ORIGINAL ARTICLE

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The presence of eggs in the nest and female choice in common freshwater gobies (*Rhinogobius brunneus*)

Abstract In many species of fishes with paternal care, females prefer to spawn with males who are already guarding eggs. We studied the effect of egg presence on female mate choice in common freshwater gobies, *Rhinogobius brunneus* sp. OR. In our tests, females did not prefer males with eggs, suggesting egg presence per se may not act as a cue to attract females. We also examined the effects of brood size on paternal care and offspring survival to look for possible benefits females could obtain when choosing males with eggs. Both fanning by egg-guarding males and egg survival increased with brood size. The presence of neighboring males did have a significantly negative effect on males' parental activity, which subsequently results in a lower level of egg survival than in solitary egg-guarding males. This result provides a partial explanation for the result of eggs not attracting females to mate.

Keywords Female choice · Paternal care · Sexual selection · *Rhinogobius brunneus* · Gobiidae

Introduction

In many fish species with paternal care, females have been reported to prefer males who are already guarding eggs (Ridley and Rechten 1981; Marconato and Bissaza 1986; Unger and Sargent 1988; Knapp and Sargent 1989; Kraak and Groothius 1994; Forsgren et al. 1996; Goulet 1998). Such preference is also believed to have been the driving force behind the evolution of egg-mimicking ornaments in the dorsal fin of male fantail darter, *Etheostoma flabellare* (Knapp and Sargent 1989), and of

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alloparental care, in which males care for eggs of other males by kidnapping the eggs (e.g., three-spined stickleback, *Gasterosteus aculeatus*; Rohwer 1978) or taking over the nest (e.g., tessellated darter, *Etheostoma olmstedi*; Constantz 1985; fathead minnow, *Pimephales promelas*; Unger and Sargent 1988; damselfish, *Chrypsiptera cyanea*; Gronell 1989).

Although there still is controversy over the benefits received by females when spawning in nests with eggs, four categories of hypotheses have been put forward to explain why females should show such a preference. (1) Per capita egg loss from predation or cannibalism may be reduced by the presence of other eggs (the selfish-herd hypothesis; Rohwer 1978; Ridley and Rechten 1981; Unger and Sargent 1988). (2) Males are expected to increase their share of care as the size of their broods increases (the parental-investment hypothesis; Coleman et al. 1985; Sargent 1988; Petersen and Marchetti 1989; Hoelzer 1992). (3) The presence of eggs may indicate that males have been successful in caring for broods (the phenotypic-quality hypothesis; Ridley and Rechten 1981). (4) By copying the choices of others, a female may produce offspring of good genetic quality or "sexy sons" (the mate choice copying hypothesis; Losey et al. 1986). None of these hypotheses are mutually exclusive, but increased egg survival (hypotheses 1 and 2) is the explanation favored by most workers who have demonstrated female preference for males with eggs (see Jamieson 1995 for review).

The costs of spawning in nests containing many eggs can sometimes outweigh the benefits described above. The added oxygen consumption of a second clutch may exceed the additional egg ventilation that males are able to provide for their brood, especially under conditions of low dissolved oxygen (e.g., Reynolds and Jones 1999). In addition, in some species, parental males may invest more heavily in older eggs, because, from the male's perspective, the eggs close to hatching have greater reproductive values (Sikkel 1989; Jamieson 1995). In such a situation, eggs deposited late in a nest can suffer greater mortality than those laid earlier, and females may avoid

males with eggs in late stages of development (e.g., Petersen and Marchetti 1989; Sikkel 1989, 1994).

Not all fish with paternal care exhibit preference for males with eggs. For instance, female sand gobies, *Pomatoschistus minutus*, do not show preference for males with eggs to males without eggs, possibly due to the shortage of nest sites in their breeding grounds (Lindström and Kangas 1996). In an environment with few nest sites available, most nests are soon likely to become full of eggs, and a female who spawns in an empty nest can expect her eggs to enjoy the benefits of being with many other females' eggs within a reasonably short time. Yet Forsgren et al. (1996) were able to demonstrate egg preference in this species, presenting data suggesting that this preference can be explained by increased hatching success of larger brood.

