Female cannibalism and male courtship tactics in threespine sticklebacks

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Summary. Female threespine sticklebacks (*Gasterosteus aculeatus*) frequently raid male nests and eat all the eggs therein. We tested the hypothesis of Vickery et al. (1988) that females prefer to raid nests containing large numbers of eggs than ones with smaller numbers of eggs. This hypothesis is based on the finding that females spawning in nests containing many eggs will have reduced hatching success because of egg crowding. By consuming the male's eggs and forcing him to rebuild his nest, raiding females might obtain a new opportunity to spawn under better conditions. Our results were consistent with the first prediction of this hypothesis that females were more likely to spawn in nests containing fewer eggs than in nests with many eggs. However, this may be the result of males becoming less receptive to females as the number of eggs in their nests increases. Prediction 2 was that females should raid those nests containing the most eggs. Contrary to this prediction, males defending only one clutch were as likely to have their nests raided by groups of females as males defending several clutches of eggs. Female cannibalism is therefore unlikely to have evolved as a means of gaining access to a male defending a small number of eggs. We also examined the tactics used by males to counter female raids. Most raids occur when the male is courting, and nests are more vulnerable to shoals of females than to single females. Therefore, we hypothesized that males with eggs preferentially court a single female rather than large groups of females, and that males without eggs court both groups indiscriminately. We also predicted that males restrict the number of females they mate with when risk of having their nest raided is high. Our results indicate that: (1) both males with eggs and those without eggs minimize the risk of female cannibalism by courting solitary females rather than groups of females and (2)

males limit the number of females that lay eggs in their nest when several potentially raiding females are present.

Introduction

Cannibalism of eggs in fish is a widespread phenomenon (Dominey and Blumer 1984) that is receiving increasing attention from behavioral ecologists (Rohwer 1978; Loiselle 1983; Dionne 1985; Whoriskey and FitzGerald 1985; Schwanck 1986; DeMartini 1987; FitzGerald and van Havre 1987; Meffe and Crump 1987; Mrowka 1987; Hoelzer 1988; Vickery et al. 1988; Hyatt and Ringler 1989; Peterson and Marchetti 1989). Female threespine sticklebacks (*Gasterosteus aculeatus*) often form shoals to raid nests and consume all the eggs therein (Snyder 1984; Whoriskey and FitzGerald 1985; Foster 1988; Ridgway and McPhail 1988). Raids are initiated by a single female but are usually successful only if other females join to form a large group. Nest raiding and egg cannibalism by females is common despite high levels of food (e.g., Whoriskey and FitzGerald 1985). This observation led Vickery et al. (1988) to propose that egg cannibalism provides advantages other than nutrition to female sticklebacks. They suggested that by raiding nests, females gain opportunities to spawn under better conditions. They argued that if eggs are crowded in a nest, this causes oxygen depletion and results in increased egg mortality (see Reebs et al. 1984). Consequently, females should cannibalize the eggs rather than spawn in such a high-egg nest if sites or mates are in short supply. Presumably, a female's reproductive success will be higher if she mates with a male after he has rebuilt his nest (i.e., the nest will contain fewer eggs).

The frequency of nest-raiding and egg-cannibalism by gravid females poses a serious risk to the male. A guardian male can generally defend his nest against groups of fewer than six or seven females by biting and chasing them off his territory (Ridgway and MacPhail

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1988). Males also use decoy displays to lure the groups of females away from their nests (Whoriskey and Fitz-Gerald 1985; Ridgway and McPhail 1988; Foster 1988). However, males must court to obtain eggs. Paradoxically, most raids on nests occur when the male is courting rather than performing other nest-directed activities such as fanning the eggs (Rohwer 1978; F.G. Whoriskey, personal communication; personal observation). Hence, males should have evolved courtship tactics to minimize egg losses to cannibalistic females. For instance, males with eggs should court single females rather than females in shoals because shoals pose bigger risks (decoy displays are not always successful); however, those without eggs should court all ripe females irrespective of shoal size. Furthermore, since most raids on nests occur when the males are courting, there should be an optimum number of clutches above which it does not pay males to allow additional spawnings. This is so because when males court, they may loose the entire brood to marauding females. Thus, males should stop courting when $N = (N+1)(1-R)$ where N is the number of clutches in the males nest and R is the probability of having their nest raided by females while courting. Hence, males should limit the number of clutches in their nest when the probability (R) of having their nests raided by females is high (i.e., when enough females are present to form a large raiding group).

