

Effect of clutch size on male egg-fanning behavior and
hatching success in the goby, Eviota prasina (Klunzinger)

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Abstract

In fish that exhibit paternal care, the females often choose their mates on the basis of male traits that are indicative of the parental ability of the males. In a marine goby, Eviota prasina, males tend their eggs within their nests until hatching, and females prefer males that have longer dorsal fins and exhibit courtship behavior with a higher frequency as their mates. In order to clarify the relationship between these sexually selected traits and the parental ability of males of E. prasina, the factors affecting the hatching success of eggs within male nests and the male parental care behavior were examined in an aquarium experiment. Females spawned their eggs in male nests and the clutch size of females showed a high individual variation (range = 88-833 eggs). The hatching success of eggs within male nests showed a positive correlation with the time spent by males in fanning eggs and the clutch size. In contrast to the prediction, however, the hatching success did not show a significant correlation with the sexually selected traits, i.e., the male dorsal fin length and the frequency of courtship displays. Moreover, multiple regression analysis indicated that the time spent by the males in fanning was the most important factor affecting the survival rate of the eggs. The time spent by males in fanning behavior was influenced by the clutch size within their nests; the fanning behavior

of males occurred with a higher frequency when they tended larger clutches. Males are required to invest a greater effort in egg-tending behavior to achieve a higher hatching success when they receive larger clutches, probably due to the greater reward for their parental behavior. Based on their mate choice, females may obtain other benefits such as high quality offspring.

Keywords: Clutch size; Eviota; Goby; Hatching success; Parental care; Sexually selected trait

1. Introduction

Parental care provided to the offspring often results in an increase in the survival rate of the offspring and the reproductive success of the parents (Clutton-Brock, 1991). However, care-behavior toward the offspring also incurs some costs to the parents, such as energy and time investment, as well as the limitation on foraging activity (Clutton-Brock, 1991; Smith and Wootton, 1995; Mock and Parker, 1997). Therefore, high investment in parental care for the current offspring may reduce the future reproductive opportunity of the parents (Trivers, 1985; Mock and Parker, 1997; Sargent, 1997). Under certain circumstances, it is believed that as a consequence of the trade-off between the current and the future reproductive success, the parents invest as little effort as possible in the current offspring (Rohwer, 1978; Stearns, 1992; Sargent and Gross, 1993; Mock and Parker, 1997; Sargent, 1997; Roff, 2002). It is also known that the degree of parental investment in the current offspring is affected by several factors such as the reproductive value of the parents, age of the offspring, and physical condition of the parents (Trivers, 1985; Sargent, 1997; Takegaki and Nakazono, 1999; Lissåker et al., 2003; Manica, 2003; Pampoulie et al., 2004).

In fishes that exhibit paternal care, filial cannibalism or offspring desertion by male parents has

often been reported (Rohwer, 1978; Petersen, 1990; Marconato et al., 1993; Okuda and Yanagisawa, 1996; Lindström and Sargent, 1997; Lindström, 1998; Manica, 2002; Payne et al., 2002). When males exhaust their energy through parental care, desertion or cannibalization of their offspring is necessitated in order to recover their own condition and to increase their lifetime reproductive success (Marconato et al., 1993; Kraak, 1996; Okuda and Yanagisawa, 1996; Kvarnemo et al., 1998). In species exhibiting paternal care, the number of offspring in the current brood also influences the degree of paternal care investment (Sargent, 1988; Ridgway, 1989; Kraak, 1996; Lindström, 1998; Suk and Choe, 2002). Since the presence of a greater number of current offspring will result in a higher reproductive success of the male parents, greater effort in caring for the offspring by males with a greater number of offspring is considered to be an adaptive process (Coleman et al., 1985; Sargent, 1988; Sargent and Gross, 1993).

Several theories of sexual selection predict that females choose mates having a higher probability of exhibiting a greater investment in paternal care (Hoelzer, 1989; Andersson, 1994; Dugatkin and FitzGerald, 1997; Kokko et al., 2003). In many fishes, females prefer males that exhibit parental care to a greater degree and have a higher hatching success of their broods (Downhower and Brown, 1980; Unger and Sargent, 1988; Knapp and Kovach, 1991;

Forsgren et al., 1996; Forsgren, 1997). In some cases, sexually selected traits of males are often thought to be a reliable indicator of the parental ability of the males, and females assess the males' parental ability on the basis of these traits (Downhower and Brown, 1980; Côte and Hunte, 1989; Knapp and Kovach, 1991).