We studied female choice of common freshwater gobies, *Rhinogobius brunneus* sp. OR (*Rhinogobius brunneus* species complex), which is a small freshwater fish abundant in upstream rivers of East Asia (Kim 1995). During the breeding season, a male builds a nest under a rock and courts females, who attach their eggs in a single layer (typically 600–1,700 eggs) to the ceiling of the nest. After depositing eggs the female leaves the nest, while the male remains and provides care, which includes fanning and defending for 3–5 days until planktonic larvae hatch from the eggs (Kawanebe and Mizuno 1989). Elsewhere we reported that females preferred males with the larger first dorsal fin and males showing more active courtship (H.Y. Suk and J.C. Choe, unpublished data). This result, however, might represent only part of mate-choice cues used. We wanted to confirm whether signals other than secondary sexual traits, which might also reveal mate quality, could affect female preference in this species.

We conducted another series of female choice tests: (1) to address whether female common freshwater gobies prefer males whose nests contain eggs, and (2) to determine the effect of female choice, that is brood size, on offspring survival and paternal investment. Specifically, we varied the neighboring male density around a parental male to simulate field conditions, and then compared the consequences of parental behavior and egg survival in these varying situations.

Methods

Subjects

The study was conducted from late April to late May, 1999–2000 in the laboratory at Seoul National University, Korea. Males and females for the laboratory study were collected using push-nets from Gapyong Stream (North Han River; Gapyong-eup, Gyonggi-do, Republic of Korea; 37°55′N, 127°37′E). Most fish were captured in early March, and most likely had no breeding experience. We separated them by sex in six large holding tanks (110–160 l) with under-gravel filtration systems. The tanks and all laboratory setups had a natural dark:light cycle and water temperature was maintained at 14.5±3.24°C. The fish were fed frozen chironomid larvae twice daily.

Female choice test

We studied the importance of egg presence (and developmental stage) in female mate choice using the binary choice procedure similar to the one described in Lindström and Kangas (1996) and Forsgren et al. (1996). Tests were carried out in test aquaria (L80×D60×H45 cm), each divided by a clear Plexiglas partition into equal male and female areas. The male area was further divided into two halves by a removable opaque divider, separating pairs of males and allowing each male time to acclimate. Each male of a pair matched for total body length (to the nearest millimeter), wet weight (g), and body-color pattern was allocated randomly to the left or right compartment, with a ceramic tile measuring 25×25×0.75 cm as a nest site, and was given 1 day to build a nest. One female was introduced into each compartment and they were left alone until they had spawned. Once the female had spawned (day 1), she was removed. Both nests were briefly lifted out of the water and one of them, randomly chosen, was substituted with an empty nest. The other male was allowed to keep his eggs by returning his nest. After 1 day (day 2) or 3 days (day 4; i.e., approximately 1 day before hatching) we introduced a second female (test female), restrained under a clear Plexiglas cage with many drainage holes, into her area. After 1 h, the opaque divider and the clear Plexiglas partition were removed, and the female was released from the cage so that she can spawn with the male of her choice. When the second female had spawned, we recorded which of the males she had chosen.

We recorded male courtship activities for 1 h per replicate while a test female was in the cage. We measured: (1) the % time near the female, and (2) the frequency of attempts to lead a female to the nest (leading rate per minute). Before female choice was determined, two criteria had to be met. First, both males had to court actively. Second, both males had to attempt to lead the female to their nests at least once.

Offspring survival and brood size

We investigated whether paternal care and egg survival are related to brood size. Tests were carried out in test aquaria (150×60×45 cm) divided into three equal chambers by two glass partitions with many drainage holes. A ceramic tile (25×25×0.75 cm) was provided in the central chamber, while the end chambers contained some rocks. Each male was allowed to build a nest in the central chamber for 1 day and was randomly given one (N=21), two (N=14), or three females (N=7) to spawn with, resulting in a broad range of brood sizes. The brood sizes obtained ranged from 692 to 4,182 eggs. The females and glass partitions were removed after spawning; the nest was briefly taken away, and the area covered with eggs outlined with a pencil. We traced the outline onto a clear piece of acrylic and counted the number of eggs in 1 cm² at the center (N=1), halfway between the center and perimeter (N=5), and perimeter of the egg clutch (N=5) under a dissecting microscope. The outlines of egg clutches were transferred to tracing paper and digitized to obtain areas. We estimated the number of eggs in each nest as the product of the egg-clutch area and the average density count. This procedure was repeated until hatching (day 5). Between spawning and hatching, the males were left undisturbed, apart from being fed once a day. We estimated hatching success by comparing the number of eggs just after spawning with the number just before hatching.