In this paper we present results of five experiments designed to examine the adaptive value of female cannibalism and the courtship tactics used by males to reduce egg raiding by females. Our five predictions are: (1) females prefer to spawn in nests containing few eggs rather than in nests containing many eggs, (2) nests with many eggs are more likely to be raided than those with few eggs, (3) males with eggs court single females rather than females in shoals, (4) males with no eggs should court all ripe females irrespective of shoal size, and (5) males should mate with fewer females when the risk of having their nest raided is high (i.e., when several females are present) compared to when the risk is low.

Methods

Male and female threespine sticklebacks were collected in May and June 1988 and 1989, by seining tide pools located on the Isle Verte Ecological Reserve, Québec, Canada (FitzGerald 1983). After capture, the fish were transported in large aerated buckets to a laboratory at Laval University where the experiments were conducted. Fish were kept in brackish (20‰) water stock aquaria under a 16:8 light:dark photoperiod. Water temperature ranged between 15° and 20° C. Fish were fed *ad libitum* twice daily with commercially prepared freeze-dried food (Hagen, Montréal).

Experiment 1. To determine whether females prefer to spawn in nests with few eggs rather than in nests with many eggs, we partitioned the 102-l test aquaria (90 × 30 × 37.5 cm deep) into three equal chambers. Small perforations in the transparent plexiglass partitions allowed water to circulate between chambers, permitting olfactory and visual contacts among fish in the different chambers. A small charcoal filter and an air stone placed in the central chamber cleaned and aerated the water. The end chambers of the test aquarium were covered with sand, and pieces of filamentous green algae (*Enteromorpha sp.*) were distributed throughout these

chambers to provide nesting material. Otherwise, experimental conditions were the same as holding ones.

Forty-four pairs of males of similar size (mean total length ± SD = 71.7 mm ± 2.9; mean weight ± SD = 3.8 g ± 0.4) were transferred to the experimental aquaria. One male was introduced into each end chamber. A few hours after introduction, males displayed their breeding colors and started characteristic nest-building behavior (Wootton 1976). They were receptive to females within 24 h from introduction into the aquarium as indicated by their courtship responses.

Prior to mating we removed the doors and allowed males to interact until they established their territorial boundary. The partitions were then replaced and one of the two males was randomly selected to mate with a predetermined number (1–5) of females. This was done by placing a gravid female into the paternity chamber and removing her immediatly after spawning. A single female was introduced into the paternity chamber every 2 h until the male had spawned with the assigned number of females. Although we did not count the eggs in a male's nest, we used similar sized females so clutches should have been approximately equal (i.e., about 360 eggs per clutch; Craig and FitzGerald 1982). The other male was not given any eggs.

About 12 h later, we introduced a gravid female into the central chamber. The test female was given 10 min to acclimate before testing. The partitions were then removed and we recorded in which of the two nests the females spawned. Most females laid eggs within 10 min following the removal of the partitions. Forty-four females were tested and each was tested only once.

Experiment 2. To examine whether nests with many eggs were more likely to be raided than those with few eggs, we introduced a male into a small wading pool (1.5 m in diameter) filled with 20 cm of water. Suitable substrate (sand) and nesting material (algae) were always available. The male was given either 1, 2, or 4 clutches of eggs by allowing him to spawn with different numbers of females. Females were removed immediately after spawning. The following morning 10 gravid females were introduced into the pool for 3 h or until a raid was made. All females were fed *ad libitum* before the experiment but not during the experiment. This experiment was replicated 41 times.

Experiment 3. To examine male courtship tactics, 20 males were randomly selected from the stock tanks and placed individually into 38 l aquaria (50 × 25 × 30 cm deep). The aquaria were divided into three aqual chambers by two glass partitions. Suitable substrate and nesting material were available in the central chamber. The end chambers were left empty. All three chambers were aerated and cleaned through small filters and air stones. Half of the males were given a clutch of eggs by allowing them to spawn with one female and half were not given any eggs. Again we used similar sized females so that males defended approximately equal numbers of eggs.