Eviota prasina is a small gobiid fish that is distributed over the shallow reef zone of the Indo-Pacific Ocean (Nakabo, 1993). Apparent sexual dimorphism is observed in E. prasina; males have larger body sizes and longer dorsal fins than females (Sekiya and Karino, 2004). Males of E. prasina maintain their nests within the holes or crevices of the substrate. Females visit the male nests and spawn eggs within the nests. After spawning, only the males tend the eggs until hatching (Sunobe and Nakazono, 1999). During this period, males seldom move out from their nests (Sunobe and Nakazono, 1999). Thus, they may starve during the egg-tending period. Females of E. prasina prefer males having longer dorsal fins and exhibiting a higher courtship frequency as their mates (Sekiya and Karino, 2004). Sekiya and Karino (2004) have suggested that these secondary sexual male traits may indicate the parental care ability of the males and that females can acquire a higher survival rate of their offspring under the care of males with longer dorsal fins and higher courtship frequency. However, the relationships between these sexually selected traits of the males in

addition to other factors and male parental behavior or hatching success of eggs under the care of the males have not been examined. In the present study, we investigated the influence of these sexually selected male traits, body sizes, and clutch size in the male nests on the frequency of paternal care behavior and hatching success of the eggs in E. prasina in an aquarium experiment.

2. Materials and methods

In March and September 2001 and March and July 2002, we collected fish specimens from the fringing reef of Sesoko Island (26°38'N, 127°52'E), Okinawa, southern Japan. The collected specimens were reared in 40 l aquariums with circulating water maintained at 27-31 °C under 14 h light and 10 h dark photoperiods in a laboratory at the Tokyo Gakugei University. The fish were fed commercial fish food (Tetra Marine; Tetra Werke, Germany) and newly hatched brine shrimp nauplii. The sex of all the fish was determined on the basis of the shape of the urogenital papilla. The fish were individually marked by subcutaneous injections of fluorescent elastomer (Northwest Marine Technology, USA).

2.1. Measurements of male traits and hatching success

We used small experimental aquariums (35 × 22 × 25 cm height) for examining the parental behavior of individual males and the hatching success of eggs in their nests. A gravel layer with a thickness of 2-3 cm was spread at the bottom of the aquarium and opaque boards were placed on three sides of the aquarium to minimize external disturbance. The aquarium was filled with seawater that was circulated and maintained at 28-30 °C. An 18-watt daylight lamp was placed 10 cm above the aquarium, which was kept under 14 h light and 10 h dark photoperiods. We divided the aquarium into two compartments by using an opaque divider and placed an opaque acrylic pipe (diameter of 1.0 cm and length of 7.0 cm) at the bottom of one of the compartments, which functioned as the nest. The inside of the pipe was covered by a thin plastic sheet with 1 × 1 mm mesh lines.

A test male was introduced into the compartment containing the nest. Immediately prior to the introduction, we measured the body size (standard length) and the longest part of the first dorsal fin of the male to the nearest 0.1 mm by using a vernier caliper. At the time of measurement, the fish was anesthetized using a 2-phenoxyethanol solution. Following a 24-h acclimation period, we confirmed that the test male used the pipe as his nest based on the nest-cleaning behavior. We then introduced a female into the other compartment of the aquarium. We chose a female with a swollen abdomen containing ripe eggs. After a 10-min

acclimation period, we removed the opaque divider from the aquarium and observed the test male and the female during a 30-min observation period. During the observation period, we counted the number of male courtship displays toward the female. The male of E. prasina exhibited a typical motor pattern as courtship display (Sunobe, 1998); the male approached the female by simultaneously moving the pectoral fins and erecting the dorsal fin, and soon after reaching the female, reversed his direction and led the female to the nest. We counted this sequence of the male approaching and leading behavior as one courtship display (Sekiya and Karino, 2004). All the test fish were fed 1 h prior to the courtship observation.

After the courtship observation, the male and the female were maintained in the aquarium until spawning. The female was removed from the aquarium soon after spawning. If the female did not spawn until the next day, we exchanged the female for another female and repeated the male courtship observation after a 24-h period of maintaining a single-male condition. Consequently, in this experiment, a test male obtained a single clutch from a female.

