratory study, van den Assem (1967) found that males with larger territories had more eggs in their nests than males with smaller territories, suggesting that a male should defend the largest territory possible to maximize its reproductive success. However, results of three studies, two from the laboratory (Sargent 1982; Sargent and Gebler 1980) and one from the field (FitzGerald 1983), have challenged this view, finding no relation between territory size and eggs per nest. Sargent and Gebler (1980), Kynard (1978), and Moodie (1972) found that nests hidden in cover, and hence protected from egg predators, contained more eggs than those established in the open. Thus nest site topography may be a more important

determinant of reproductive success than territory size.

Space for nest sites may be a limiting resource for this species in nature, as both Wootton (1976) and FitzGerald (1983) reported that many nonterritorial males were present on the breeding grounds during the reproductive season. However, Whoriskey (1985) and FitzGerald and Whoriskey (1985) experimentally manipulated adult fish densities in tide pools at Isle Verte, Québec, and found that up to two-thirds of the males present did not nest despite the availability of apparently suitable space. This suggests that the non-nesting males were not ready to spawn.

The previous discussion indicates that in natural populations of threespine sticklebacks, two factors remain poorly understood: (i) what constitutes a good nest site, and (ii) what determines the number of males that become territorial, breeding individuals.

Here, we report the results of a male removal experiment which we conducted on a field population of *G. aculeatus*. We hypothesized that certain nest site characters offered significant protection to nests independent of the presence of resident males. Sites possessing these characters would be relatively invulnerable to attacks by egg predators, while others that lacked these characters would be vulnerable and subjected to rapid destruction. We believed that nest site characters alone could be an important determinant of male reproductive success (the number of eggs per nest) because previous studies with this population failed to establish a correlation between measures of male quality (male size, color, and aggressive behavior) and male reproductive success (FitzGerald and Whoriskey 1985; Whoriskey 1985; FitzGerald 1983). To test this hypothesis we recorded the site characteristics and incidences of predatory attacks on nests from which we had removed the male. We then compared the site variables of nests that were destroyed with those that were not, to see if any significant differences existed. We assumed that any such differences would indicate a factor providing protection against egg predators. Our second objective was to determine if space was the factor limiting the number of males breeding at Isle

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Verte. Because surplus males were present in the breeding population we predicted that if territorial males were removed, the open territories would be rapidly reoccupied.

Materials and methods

The site

We worked in the *Spartina patens* marsh-grass zone of the Isle Verte National Wildlife Area, near Rivière du Loup, Québec. The site contains an extensive network of tide pools ranging in size from 1 to 1000 m² in surface area, although most pools range from 10 to 50 m². Pool substrate (mud) and relief (flat) are homogeneous both within and among pools (Ward and FitzGerald 1983). Further details of the site and its fauna can be obtained from Reed and Moisan (1971) and Ward and FitzGerald (1983). Additional information on the fish community of the St. Lawrence estuary near the site is given by Dutil and Fortin (1983).

Fishes in the pools

Three species of sticklebacks, the threespine, the blackspotted (*Gasterosteus wheatlandi*), and the ninespine (*Pungitius pungitius*), migrate from the St. Lawrence estuary to breed in the marsh's tide pools during May and June of each year. With the exception of rare occurrences of mummichogs (*Fundulus* sp.) and fourspine sticklebacks (*Apeltes quadracus*), these are the only fishes in the pools. The territorial systems of *G. aculeatus*, *G. wheatlandi*, and *P. pungitius* are similar (Wootton 1976). For this reason, and because of time constraints, we worked only with *G. aculeatus*.

Removal experiments

Our study was conducted during three periods within the peak of stickleback breeding activity at this site: (i) 11–17 June 1983; (ii) 28 May–3 June 1984; and (iii) 13–19 June 1984. Densities of *G. aculeatus*, *G. wheatlandi*, and *P. pungitius* (adults of both sexes) were estimated to be 3, 5, and 3 adults/m², respectively, during the study period in 1983. Natural densities (fish per square metre) were not estimated in 1984, but in the 2 previous years they ranged from 2 to 7 for *G. aculeatus*, from 1 to 5 for *G. wheatlandi*, and from 3 to 6 for *P. pungitius* during the period 28 May–19 June (Whoriskey 1985). During 1983, 34 males were removed from their nests and in 1984, 12 males were removed during each of the two study periods.