Two hours later, we simultaneously placed a single gravid female and a group of 10 females in each of the two end chambers of the aquaria. After an initial warm-up period of 1 min, we counted the frequency of courtship bouts (zig-zags/3 min) directed at the female in the end chambers A zig-zag was defined as a sideways lead toward the female behind the glass partition immediately followed by another sideways leap away from her (Rowland 1982). The number of zig-zags performed per unit of time is considered as a reliable measure of male courtship (Sevenster 1961; Sevenster-Bol 1962). This test was conducted on each male for 5 consecutive days. Preliminary analyses showed no habituation effects to the females, and consequently we pooled data for the 5 days to give a single behavioral score for each male.

Experiment 4. The procedure of experiment 4 was similar to that of experiment 3 except that we used static models of courting females (head-up posture; Tinbergen 1951) instead of life females, and we conducted sequential rather than simultaneous presentations. The models were prepared by sacrificing gravid females and

spraying them with resin (Helfman 1983). The model was then hooked by her back to a coat hanger that was placed on top of the aquaria. The same models were used throughout the experiment. The courtship responses to the models appeared identical to that of the live females (see also Rowland 1982, 1989). Fifty-six males with eggs and 54 males without eggs were tested once daily for 3 min on each of the 5 consecutive days. Each male was presented separately to the single female and the group of 10 females. The order of presentation of the models (1 vs 10) was randomized. Our measure of courtship was scored as described above.

Experiment 5. To examine whether males limit the numbers of females they mate with when the risks of having their nest raided is high, we randomly selected 21 males from the stock aquaria. These males were individually placed in 102-l ($90 \times 30 \times 37.5$ cm deep) aquaria separated at mid-length by plexiglass partitions similar to those used in experiment 1. Again suitable substrate and nesting material were available in the aquaria. Twenty-four hours after introduction into a test aquarium, a gravid female was introduced into the male chamber, and 5, 10, or 15 females were placed into the second chamber. Every 2 h from 0800 to 2000 hours, we examined whether the female in the male chamber had spawned. The female was then removed (whether she had spawned or not), and a new gravid female was introduced into the male chamber. At the end of the experiment we counted the total number of females that had laid eggs in the male's nest. We used similar sized females to obtain clutches of approximately equal sizes. This experiment lasted 3 days; it was stopped sooner if the male clearly refused to court the females. Males that entirely covered their nest with sand and constantly chased females away were considered unreceptive. These unreceptive males often lost their nuptial coloration.

Results

Experiment 1. Do females preferentially spawn in nests with few eggs as opposed to many eggs?

To examine whether females prefer males with few eggs in their nest as opposed to those having many eggs,

Table 1. Frequency of raids by gravid females on nests containing 1, 2, and 4 clutches of eggs in experiment 2

Number of clutches

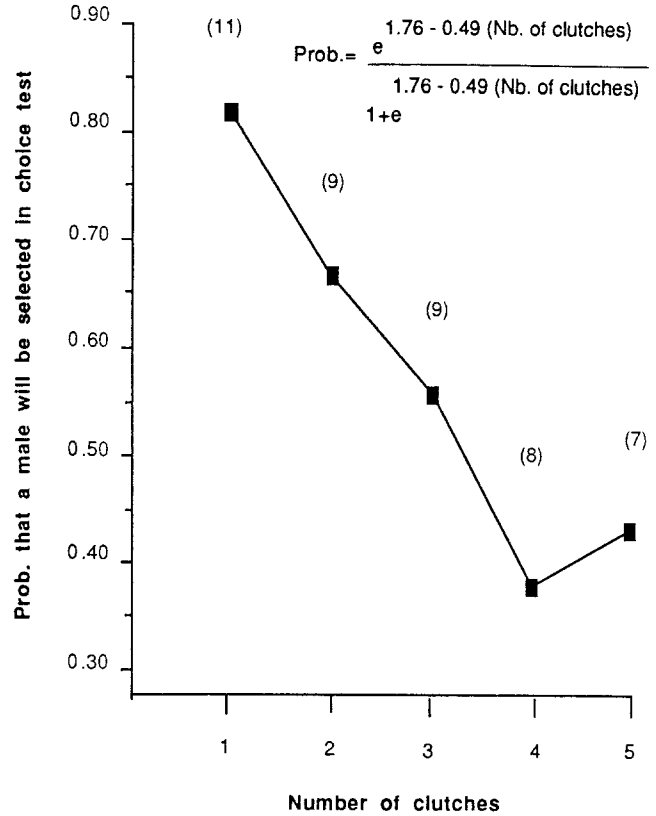| 1	2	4
9/15 (60%)	6/15 (40%)	4/11 (36%)
	$X^2 = 1.81$, $df = 2$, $P > 0.40$	

