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## [SHORT COMMUNICATION]

## Female Preference for Nest Size in the Stream Goby *Rhinogobius* sp. DA

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**ABSTRACT**—Females of the stream goby *Rhinogobius* sp. DA with paternal care favor males courting in fast water currents, whereby they mate males of high parental ability. Here we examined female choice of male nest size of this goby in laboratory. The dichotomous choice experiment clearly indicated that females prefer large nests. Spawning at large nests seems to improve egg survival rates in natural habitats in this goby. We discuss the possibility of multiple criteria in mate choice of this goby.

**Key words:** *Rhinogobius*, female choice, nest size, paternal care, multiple criteria

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### INTRODUCTION

Female mate choice may evolve whenever access to males of a certain quality or resources provided by males limits the reproductive success of females (Andersson, 1994). Females choose mates based on various criteria such as male morph traits, physical conditions, courtship behaviors, or conditions of resources males possess (Andersson, 1994). Recently, the importance of consideration of multiple criteria in female choice has been addressed (Pomiankowski and Iwasa, 1993; Kraak *et al.*, 1999; Widemo and Sæther, 1999). However, most empirical studies of female choice focused on a single criterion partly because of the difficulty of discrimination among the criteria (Thompson, 1986; Hastings, 1988).

*Rhinogobius* sp. DA (Dark type) (Kawanabe and Mizuno, 1989) is a small fish living in deep pools of short streams with relatively steep gradients in Japan (Mizuno *et al.*, 1979). After making nest under stones on the shallow riffle, a male attracts a female by courtship displays in the deep pool and lead to his nest (Takahashi and Yanagisawa, 1999; Takahashi, 2000a). Thereafter, the female deposits her clutch of eggs in a single layer on the nest ceiling, and the male attends to the eggs until hatching. Females have no choosiness of male body size, and prefer males courting

in fast currents in the pool (Takahashi and Kohda, 2001; Takahashi, 2000b), and such males have good parental ability based on sufficient energy stock (Takahashi, 2000b).

In *Rhinogobius* sp. DA the number of eggs in nests is positively related to the nest size, and males compete for large nests (Takahashi *et al.*, 2001). Some nests contain a large amount of eggs, which are much more than the female fecundity (Takahashi and Yanagisawa, 1999), indicating that some males can fertilize eggs from more than one female in a single breeding cycle. Females spawn a whole clutch at a time (D. Takahashi, personal observation), and a brood of a nest contains eggs at the same developmental stage regardless of brood size (Takahashi and Yanagisawa, 1999). Hence, males with larger nests may mate with more females simultaneously. In preliminary observations, we noted that females seem to have choosiness not only of male courting ability in water currents but also his nest characters. If females preferentially spawn eggs in large nests, the preference partly explains the male-male competition for large nests, and provides an evidence of female choice based on multiple criteria. Field observations on the female preference for nest in this goby, however, are quite difficult because riffles in which the *Rhinogobius* males make their nests are inconspicuous and often in turbulent water (Takahashi and Yanagisawa, 1999). The aim of the present study is to experimentally examine female choice of large nests in this goby under the laboratory condition.

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## MATERIALS AND METHODS

A total of 190 adult fish of *Rhinogobius* were collected from the Kashiwa River (3.2 km long), southwestern Shikoku Islands, Japan (33°1'N, 132°30'E) in a few months before breeding season (early April - early July) in 1998. We stocked about 20 fish of the same sex in 9 tanks [60x28x30 cm (H)] supplied with filtering water. The tank contained PVC pipes and artificial waterweeds as refuge sites. They were adequately fed daily with defrosted bloodworm and flake food (TetraFin, Tetra Co) during the experiments. The stock tanks and experiment tanks (see below) were kept in 14L10D photoperiod by fluorescence lights and in water temperature ca. 18°C. The conditions of the experiment tanks are similar to the natural habitat during the breeding period.

Female preference for nest size was examined in experiment tanks [60x28x30 cm (H)] with gravel bottom of 3 cm thick. A transparent PVC board separated the tank into two male-compartments (30x14 cm) and a female-compartment (30x28 cm) where air-stone was set for aeration (Fig. 1). Two males separated by the opaque board could not see each other. Males are larger than females, and only females could pass through the slits of 0.75 cm width at the bottom of the transparent board. Small (10x10 cm) and large (20x10 cm) clay tiles were put in two male-compartments (Fig. 1). The undersurface area of small tile was twice as large as the mean area of egg clutch in male nests of this fish in the field (50.9 cm<sup>2</sup>), and that of large one was larger than the maximum size (152.9 cm<sup>2</sup> of multiple clutches). Males made a nest hole under the tile as large as possible: small nests under small tiles and large ones under large tiles.