To select a male for removal, the observer crept up to the edge of a pool and watched the males present until one was spotted fanning its nest. This male was captured with a dip net and its nest was gently touched (without causing any damage) to determine if there were eggs present. If the nest was empty it was excluded from the study, but if it contained eggs we returned to observe it at 1-h intervals during daylight periods over the next 24 h to see if it had been attacked by conspecific and (or) heterospecific egg predators. Initially, we intended to stay with each removal nest and identify the egg predators, but this proved impractical as 12 h or more could pass before the nest was raided. Because of the gap in observations that occurred between our last evening and first morning observations, the survival times of 11 nests that were destroyed at night could not be accurately determined. These nests were excluded from our calculation of median nest survival time. Nests that were not attacked within 24 h following male removal were considered to have a low risk of predation, and we halted our systematic verifications of nest state. However, in some instances we made casual observations of the surviving nests during the next 4 days to see if they were attacked.

During 1983, no control nests were established, but in 1984, 22 removals were paired with a control nest from the same pool. Control males were captured and their nests were touched to determine if eggs were present, but the male was returned to the pool. Only nests with eggs were used as controls, and all males that were returned to the pools reoccupied their territories. Removal and control nests were separated by a distance of at least 7 m, and a maximum of two nests were observed in any given pool.

Nest site characters

For both the control and removal nests, we measured nest depth,

distance to the pool bank, and nearest neighbor distance, and the species of the nearest neighbor was identified. We also recorded the number and species of all males nesting within a 1-m radius of the test male's nest, as overlapping defense of nest sites by several neighbors may result in each male having better protection against egg predators than it would if it nested alone (Dominey 1981; Gross and MacMillan 1981; Itzkowitz 1978; Loiselle 1977). The degree of cover of each nest was determined as 0%, cover absent, nest visible to intruders on all sides; 25%, nest visible on three sides; 50%, nest visible on two sides; 75%, nest visible on one side; and 100%, nest in dense algae, not visible to intruding fish or those swimming near the water's surface but sometimes visible to the observer from above (see FitzGerald 1983). The material used to construct the nest (straw, algae, or both) was also noted because choice of nest material may aid in camouflaging the nest.

The selected nests showed a wide range of nest site characters (nest depth, 7–30 cm; distance to the pool bank, 5–158 cm; nearest-neighbor distance, 20–300 cm; number of neighbors nesting within 1-m radius, 0–7; cover 0–100%) but there were no significant differences in these variables between the control and the removal nests (Mann–Whitney *U*-test, $p > 0.10$).

Territorial observations

Before removing a male from a nest, we mapped its territory by observing the distance from the nest to the borders it defended against intruders. This permitted us to determine if the territory was reoccupied after a male was removed, or if neighbors expanded their territorial boundaries to occupy newly available space. Sites were checked for reoccupancy 1, 3, and 5 days after male removal. Most fish spend only 9–15 days in the marsh (Whoriskey 1985) and the parental cycle takes approximately the same amount of time to complete at this site, so if space was limiting we expected the territories to be rapidly reoccupied.

Statistical analysis

All statistical procedures follow Zar (1974). Data from some of our variables were non-normal and violated conventional assumptions of homogeneity of variance. We could not find a suitable transformation to render these data normal, so nonparametric statistics were used in the analysis. No significant differences were found between the two years in the survival times of nests from which males had been removed, so data from both years were pooled for comparison with the 1984 controls. The computations were done with Statistical Analysis System programs (Helwig and Council 1979).

Results

All 22 control nests survived the 24-h experimental period while only 11 of the 58 nests from which males were removed were still intact at the period's end. Both the median survival times and the percent of nests surviving differed significantly between the controls and the removals (median survival times, hours: for controls, 24.0 ($n = 22$), for removals, 4.4 (range 0.2–24.0, $n = 47$), Mann–Whitney *U*-test, $p < 0.05$; percent of nests intact after 24 h: for controls, 100% ($n = 22$), for removals, 19% ($n = 58$), χ^2 , $p < 0.001$).

We tested for significant differences in nest site variables between nests that were destroyed after the male was removed ($n = 47$) and those that survived the 24-h period despite the loss of paternal care ($n = 11$). There were no significant differences between these two groups in their depths, distances to the pool bank, distance to the nearest neighbor, species of nearest neighbor, percent cover, or the numbers and species of males nesting within a 1-m radius of the subject nests (Mann–Whitney *U*-tests, $p > 0.10$).

While we did not systematically observe the attacks by egg predators on the undefended nests, on seven occasions we witnessed a nest's destruction during our hourly verifications.

In six of seven instances the nest was raided by a group of conspecific females, while the seventh attack was by a male *G. aculeatus* whose nest was empty. Twelve minutes after the test male was removed, this fish stole eggs from the nest and returned them to its own nest where it started fanning.

Only 3 of the 58 (5%) vacated territories were reoccupied during the next 5 days. On two occasions new *G. aculeatus* males moved into the territories and constructed their nests. These males occupied 25% and 75%, respectively, of the area of the old territories. The third reoccupation occurred when a neighboring male *G. aculeatus*, which was guarding fry, expanded its territory and started attacking intruders near the boundaries of the removed male's territory. This defense continued for 3 days, after which the fry dispersed and the male left the site. On the 5th day of observation this territory was unoccupied.