Five to seven days (mean 5.6 days \pm 0.7 S.D.) after spawning, the eggs in the nests hatched during the night. The eggs within the nest were checked on a daily basis and the day of hatching was determined on the basis of the progress of development of the embryo, such as the degree

of eye development. We counted the number of eggs in the male nest immediately after spawning as well as immediately prior to hatching. In order to count the number of eggs, the plastic sheet inside the nest was removed and the sheet with eggs was photographed using a digital camera (Nikon Coolpix 950, Japan). Following this, the sheet with the eggs was returned to the nest. This procedure for counting the number of eggs did not appear to affect the parental behavior of males. The males soon returned to their nests and continued their egg-tending behavior.

The digital image of the plastic sheet with eggs was uploaded onto a Macintosh computer and the number of eggs on the sheet was counted using Photoshop 6.0 software (Adobe, USA). Since the eggs of E. prasina were rather large in size (mean diameter = $0.61 \text{ mm} \pm 0.04 \text{ S.D.}$, $n = 34$ clutches) and were spawned in a scattered fashion on the nest substrate, the number of eggs in the nest could be precisely counted using this procedure. We estimated the hatching success (%) by comparing the number of eggs immediately after spawning (clutch size) with the number of eggs immediately prior to hatching. In some cases, all the eggs in the nests disappeared before hatching (mean $2.8 \text{ days} \pm 1.2 \text{ S.D.}$ after spawning). Since no other egg predators existed in the aquariums, we attributed this early disappearance of eggs to filial cannibalism by the male parents.

2.2. Observation of male parental behavior

We observed the male behavior during the afternoons, for 15 min daily, from the spawning until the hatching stage or the disappearance of eggs due to filial cannibalism. Males of E. prasina fanned their eggs by moving their pectoral and other fins within their nests. We measured the time spent by the males exhibiting the fanning behavior within their nests. Occasionally, males pecked their eggs with their mouths and the time spent by the males in exhibiting this pecking behavior was recorded. The time spent in fanning and pecking by males was recorded using a continuous sampling procedure during the 15-min observation period. The mean values of the time spent by the males for both fanning and pecking the eggs were calculated for further analysis. All the test males were used only once during the experiment.

2.3. Data analysis

To examine the factors affecting the hatching success of eggs, we conducted multiple regression analysis with a stepwise removal procedure (the level of the removal from the model; $F < 4.0$; Grafen and Hails, 2002) using the hatching success as the dependent variable and the male body size, dorsal fin length, courtship frequency, the time spent in fanning, time spent in pecking by the males, and

clutch size as independent variables. In order to ascertain the determinant factor for male parental effort, we also performed multiple regression analysis with the time spent in fanning by the males as the dependent variable and the male body size, dorsal fin length, courtship frequency, and clutch size as independent variables.

In addition, to examine the factors affecting the hatching success and male parental effort, we performed multiple regression analyses by eliminating the data on males with clutches in which all the eggs disappeared before hatching. This procedure was performed because it was possible that the eggs in these unsuccessful clutches were of a lower quality due to an artifact of captivity (e.g., over-ripened eggs) and thus, males either ate them or did not exhibit the usual parental care. If similar results can be obtained by reanalysis, using data that excludes the data on unsuccessful clutches along with those of original data, the factors affecting the hatching success and male parental effort might be clearly demonstrated.

Data on the hatching success were arcsine-transformed and the remaining data were transformed as $\log(\text{original value} + 1)$ because some data contained 0-values prior to the analysis (Sokal and Rohlf, 1973). All these transformed data were normally distributed. StatView 5.0 (SAS Institute, USA) was used for the analysis.

3. Results

Thirty-four males spawned with females. Following the spawning period, eggs were obtained in the male nests. The clutch size of individual females varied (range = 88-833, mean \pm S.D. = 366.7 ± 179.5) and was positively correlated with the body size of the females ($r = 0.52$, $n = 34$, $p = 0.002$; Fig. 1). In 6 of the 34 cases, males cannibalized all the eggs in their nests before the eggs could hatch. Males that exhibited total filial cannibalism had smaller clutch size (mean = 210.0 eggs ± 102.5 S.D.) as compared with other males (400.2 ± 175.6 ; t -test; $t = -3.1$, $df = 32$, $p < 0.001$).