We repeated the above experiment at a higher male-density. Three males were placed into each of the two end chambers. We matched the total body length of these non-nesting males for equal or less than an egg-guarding male to prevent taking over the nest or egg predation by neighbors. Each male was given one (N=17), two (N=12), or three females (N=8) to spawn with. Brood sizes ranged from 582 to 3,997 eggs. After spawning, the females and the glass partitions were removed. The experimental procedure was identical to the above.

We observed the care-giving males during the afternoon, for 15 min every day until hatching. Males fan their eggs by moving the pectoral fins, alternately pushing fresh water over the eggs in

the ceiling of the nest. This behavior was recorded as fanning. Guarding males also left their nests occasionally to forage or deter conspecifics around their nests. These behaviors were recorded together as time spent outside the nest. We also measured the duration of each fanning bout. A fanning bout was considered to start when the male's pectoral fins began to beat, and to end when the male stopped the fanning.

Results

Female choice test

When test females were introduced on day 2, they spawned in 8 of the 19 replicates with the male whose nest already contained the eggs (χ^2-test: $\chi^2=0.473$, $df=1$, $P=0.491$). However, females avoided males guarding eggs that were about to hatch. Most test females (11 of the 14 replicates) spawned in empty nests on day 4 (χ^2-test 2×2 contingency table: $\chi^2=3.897$, $df=1$, $P=0.048$).

The presence and developmental stage of eggs affected male courtship activity. On day 2, males lacking eggs spent more time near females (mean±SE=75.31±4.68%) than males with eggs (58.75±5.76%), whereas there was no difference between them on day 4 (males without eggs: 71.72±4.58%; males with eggs: 71.38±4.50%; 2-way ANOVA: $F_{1,32}=27.58$, $P<0.001$). We did not find, however, any significant difference in leading rate per minute between them on both day 2 (males without eggs: mean±SE=3.29±0.32; males with eggs: 3.14±0.37) and day 4 (3.72±0.36; 3.71±0.33, respectively; 2-way ANOVA: $F_{1,32}=27.58$, $P=0.252$).

Offspring survival and brood size

The total fanning time by egg-guarding males increased with brood size under both treatments (when they were alone: $r=0.821$, $N=42$, $P<0.001$; when accompanied by non-nesting males: $r=0.695$, $N=37$, $P<0.001$; Fig. 1). We found no significant relationship between brood size and fanning-bout frequency (when they were alone: $r=0.063$, $P=0.692$; when accompanied by non-nesting males: $r=0.308$, $P=0.064$) or total time spent outside nest ($r=0.069$, $P=0.667$; $r=-0.120$, $P=0.484$, respectively). However, mean fanning-bout duration was positively correlated with total fanning time ($r=0.842$, $P<0.001$; $r=0.702$, $P<0.001$, respectively), as well as brood size ($r=0.633$, $P<0.001$; $r=0.505$, $P=0.012$, respectively). Males exhibited higher mean fanning in the treatment without non-nesting males (Fig. 1; Table 1; ANCOVA: $F_{1,76}=84.430$, $P<0.001$). Non-nesting males disrupted the fanning of parental males by their movements and approaching nests. Parental males in treatment with non-nesting males chased other males around their nests or appeared to be vigilant, which resulted in significantly shorter bouts of fanning.