Temporary occupation of two vacated territories occurred during the first 24 h following male removal. In the first instance a new male moved into the territory within 2 h of the resident being captured, left the old nest intact, and started building a new nest on top of it. Our observation of this male was interrupted by darkness, and the next morning the old nest was destroyed and the new male was gone. The site was not reoccupied during the next 4 days. In the second temporary reoccupation, a new male "adopted" and started fanning the old nest about 1 h after the owner was removed. This continued for 3 h, after which the nest was destroyed by unidentified raiders; the territory remained unoccupied for the next 4.5 days.

Discussion

No control nests were destroyed during the 24-h test period, and there were no significant differences in nest site variables between nests that were raided following male removal and those that were not. This suggests that nest site variables *per se* offer little direct protection to a male's nest. However, other studies at the site have found a consistent positive correlation between percent cover and the number of eggs per nest for *G. aculeatus* (Whoriskey 1985; FitzGerald 1983). A hidden nest may increase the probability that a female will spawn in the nest and (or) increase the effectiveness of male defense.

We were also surprised by the relatively long period (median 4.4 h) between male removal and nest destruction. With the immediate risk of predation so low, males may be free to abandon temporarily their nest sites and pursue other activities such as foraging or attracting females. In contrast to the pattern for the threespine stickleback, nests in bluegill sunfish colonies were attacked on average 1.7–5.7 min after males were removed (Gross and MacMillan 1981).

One nest that we followed sporadically after its initial 24-h observation period was still intact 5 days after the male was captured, a time period equivalent to 30–50% of this site's 9- to 15-day egg development interval. We do not know if the eggs can hatch in the absence of parental fanning (aeration), but if they can, males killed by predators or other causes during the parental cycle may occasionally realize some reproductive success.

Only 5% of the territories from which males were removed were reoccupied by new males or by neighbors expanding their territorial boundaries. Because the nests we observed at this site all contained eggs, we assumed that they occupied "high-quality" sites. Our observation that these sites remained vacant after male removal indicates that suitable nest sites are not a

limiting resource in this population. The nonterritorial males in this population, for unknown reasons, do not appear to be capable of breeding.

In two instances of male removal, we observed unusual behavioral patterns in the males that next used the sites. In the first instance a male temporarily adopted the available nest, and fanned it for about 3 h, at which point the nest was destroyed by unknown raiders. Adopting another (probably unrelated) egg clutch would bring no gain to the adopting male's fitness, unless the presence of eggs in a male's nest stimulates other females, which would ordinarily resist, to spawn with it (see below). However, the adoption would cost a male time and energy. The male in question may have recently lost its nest but may have been experiencing an internal fanning drive (van Iersel 1953) so strong that when presented with the stimulus of the nest, it adopted the nest and started fanning it. Another possibility is that this was a maladaptive behavior brought about by senescence or parasitism although we have no data at present to support this suggestion.

In the second instance a male stole eggs from an unprotected nest and placed them in its own nest, as described previously in laboratory studies by van den Assem (1967). Rohwer (1978) proposed an explanation for the egg-stealing behavior. He suggested that females seek out nests already containing eggs when they are ready to spawn. Females prefer to spawn in full nests because the resident male has demonstrated its capacity to protect its eggs against predation, and because the female's risk of losing some of its eggs to filial cannibalism is reduced. Filial cannibalism presumably occurs when a male, forced into a fast by time-consuming parental duties, eats a small portion of the eggs it is guarding to maintain itself in sufficiently good physical condition to undertake future reproductive cycles. Because of the large number of eggs (from several females) present in full nests, the probability of a given female's eggs being eaten would be reduced compared with its losses if it spawns in an empty nest. Males with empty nests presumably have a difficult time convincing females to spawn with them, so they steal eggs from other nests and place them in their own to make their nests acceptable spawning sites for the females. The male we observed stole a large quantity of eggs from an unprotected nest, but abandoned its own nest the next day. Thus it derived little benefit from its newly acquired eggs. If the male described in the previous paragraph also adopted the nest with eggs to have the stimulus necessary to convince females to spawn in a nest, it too gained little benefit from its new eggs as the nest was rapidly destroyed.

Thus there is no evidence that space for territories is a limiting resource for male *G. aculeatus* at the fish densities encountered at the study site during 1983 and 1984. Furthermore, nest site locations offered little defense against egg predation in the absence of the resident male. A combination of male quality and nest site location may be the best predictor of individual reproductive success. A good site could increase male reproductive success both by increasing the effectiveness of nest defense and by helping to convince females to spawn more often in the quality nests (Sargent 1982). The relative contributions of each of these factors still needs to be elucidated for the Isle Verte sticklebacks.

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