In simple regression analysis, the time spent by the males in fanning and the clutch size positively correlated with the hatching success of the eggs (Table 1, Fig. 2a). Hatching success was negatively correlated with the time spent by the males in exhibiting pecking behavior (Table 1, Fig. 2b). Therefore, the pecking behavior of the males appeared to be an egg-cannibalizing behavior and not an egg-tending behavior. Male body size, dorsal fin length, and courtship frequency did not significantly correlate with the hatching success (Table 1). In the results of multiple regression analysis, only the time spent by the males in fanning entered the final model, while the other

factors were removed (Table 1).

Even when the unsuccessful clutches were excluded from the analysis ($n = 28$), similar results were observed (Table 2). The hatching success of eggs was positively correlated with the time spent by males in fanning, and only the time spent in fanning was used in the final model of the multiple regression analysis (Table 2). These results indicate that egg survival within male nests is primarily determined by the time spent by the males in fanning by.

Simple regression analysis between the time spent by the males in fanning and the clutch size, when all clutches were included in the analysis ($n = 34$), was found to result in a significantly positive correlation coefficient (Table 3, Fig. 3). The relationships between the time spent in fanning and the male body size, dorsal fin length, and courtship frequency were not significant (Table 3). After the multiple regression analysis with the stepwise removal procedure, only the clutch size entered the final model, and it positively correlated with the time spent by the males in fanning behavior (Table 3). In the result of analysis of data after elimination of the unsuccessful clutches ($n = 28$), both clutch size and courtship frequency were used in the final model of the multiple regression analysis (Table 4). The clutch size also positively correlated with the time spent by males in fanning (Table 4). In contrast to our prediction, the courtship frequency

of males negatively associated with the time spent in fanning; however, the relationship was not found to be significant ($p = 0.054$).

4. Discussion

The results of the present study indicate that the clutch size in the nest determines the parental effort of male E. prasina, which is similar to that observed in some other fishes exhibiting paternal care (Sargent, 1988; Ridgway, 1989; Karino, 1997; Lindström, 1998; Suk and Choe, 2002). The clutch size of the females showed high individual variation in E. prasina. Therefore, the males may acquire greater reproductive success by fanning their eggs with a higher frequency, when they obtain larger clutches on spawning with females carrying many eggs. On the other hand, when the females spawned only a small number of eggs in the male nests, the males would invest considerably less effort in parental behavior and often cannibalize their eggs as is observed in other fishes exhibiting paternal care (Forsgren et al., 1996; Kraak, 1996; Kvarnemo et al., 1998; Manica, 2002). In Okinawa, the reproductive season of E. prasina extends from March to November (Sekiya and Karino, 2004). This long reproductive period may ensure the acquisition of the clutches by males in the future, even if the males cannibalize their current

clutches when the clutch size is small.

Hatching success was strongly influenced by the time spent in the fanning behavior by the males of E. prasina. The time spent in the fanning behavior by the males positively correlated with the clutch size; hence, females may be able to ensure the survival of their eggs by spawning large clutches within the nests. In the fathead minnow, Pimephales promelas (Rafinesque), when the males acquire a larger number of eggs within their nests, they tend their eggs with a higher frequency and the survival rate of the eggs under the care of the males becomes high (Sargent, 1988). Therefore, female fathead minnows prefer male nests as spawning sites in which the males are already guarding the clutches of other females (Unger and Sargent, 1988). Females can enhance the hatching success of their eggs by preferring the nests with eggs as spawning sites, because the males will increase the parental investment in their broods across multiple clutches. The female preference for male nests with eggs has also been known in other fishes exhibiting paternal care (Marconato and Bisazza, 1986; Sikkel, 1989; Goldshmidt et al., 1993; Kraak and Groothuis, 1994). Hence, it is possible that the females of E. prasina also preferentially spawn their eggs within the nests in which males are already tending the eggs of other females, although female preference for nests with eggs has not yet been empirically tested in this species. Further studies are required to ascertain the

reproductive strategy of females of E. prasina.