Fig. 1. Probability that males with different numbers of eggs are chosen by females in a simultaneous choice test. Sample sizes are given in parentheses (see text for details)

we analyzed the data with a logistic regression analysis (Dixon 1985). The probability that a female will spawn with a male tending eggs decreases as the number of clutches in his nest increases ($F = 6.85$, $df = 1,42$ $P < 0.02$; Fig. 1).

Experiment 2. Are nests with many eggs more likely to be raided than those with few eggs?

Forty-six percent (19/41) of the nests were raided. The attacks were successful when at least six females formed a group. Successful raids resulted in the females eating all the eggs in the nest. Females raided all types of nests equally often, but there was a weak tendency for females to attack nests with the fewest eggs (Table 1).

Table 2. Number and percentage of males with eggs and without eggs that courted the single female and the group of 10 females in experiment 3 and 4 (see text). * One-tailed test

Experiment	Males with eggs		Males without eggs	
	1 female	10 females	1 female	10 females
Simultaneous	4/10 (40%)	0/10 (0%)	5/10 (50%)	0/10 (0%)
	* $X^2 = 2.8$, $df = 1$, $P < 0.05$		* $X^2 = 4.2$, $df = 1$, $P < 0.05$	
Sequential	33/54 (59%)	13/56 (23%)	31/54 (57%)	14/54 (26%)
	* $X^2 = 16.2$, $df = 1$, $P < 0.001$		* $X^2 = 11.0$, $df = 1$, $P < 0.001$	

Table 3. Frequency of courtship behavior per 15 min of observation time performed by 56 males with eggs and 54 males without eggs toward a model of a single female and a group of models of 10 females presented sequentially (experiment 4). * Wilcoxon matched-paired rank test

	Males with eggs		Males without eggs	
	1 female	10 females	1 female	10 females
Number of zig-zags				
Median	2	0	1	0
Interquartile distance	0–12	0–0	0–8	0–1
Range	0–46	0–26	0–56	0–61
	* $T = 559.5$, $N = 34$, $P < 0.05$		* $T = 427.0$, $N = 30$, $P < 0.05$	

Table 4. Number of females that males mated with when different sized groups of potentially raiding females were present in the aquaria. Superscripts a and b indicate treatments that differ significantly according to a Scheffe multiple comparison test

	Number of potentially raiding females		
	5	10	15
Number of mated females			
Mean	11.3[a]	3.8[b]	3.9[b]
SD	2.0	1.8	0.84
Range	9–14	2–7	3–5
Sample size	7	6	8
	$F = 51.4$, $df = 2,18$, $P < 0.001$		

Experiment 3. How do males adjust their courtship in response to different sized groups of females?: simultaneous tests

Neither those males with eggs or those without eggs courted the group of ten females and only a few males courted the single female (Table 2).

Experiment 4. How do males adjust their courtship in response to different sized groups of females? sequential tests

Males courted both the single female and the group of ten females. However, a large proportion of males courted the single female than the group of ten females (Table 2). Furthermore, both groups of males directed more zig-zags toward the single female than toward the group of females (Table 3).

Experiment 5. How do males adjust the number of clutches in their nest in response to group sizes of females?

Males mated with fewer females when large groups of females were placed in the aquaria than when only a small group was present (Table 4).