Asymmetries in eye use in relation to brain functions have been reported in some fishes (Bisazza *et al.*, 1997). In a dichotomous choice experiment, female choosiness for right- or left-side males would be affected by such asymmetries. To avoid the effect of the asymmetries, large nests were put in right male-compartments from the female compartment in 8 of 17 nest choice experiments.

Female *Rhinogobius* choose males with good physical condition over a certain threshold level (Takahashi and Kohda, 2001), and thus, such good condition males were used in the experiment. After weighing and measuring size, we introduced two sexually active males of different total length (TL) (mean difference was 14.7 mm±4.8 SD, n=17) into two male compartments. All males made nests under the tiles by the next day. Females ready to spawn exhibit a nuptial color of deep yellow on the enlarged abdomen. A nuptial female was released into the female-compartment 2 days after the male introduction. All males eagerly courted females entering his compartment. In all trials, females responded to courtships of two males, and entered both male nests before spawning. The loose of the nuptial coloration on the slender belly in the females was the sign of spawning. Females spawn a whole clutch at one nest (D. Takahashi, personal observation). Soon after checking the sign, we turned over both tiles and checked the spawning nests. Mean TL of males and females were 80.8 mm (±8.4 SD, n=34) and 62.0 mm (±3.9, n=17), respectively. The condition factors [K=body weight (mg)x100/standard length (mm)<sup>3.04</sup>; Takahashi and Yanagisawa, 1999] of males before the experiments were not so varied (mean=1.94, CV=0.11, n=30; no data in 4 males). Because the mean K of which males can court in fast water currents is 1.80 (±0.12 SD, range=1.61-1.95; Takahashi, 2000b; Takahashi and Kohda, 2001), the experiment males had so good physical conditions that they may be able to court females in the fast currents. Although courtship intensity can be a criterion of mate choice in some fish (e.g., Karino, 1995), *Rhinogobius* females do not choose males depending on the courtship intensity (Takahashi, 2000b). Therefore, in the present study, we did not consider the courtship intensity as criteria for female choice.

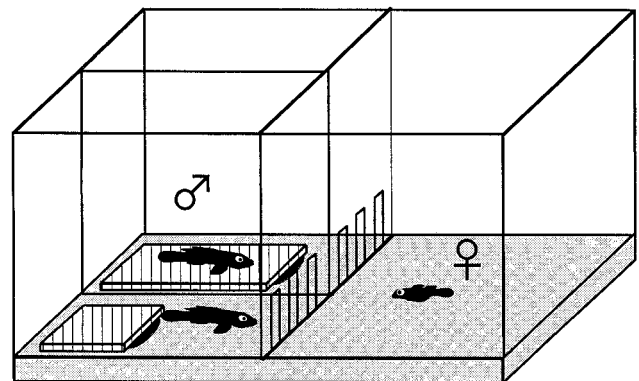
In statistical analysis, we used binomial test and multiple logis-

tic regression analysis by SPSS 6.1 (SPSS Inc.). The logistic regression analysis was used in order to clear independent effects of male body size, nest position and nest size to the female mating preference. Backward step-wise analysis was used to remove variables from the model. Following Forslund (2000), one of the males in each trial was randomly chosen as the focal male, and his spawning success in the trial (i.e., whether the trial female mated with him or not) was used as the binomial dependent variable in the logistic regression analysis. As independent variables we entered relative TL, nest position in right or left male compartment and nest size. We calculated the relative TL by first estimating the mean TL within a trial pair of males, then subtracting the mean TL from the focal male's TL, and finally dividing this deviance by the mean TL.

## RESULTS

The mean period from the female introduction to spawning was 2.7 days (±1.3 SD, n=17). Before spawning, females repeatedly visited and entered each of the two nests, and males vigorously courted the females.