In some fish species, females choose their mates on the basis of the male traits that indicate parental ability of the males (Downhower and Brown, 1980; Knapp and Kovach, 1991; Forsgren, 1997). Sekiya and Karino (2004) have documented that in E. prasina, the females prefer mates with long dorsal fins and high courtship frequency. It has been suggested that in E. prasina, these male traits reflect the body condition of the males, which in turn, indicate the parental ability of the males (Sekiya and Karino, 2004). However, the results of this study indicate that male dorsal fin length and courtship frequency do not have a positive influence on the hatching success of the eggs as well as the time spent by males in the fanning behavior. The body size was also not found to influence the hatching success and parental behavior. Based on their choice of mate depending on male dorsal fin length and courtship frequency, females may obtain benefits other than high survival rate of their eggs through frequent parental care by the males. For instance, it is possible that females may produce higher quality offspring possessing traits such as greater competitive ability, based on their mate preferences, as predicted by many sexual selection theories (Andersson, 1994; Dugatkin and FitzGerald, 1997; Kokko et al., 2003). This is because only the dominant males of E. prasina can elongate their dorsal fins within the population (Sekiya et al., unpublished data).

Furthermore, dominant males may acquire high quality nests by outperforming the other males (Bisazza and Marconato, 1988; Lindström, 1988). It is also probable that the nest quality contributes to the survival rate of eggs within the nests, similar to that observed in the case of other fishes exhibiting paternal care (Hastings, 1988; Côte and Hunte, 1989; Kvarnemo et al., 1998). It will be worthwhile to clarify the indirect effect of male dominance on the survival rate of eggs and to investigate the direct benefit (offspring survival) to the females through their mate preferences on the basis of male secondary sexual traits in E. prasina.

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References

- Andersson, M., 1994. Sexual selection. Princeton University Press, Princeton.
- Bisazza, A., Marconato, A., 1988. Female mate choice, male-male competition and parental care in the river bullhead, Cottus gobio L. (Pisces, Cottidae). Anim. Behav. 36, 1352-1360.
- Clutton-Brock, T.H., 1991. The evolution of parental care. Princeton University Press, Princeton.
- Coleman, R.M., Gross, M.R., Sargent, R.C., 1985. Parental investment decision rules: a test in bluegill sunfish. Behav. Ecol. Sociobiol. 18, 59-66.
- Côte, I.M., Hunte, W., 1989. Male and female mate choice in the redlip blenny: why bigger is better. Anim. Behav. 38, 78-88.
- Downhower, J.F., Brown, L., 1980. Mate preferences of female mottled sculpins, Cottus bairdi. Anim. Behav. 28, 728-734.
- Dugatkin, L.A., FitzGerald, G.J., 1997. Sexual selection. In: Godin, J.G.J. (Ed.), Behavioural ecology of teleost fishes. Oxford University Press, Oxford, pp. 266-291.
- Forsgren, E., 1997. Female sand gobies prefer good fathers over dominant males. Proc. R. Soc. Lond., B Biol. Sci. 264, 1283-1286.
- Forsgren, E., Karlsson, A., Kvarnemo, C., 1996. Female sand

gobies gain direct benefits by choosing males with eggs in their nests. *Behav. Ecol. Sociobiol.* 39, 91-96.

Grafen, A., Hails, R., 2002. *Modern statistics for the life sciences*. Oxford University Press, Oxford.

Goldshmidt, T., Bakker, T.C.M., Feuth-De Bruijin, E., 1993. Selective copying in mate choice of female sticklebacks. *Anim. Behav.* 45, 541-547.

Hastings, P.A., 1988. Female choice and male reproductive success in the angel blenny, *Coralliozetus angelica* (Teleostei: Chaenopsidae). *Anim. Behav.* 36, 115-124.

Hoelzer, G.A., 1989. Good parent process of sexual selection. *Anim. Behav.* 38, 1067-1078.

Karino, K., 1997. Influence of brood size and offspring size on parental investment in a biparental cichlid fish, *Neolamprologus moorii*. *J. Ethol.* 15, 39-43.

Knapp, R.A., Kovach, J.T., 1991. Courtship as an honest indicator of male parental quality in the bicolor damselfish, *Stegastes partitus*. *Behav. Ecol.* 2, 295-300.

Kokko, H., Brooks, R., Jennions, M.D., Morley, J., 2003. The evolution of mate choice and mating biases. *Proc. R. Soc. Lond., B Biol. Sci.* 270, 653-664.

Kraak, S.B.M., 1996. Female preference and filial cannibalism in *Aidablennius sphynx* (Teleostei, Blenniidae); a combined field and laboratory study. *Behav. Process.* 36, 85-98.