Discussion

Evolutionary models of cannibalism have generally assumed that cannibals acquire supplementary energy by eating conspecifics and that this energy is transformed into increased fecundity or survival (e.g., Bobisud and Potratz 1984; Skurdal et al. 1985; Stenseth 1985). But cannibalism may also provide advantages other than nutritional benefits (Polis 1981). For example, by preying on conspecifics, cannibals may eliminate potential competitors for resources including mates. Vickery et al. (1988) proposed that female threespine sticklebacks raid those nests that contain a large number of eggs to gain access to a male tending fewer eggs because they may expect higher reproductive success in these low-egg nests.

Our data are consistent with Ridley and Rechten's (1981) finding that females prefer to spawn with males having a single clutch rather than an empty nest. These authors proposed that females should spawn with males having some eggs in their nests because this will increase the chances that their eggs will survive a raid (dilution effect). However, these authors did not consider the possibility that spawning in nests with many eggs could decrease rather than increase the number of hatched eggs (see Reebs et al. 1984). This may occur because of competition for oxygen among crowded eggs. But, as predicted by the Vickery et al. (1988) model, our results indicate that females are more likely to spawn with males having few eggs than with those having several clutches of eggs. Alternatively, our results may be explained by a decreased receptivity of the males to additionnal spawns as the number of eggs in their nests increases (Blumer 1979 and references therein). For example, if losses due to oxygen depletion are substantial when a certain number of eggs within the nest is exceeded, then it will not pay a male to allow an additional spawning. However, the results of experiment 5 indicate that males do not stop courting until they have obtained about 11 clutches of eggs.

On the other hand, females may respond to subtle changes in the male's behavior. Van Iersel (1953) found that males with many eggs court females less intensively than those with few eggs. Perhaps, females choose males with fewer eggs because they court more intensively. Recently, Jamieson and Colgan (1989) showed that males that had recently spawned courted more intensively and were more successful at leading females to their nests. Their results also indicate that the presence of eggs has a priming effect on courtship behavior of male threespine sticklebacks. However, evidence for active female choice was lacking. Similarly, Ward and FitzGerald's

(1987) laboratory study revealed no conclusive evidence of active female mate choice in this species.

Contrary to our second prediction (experiment 2), we found that nests with several clutches were no more likely to be raided than nests containing few clutches. These results are to be expected if females show no preference for males with eggs, as we just argued. However, we found a weak tendency for females to attack nests with the fewest eggs. Perhaps males with few eggs are more vulnerable to female raids than those with many eggs because they court more intensively (van Iersel 1953) and/or defend their nest less intensively because these nests are of lower value since they contain fewer eggs. Furthermore, the high incidence of raiding on low-egg nests suggests that females attack nests irrespective of how many eggs they contain. Therefore, our results do not support the resource-competition hypothesis of Vickery et al. (1988). Other explanations of female cannibalism are needed.

Despite the high levels of food at our study site, females may benefit by eating eggs if they can more effectively convert into egg production than other food items (Elgar and Nash 1988) or if eggs contain essential nutrients that are lacking in other foods (Wolcott and Wolcott 1984). A prediction that follows from these two non-exclusive hypotheses is that cannibalistic females should be more fecund or their progeny more viable than females that are prevented from eating eggs, even though other food items are abundant.

High risk of raiding by females presents the male with a dilemma. He must reconcile the need to court females to obtain eggs yet avoid raiding by gravid females. As we predicted, males tending eggs minimize their chance of being raided by preferentially courting single females rather than large groups of females, whether we performed sequential of simultaneous tests. Curiously, males without eggs did not court indiscriminately. These males did not court in the presence of large groups of females.

Few males mated with females when large groups of females were present in the aquaria. This observation suggests that any courtship may be costly when large shoals of females are in the immediate vicinity. Results of our experiments are consistant with the idea that males have evolved flexible courtship behavior to minimize loss of eggs to marauding females. However, we cannot exclude alternative proximate hypotheses that may explain why the males failed to court groups of females. For example, something like the confusion effect, in schooling fish, might arise making it difficult for a male to sustain a relationship with one female if several other females are nearby.

In conclusion, we suggest that nest raiding and cannibalism by female sticklebacks did not evolve as a consequence of competition for nests containing few eggs. Tests of alternative hypotheses such as nutritional benefits are underway in our laboratory.

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