In the 17 nest choice experiments, females spawned the whole clutch in either one of the two nests. Most females spawned at large nests (14 spawnings in the large nest, 3 in the small one; binomial test,  $P < 0.05$ ). Nine females spawned in right nests, and 8 in left ( $P > 0.3$ ). Although females seemed to mate smaller males more, the female selectivity for male body size was not significant (6 matings with the large male, 11 with the small one;  $P > 0.1$ ). In the multiple logistic regression analysis, a model composed by the nest size produced the best fit (Table 1;  $\chi_3^2 = 10.06$ ,



**Fig. 1.** A diagram of the tanks used in the experiments of female choice of male nests. In fact, male compartments are divided by an opaque board.

**Table 1.** Logistic regression analysis with female preference as dependent variable and male relative total length (TL), nest position and nest size as independent variables. The table shows statistics after the first step, when all variables and the constant have been entered into the model. Sample size is 17.

Variables	B	SE	P
TL	-0.12	0.09	0.19
Nest position	0.5	1.51	0.74
Nest size	3.27	1.48	0.02
Constant	-1.47	1.42	0.3

$P < 0.05$ ;  $-2 \log \text{likelihood} = 13.44$ , goodness of fit = 13.32).

## DISCUSSION

In some fishes with paternal care, females prefer large males because of their high defending ability against nest intruders (e.g., Bisazza and Marconato, 1988) or vigorous parental care due to sufficient energy reserves (Downhower and Brown, 1980). In this study, however, there was no female selectivity for male size, as was in the previous studies (Takahashi, 2000b; Takahashi and Kohda, 2001). Males of this goby tightly close the nest entrance with gravel after spawning (Takahashi and Yanagisawa, 1999). The non-preference for large males may correspond with low risks of egg predations by the tight closing. In the Kashiwa River from which the individuals for the present experiment were collected, candidates for the egg predators are only *Rhinogobius* sp. DA, and there are few non-guarding males that eat conspecific eggs in the breeding period (Takahashi and Yanagisawa, 1999). Thus, the occurrence of hetero-cannibalism to eggs reported in some fish (e.g., Bisazza and Marconato, 1988) seems to be rare in this fish. Furthermore, in *Rhinogobius* sp. DA male body size is unlikely to be related to parental ability (Takahashi, 2000b).

Why does female *Rhinogobius* prefer large nests? In many fish species where males care for broods in nests, large brood sizes may induce high intensity of paternal care (e.g., Coleman *et al.*, 1985; Sargent and Gross, 1993). It is also reported that a large brood with multiple clutches may have dilution effect of filial cannibalism on each female's own eggs (Rohwer, 1978; Unger and Sargent, 1988; Kraak and Groothuis, 1994). These reports suggest that survival of eggs increases with the number of females spawning in a nest, and that females should prefer males having nests where other females have already spawned or will spawn. The two aspects of the intensive male care and dilution effect might be of the case in a freshwater goby of which females prefer large nests which tend to have large brood size (Bisazza *et al.*, 1989). Similarly, in our goby larger nests have larger broods (Takahashi *et al.*, 2001). Female *Rhinogobius* should receive those benefits in relation to their egg survival by the choice of large nests. The female preference for large nests will make males compete for such nests, and may indirectly cause the sexual size dimorphism of this fish (Takahashi *et al.*, 2001).

In natural habitats, *Rhinogobius* females first encounter males in deep stream pools, and then are led to the male nests in riffles (Takahashi and Yanagisawa, 1999; Takahashi, 2000a). If the male size is strictly related to the nest size, the former can be an accurate information of the latter. In fact, male sizes are roughly related to nest sizes (Takahashi *et al.*, 2001), but females do not choose large males in the pool (Takahashi, 2000b; Takahashi and Kohda, 2001). These observations and the present study strongly suggest that females directly check the nest size. Thus, *Rhinogobius* females may have two criteria in the procedure of

the mate choice. At first females may choose males of sufficient energy stock (i.e., good parental care) through the dancing ability in fast current in deep pools (Takahashi and Kohda, 2001), and after arriving at nest sites in riffle females may examine the size of the nest the male possesses.

Both of the male quality and nest quality may more or less affect brood hatching rates in many fish species of which males care for offspring in nests. Many studies of female choice in such fishes have focused on either male traits [e.g., body size (Bisazza and Marconato, 1988) and intensity of courtship (Karino, 1995)] or nest quality [e.g., nest size (Bisazza *et al.*, 1989) and presence of eggs (Kraak and Groothuis, 1994)]. The finding of the present study implies that multiple criteria in female choice should be taken into consideration in fishes with paternal males of nest care for deeper elucidation of their sexual selection patterns.

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