- Kraak, S.B.M., Groothuis, T.G.G., 1994. Female preference for nests with eggs is based on the preference of the eggs themselves. *Behaviour* 131, 189-206.
- Kvarnemo, C., Svensson, O., Forsgren, E., 1998. Parental behaviour in relation to food availability in the common goby. *Anim. Behav.* 56, 1285-1290.
- Lindström, K., 1988. Male-male competition for nest sites in the sand goby, Pomatoschistus minutus. *Oikos* 53, 67-73.
- Lindström, K., 1998. Effects of costs and benefits of brood care on filial cannibalism in the sand goby. *Behav. Ecol. Sociobiol.* 42, 101-106.
- Lindström, K., Sargent, R.C., 1997. Food access, brood size and filial cannibalism in the fantail darter, Etheostoma flabellare. *Behav. Ecol. Sociobiol.* 40, 107-110.
- Lissåker, M., Kvarnemo, C., Svensson, O., 2003. Effect of a low oxygen environment on parental effort and filial cannibalism in the male sand goby, Pomatoschistus minutus. *Behav. Ecol.* 14, 374-381.
- Manica, A., 2002. Alternative strategies for a father with a small brood: mate, cannibalise or care. *Behav. Ecol. Sociobiol.* 51, 319-323.
- Manica, A., 2003. The effect of brood size and age on partial filial cannibalism in the scissortail sergeant. *J. Fish Biol.* 63, 37-47.
- Marconato, A., Bisazza, A., 1986. Males whose nests contain

- eggs are preferred by female Cottus gobio L. (Pisces, Cottidae). Anim. Behav. 34, 1580-1582.
- Marconato, A., Bisazza, A., Fabris, M., 1993. The cost of parental care and egg cannibalism in the river bullhead, Cottus gobio L. (Pisces, Cottidae). Behav. Ecol. Sociobiol. 32, 229-237.
- Mock, D.W., Parker, G.A., 1997. The evolution of sibling rivalry. Oxford University Press, Oxford.
- Nakabo. T., 1993. Fishes of Japan with pictorial keys to the species. Tokai University Press, Tokyo (in Japanese).
- Okuda, N., Yanagisawa, Y., 1996. Filial cannibalism by mouthbrooding males of the cardinal fish, Apogon doederleini, in relation to their physical condition. Env. Biol. Fish. 45, 397-404.
- Payne, A.G., Smith, C., Campbell, A.C., 2002. Filial cannibalism improves survival and development of beaugregory damselfish embryos. Proc. R. Soc. Lond., B Biol. Sci. 269, 2095-2102.
- Petersen, C.W., 1990. The occurrence and dynamics of clutch loss and filial cannibalism in two Caribbean damselfishes. J. Exp. Mar. Biol. Ecol. 135, 117-133.
- Pompoulie, C., Lindström, K., St. Mary, C.M., 2004. Have your cake and eat it too: male sand gobies show more parental care in the presence of female partners. Behav. Ecol. 15, 199-204.
- Ridgway, M.S., 1989. The parental response to brood size

- manipulation in smallmouth bass (Micropterus dolomieu). Ethology 80, 47-54.
- Roff, D.A., 2002. Life history evolution. Sinauer Associates, Sunderland.
- Rohwer, S., 1978. Parental cannibalism of offspring and egg raiding as a courtship strategy. Am. Nat. 112, 429-440.
- Sargent, R.C., 1988. Paternal care and egg survival both increase with clutch size in the fathead minnow, Pimephales promelas. Behav. Ecol. Sociobiol. 23, 33-37.
- Sargent, R.C., 1997. Parental care. In: Godin, J.G.J. (Ed.), Behavioural ecology of teleost fishes. Oxford University Press, Oxford, pp. 292-315.
- Sargent, R.C., Gross, M.R., 1993. Williams' principle: an explanation of parental care in teleost fishes. In: Pitcher, T.J. (Ed.), Behaviour of teleost fishes, 2nd ed. Chapman and Hall, London, pp. 333-361.
- Sekiya, Y., Karino, K., 2004. Female mate preference in goby Eviota prasina: do secondary sexual traits influence female choice? Zool. Sci. 21, 859-863.
- Sikkel, P.C., 1989. Egg presence and developmental stage influence spawning-site choice by female garibaldi. Anim. Behav. 38, 447-456.
- Smith, C., Wootton, R.J., 1995. The costs of parental care in teleost fishes. Rev. Fish. Biol. Fish. 5, 7-22.
- Sokal, R.R., Rohlf, F.J., 1973. Introduction to