Eggs survived better when there were no neighboring males (Fig. 2; Table 1; ANCOVA, the effect of neighboring males: $F_{1,75}=7.283$, $P<0.01$, factor-covariate interaction: $F_{1,75}=10.103$, $P<0.01$). Egg survival through day 4 strongly increased with increasing brood size only in the absence of non-nesting males (when they were alone: $r=0.824$, $N=42$, $P<0.001$; when accompanied by non-nesting males: $r=0.305$, $N=37$, $P=0.066$; Fig. 2). The absolute egg losses of solitary males were independent of brood size ($r=0.260$, $P=0.096$) while, in the treatment of non-nesting males, absolute egg losses were correlated significantly with brood size ($r=0.879$, $P<0.001$). With non-nesting males, furthermore, the egg survival did not differ among males with different numbers of egg clutches (1 clutch: median, range=39.33%, 0–54.92%; 2 clutches: 51.32%, 23.89–67.19%; 3 clutches: 50.55%, 29.47–61.40%; Kruskall-Wallis test, $K=4.975$, $df=2$, $P=0.112$; when they were alone: 48.29%, 24.16–59.27%; 58.52%, 18.95–75.67%; 74.44%, 43.21–88.74%, respectively; $K=13.429$, $df=2$, $P<0.01$).
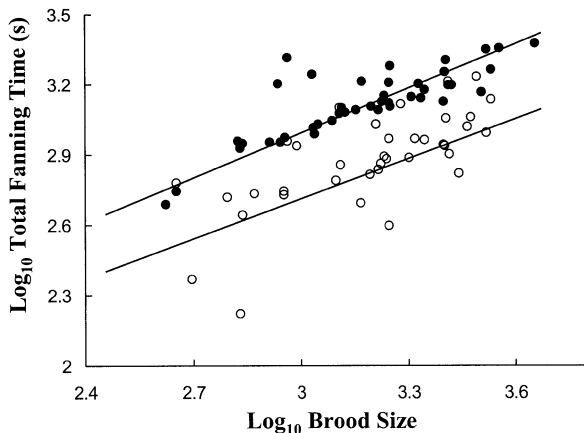

Fig. 1 Total paternal fanning summed over days 1–4 versus brood size. Total fanning increased with brood size in both treatments [without non-nesting males (*filled circles*):$y=0.507x+1.506$, $R^2=0.674$, $t=9.087$, $df=40$, $P<0.001$; with non-nesting males (*unfilled circles*): $y=0.594x+0.977$, $R^2=0.483$, $t=5.703$, $df=35$, $P<0.001$]. Total paternal fanning and brood size were transformed by $\log_{10}$

Table 1 Comparison of paternal fanning activities between males with and without non-nesting males (Bonferroni correction; α was set at 0.008 after correction). Values are mean±SE

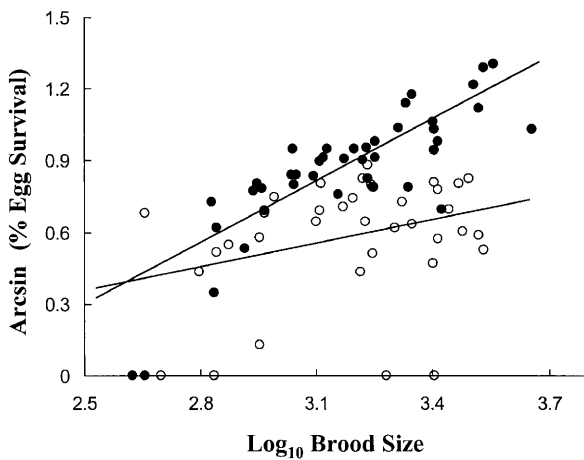| Variable	Without non-nesting males ($N=42$)	With non-nesting males ($N=37$)	t	P
Brood size (egg number)	1739.02±144.28	1793.49±136.57	–0.272	0.786
Total time fanning (s)	1380.40±67.71	823.68±56.33	6.223	<0.001
Mean fanning bout (s)	115.41±8.10	73.46±5.93	4.085	<0.001
Fanning bout frequency (no./h)	12.69±0.41	11.76±0.46	1.528	0.131
Total time spent outside nest (s)	487.10±54.75	828.26±67.07	–3.976	<0.001
Egg survival (%)	57.48±3.30	40.51±2.46	4.033	<0.001

Fig. 2 Egg survival until the end of day 4 versus brood size. Egg survival increased with brood size in both treatments [without non-nesting males (*filled circles*): $y=0.917x–2.058$, $R^2=0.678$, $t=9.182$, $df=40$, $P<0.001$; with non-nesting males (*unfilled circles*): $y=0.316x–0.430$, $R^2=0.093$, $t=1.848$, $df=35$, $P=0.066$]. Egg survival was transformed by arcsin square root

Discussion

Our first experiment clearly showed that females did not prefer spawning with males having eggs in their nests to those with empty nests. This suggests that egg presence in nests per se may not be used as an important cue in attracting females in *Rhinogobius brunneus* sp. OR. This result seems to be reinforced by our preliminary investigations in our field site, where it was observed that most nests (91.3%) contained only one egg clutch in the breeding population of *R. brunneus* sp. OR (unpublished data). These results are in a sharp contrast to some previous studies, however, which have shown that females choose mates on the basis of eggs in the nests (see Ridley and Rechten 1981; Marconato and Bissaza 1986; Unger and Sargent 1988; Knapp and Sargent 1989; Kraak and Groothius 1994; Forsgren et al. 1996; Goulet 1998; but see Lindström and Kangas 1996; Reynolds and Jones 1999). In addition, contrary to our finding, Ito and Yanagisawa (2000) found that males of *R. brunneus* sp. LD, another member of the *Rhinogobius brunneus* complex, frequently defended more than one clutch in their nests during the breeding season.