- biostatistics. Freeman and Company, San Francisco.
- Stearns, S.C., 1992. The evolution of life histories.
Oxford University Press, Oxford.
- Suk, H.Y., Choe, J.C., 2002. The presence of eggs in the nest and female choice in common freshwater goby (Rhinogobius brunneus). Behav. Ecol. Sociobiol. 52, 211-215.
- Sunobe, T., 1998. Reproductive behavior in six species of Eviota (Gobiidae) in aquaria. Ichthyol. Res. 45, 409-412.
- Sunobe, T., Nakazono, A., 1999. Alternative mating tactics in the gobiid fish, Eviota prasina. Ichthyol. Res. 46, 212-215.
- Takegaki, T., Nakazono, A., 1999. Responses of the egg-tending gobiid fish Valenciennea longipinnis to the fluctuation of dissolved oxygen in the burrow. Bull. Mar. Sci. 65, 815-823.
- Trivers, R., 1985. Social evolution. The Benjamin/Cummings Publishing Company, New York.
- Unger, L.M., Sargent, R.C., 1988. Allopaternal care in the fathead minnow, Pimephales promelas: females prefer males with eggs. Behav. Ecol. Sociobiol. 23, 27-32.

Table 1.

Effects of morphological traits and behaviors of males as well as those of clutch size in the male nests on the hatching success of the eggs ($n = 34$). Correlation coefficients (r) from simple regression analyses and standard coefficients (β) of both the full model and final model of the stepwise removal procedure of multiple regression analyses are shown

Independent variables	Simple regression r	Multiple regression	
		Full model β	Final model β
Male body size	0.02	0.04	-
Male dorsal fin length	-0.19	-0.09	-
Courtship frequency of males	-0.10	-0.08	-
Time spent by males in fanning	0.72***	0.55**	0.72***
Time spent by males in pecking	-0.34*	-0.12	-
Clutch size	0.50**	0.20	-
Constant		-1.18	-0.40
Adjusted multiple R^2		0.47***	0.51***

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Table 2.

Effects of morphological traits and behaviors of males as well as those of clutch size in the male nests on the hatching success of the eggs in which data of unsuccessful clutches were excluded ($n = 28$). Correlation coefficients (r) from simple regression analyses and standard coefficients (β) of both the full model and final model of the stepwise removal procedure of multiple regression analyses are shown

Independent variables	<u>Simple regression</u> r	<u>Multiple regression</u>	
		<u>Full model</u> β	<u>Final model</u> β
Male body size	0.29	0.20	-
Male dorsal fin length	0.03	-0.11	-
Courtship frequency of males	-0.24	-0.23	-
Time spent by males in fanning	0.45*	0.18	0.45*
Time spent by males in pecking	-0.29	-0.23	-
Clutch size	0.27	0.22	-
Constant		-1.96	-0.40
Adjusted multiple R^2		0.12	0.17*

* $p < 0.05$.

Table 3.

Effects of male morphological traits and clutch size on the time spent by males in fanning behavior ($n = 34$). Correlation coefficients (r) from simple regression analyses and standard coefficients (β) of both the full model and final model of the stepwise removal procedure of multiple regression analyses are shown

Independent variables	<u>Simple regression</u> r	<u>Multiple regression</u>	
		<u>Full model</u> β	<u>Final model</u> β
Male body size	0.03	0.14	-
Male dorsal fin length	-0.21	-0.22	-
Courtship frequency of males	-0.14	-0.21	-
Clutch size	0.50**	0.51**	0.50**
Constant		-4.62	-2.41
Adjusted multiple R^2		0.24*	0.22**

* $p < 0.05$, ** $p < 0.01$.

Table 4.

Effects of male morphological traits and clutch size on the time spent by males in fanning behavior in which data on males with unsuccessful clutches were excluded ($n = 28$). Correlation coefficients (r) from simple regression analyses and standard coefficients (β) of both the full model and final model of the stepwise removal procedure of multiple regression analyses are shown

Independent variables	<u>Simple regression</u> r	<u>Multiple regression</u>	
		<u>Full model</u> β	<u>Final model</u> β
Male body size	0.27	0.24	-
Male dorsal fin length	0.02	-0.12	-
Courtship frequency of males	-0.29	-0.31	-0.36†
Clutch size	0.34†	0.39*	0.40*
Constant		-4.78	-0.85
Adjusted multiple R^2		0.16	0.18*

† $p < 0.1$, * $p < 0.05$.

Figure captions

Fig. 1. The relationship between female body size and the clutch size. A line indicates the regression line. Data were log-transformed.

Fig. 2. The influences of the time spent by males in fanning (a) and the time spent in pecking eggs (b) on the hatching success of eggs under the care of the males. A line indicates the regression line. Data of the hatching success were arcsine-transformed and other data were log-transformed.

Fig. 3. The relationship between the clutch size in male nests and the time spent by the males in fanning eggs. A line indicates the regression line. Data were log-transformed.



Fig. 1. (Karino and Arai)

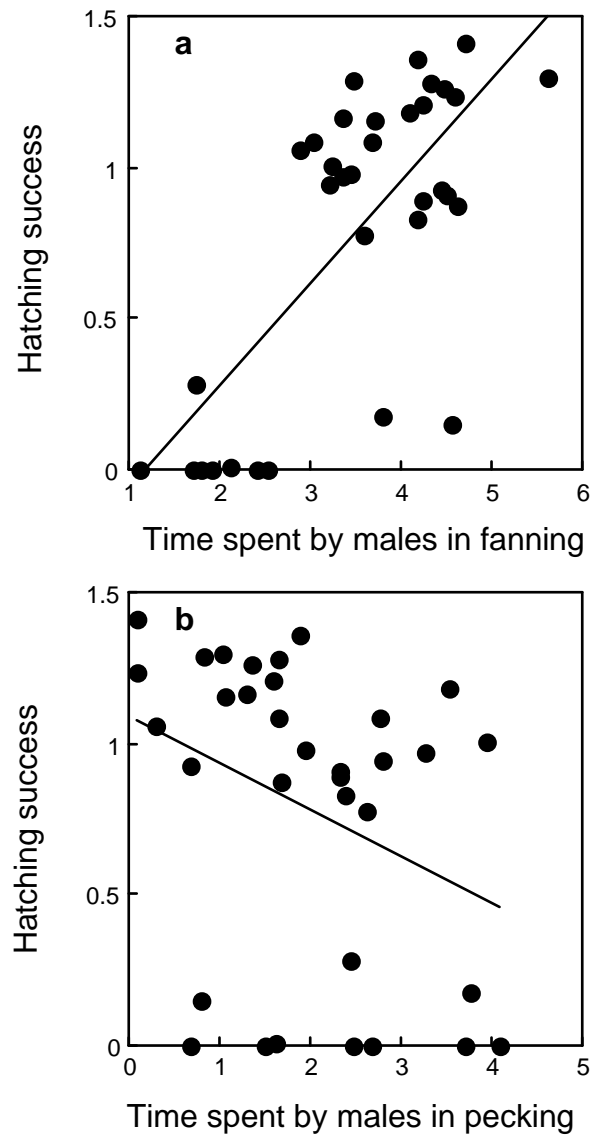


Fig. 2. (Karino and Arai)

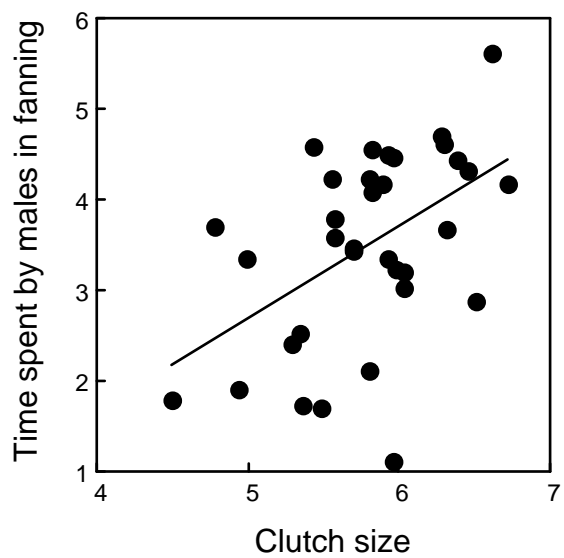


Fig. 3. (Karino and Arai)