Why did *R. brunneus* sp. OR in this study not exhibit a preference for males guarding eggs? There may be potential disadvantages associated with spawning in nests with large numbers of eggs. We have already mentioned oxygen deficiency (e.g., Reynolds and Jones 1999) or nest-site shortage (e.g., Lindström and Kangas 1996); these, however, hardly seem to be plausible explanations for our findings. The fish we studied live in the upstream regions of the North Han River, where constantly running water provides sufficient oxygen for the eggs, and nest-site substrates are available in excess on the breeding grounds (unpublished data).

R. brunneus sp. OR females have previously been shown to prefer males with longer first dorsal fins and with higher courtship intensities (H.Y. Suk and J.C. Choe, unpublished data). In each replicate of the female choice tests, we tried to match two males for the length of the first dorsal fin and total body length. Egg-guarding males had less time to attract females for spawning than males with empty nests in most replicates. However, our data did not appear to have resulted from the differences in males' behavior, since there was no significant difference in leading rate of males between males with and without eggs. During active courtship of males, the only signal that females use to distinguish between potential mates is leading display rate (H.Y. Suk and J.C. Choe, unpublished data). While eggs did not attract females to mate, clearly the developmental stages of eggs influenced female choice patterns. Females appeared to avoid nests with old eggs (i.e., approximately 1 day before hatching), as documented in other species of fish (Petersen and Marchetti 1989; Sikkel 1989, 1994).

In the second experiment, we examined the effects of brood size on paternal investment and offspring survival to determine possible benefits females could obtain when choosing males with eggs in the nest. Paternal fanning and egg survival both increased with brood size in solitary paternal males, consistent with parental-investment theory (Coleman et al. 1985; Sargent 1988; Petersen and Marchetti 1989; Hoelzer 1992). Since a large brood has a greater reproductive value than a small brood, males should invest more, in terms of parental effort, in large broods (Sargent and Gross 1985, 1986). As discussed in the introduction, the phenotypic-quality hypothesis cannot explain the results of this experiment, because brood size was experimentally controlled and randomized over male phenotype. The dilution effect may also not be clear, since parental fanning was not held constant.

In the absence of natural agents of egg mortality, male gobies can achieve maximum egg-hatching success. Just before the breeding season, most males and females in this population move to the spawning ground where small rocks and boulders are abundantly available for nesting. In our field observations, average male-density around a nest with eggs was 2.83 individuals/m² (unpublished data). Increased male-density around the nest is thus predicted to affect behavioral trade-offs of egg-guarding males, directly impacting hatching success of their eggs. In fact, in the field, egg-guarding males frequently spend time away from fanning to chase neighboring or wandering males, appearing to be vigilant, or hiding (personal observations).

The differential male-density regime in the second experiment provides an important clue for why eggs did not attract females to mate. Egg-guarding males in a high male-density regime exhibited lower rates of paternal investment and lower % egg survival than solitary egg-guarding males. Fanning may be the most demanding part of paternal care in terms of time and energy. Stickleback males spend up to two-thirds of their time fanning eggs (van Iersel 1953). Although we never witnessed

egg predation by neighboring males, the presence of non-nesting males did have a significantly negative effect on males' fanning activity, which subsequently resulted in low levels of egg survival in multiple clutches. In such situations, egg-guarding males may be more likely to consume their eggs or may be less effective in providing care, and hence females would not always benefit by choosing nests where the male is already providing a large amount of care. Sargent and Gebler (1985) and Sargent (1985) also reported that intrasexual aggression has similar effects on egg survival in the three-spined stickleback. Unfortunately, we were not able to observe most instances of egg loss. It would be desirable to determine the relationship between hatching success and brood size in the field under natural rates of egg mortality.

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