Female within-nest spawning-site preference in a paternal brooding blenny and its effect on the female mate choice

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ABSTRACT

Female spawning-site preference within a nest and its effect on the female mate choice in a paternal brooding blenny *Rhabdoblennius ellipes*, were examined in rocky intertidal pools using artificial nests. The number of eggs deposited at the nest entrance site was lower than the number deposited at the center and at deep sites. Moreover, the within-nest difference in the egg developmental stage indicated that eggs at the nest entrance site were deposited after those at the center and deep sites. These results indicated that females prefer to spawn eggs in the center and deep sites rather than at the entrance site. Owing to the higher egg mortality rate at the entrance site, females may avoid spawning at such sites. An analysis of the nests in the study area suggested that the within-nest site preference affects female mate choice that is females avoided nests where only the entrance site was available for spawning and instead spawned in nests where the center and deep sites were available.
Introduction

In species that exhibit parental care, ability to enhance offspring survival is one of the most important traits influencing the choice of a mating partner. Offspring survival depends not only on parental behaviors such as feeding of their young (Saetre, Fossnes & Slagsvold, 1995; Buchanan & Catchpole, 2000) and anti-predator behavior (Downhower & Brown, 1980; Keenleyside, Rangeley & Kuppers, 1985; Côté & Hunte, 1989; Hoelzer, 1989; Norris, 1990; Rahman, Dunham & Govind, 2004) but also on the quality of parental resources, such as breeding territories and nests.

The location and structure of breeding territories and nests are used as indicators of mate choice because these traits affect offspring-guarding efficiency (Howard, 1978; Knapp, 1993; Jones & Reynolds, 1999; Quader, 2005). For example, in a paternal brooding gobiid fish, females prefer spawning nests with smaller entrances because such nests are less vulnerable to detection by egg predators (Jones & Reynolds, 1999). Moreover, these traits of breeding sites are affected by environmental conditions, such as water and air temperature, that influence offspring survival and development (Howard, 1978; Pleszczynska, 1978; Hoi, 1994). Nest quality can also be influenced by the presence of offspring in the nest. For example, in some fishes, females prefer males with eggs in their nests because of the low predation rate on their own eggs (i.e. the dilution effect; Rohwer, 1978; Ridley & Rechten, 1981; Kraak & Groothuis, 1994; Forsgren, Karlsson & Kvarnemo, 1996) and enhanced male parental investment with the increase in the number of eggs (Coleman, Gross & Sargent, 1985; Sargent, 1988).

Not only spawning-nest selection but also spawning-site selection within a nest could evolve if there is a critical within-nest difference in the quality for offspring survival. For example, ostrich females move their eggs to the center of all eggs cared for in a nest because the outer eggs are vulnerable to predation (Bertram, 1979). In the Japanese rosy bitterling, a freshwater fish that deposits eggs into the gills of living unionid mussels, females avoid spawning at sites of high egg density within a mussel due to the high egg mortality rate resulting from oxygen deficiency (Kitamura, 2005, 2006). Female damselfish prefer to deposit their clutches contiguously to young clutches in order to avoid filial cannibalism by the egg-tending male (Afonso & Santos, 2005), or to ensure intense parental care (Knapp, Sikkel & Vredenburg, 1995). Although within-nest spawning-site preference has been given little attention, it could be an important factor
influencing female spawning nest choice and thus mate choice, particularly in polygamous species; this is because females of such species are not always able to deposit their eggs in the preferred site within a nest due to the presence of pre-existing clutches. Indeed, in the garibaldi damselfish, females prefer to lay eggs contiguously to young clutches or on thick algal cover and avoid spawning in the nests without such spawning space (Sikkel, 1994, 1995). In some substrate-brooding fishes such as blennies, gobies and damselfish, males using larger nests have more eggs because the eggs of such species are deposited on the substrate as a single layer (e.g. Côté & Hunte, 1989; Lindström, 1992). However, in our field study of the barred-chin blenny Rhabdoblennius ellipse, using artificial nest holes (Takegaki et al., 2008), vacant space for additional egg deposition was commonly observed within each nest, particularly near the nest entrance (see 'Results'); a similar finding has been observed in the hole-nesting blenny Aidablennius sphinx (Neat & Locatello, 2002). Although it remains unclear whether the mating success of male R. ellipes is limited by nest size, the presence of vacant entrance space suggests that females of this species prefer to spawn in a particular site within a nest. Takegaki et al. (2008) demonstrated that male R. ellipes prefer to use size-assortative nests, which are selected on the basis of the nest entrance size, and these authors suggested that the assortative nest use allows effective guarding against egg predators. Therefore, it is expected that female R. ellipes avoid spawning near the nest entrance because of the high risk of egg predation. In this study, to examine the female spawning-site preference within a nest, and the effect of site-specific egg mortality on the preference, both egg-laying and egg mortality patterns within a nest were investigated in the wild using artificial nests. Furthermore, the effect of the within-nest spawning-site preference on female nest preference (i.e. mate choice) was examined.

Materials and methods

Study species
Rhabdoblennius ellipse is a small omnivorous reef fish, which is distributed in the West Pacific Ocean, including the coastal waters of southern Japan, and mainly inhabits intertidal rocky shores (Aizawa, 1993). In western Kyushu, Japan, this species breeds from late June to early October, irrespective of the lunar phases (Miyano, Takegaki & Natsukari, 2006). Males use holes made by bivalve molluscs and vacant shells of the
vermetid gastropod *Serpulorbis imbricatus* as spawning nests. Spawning occurs in pairs, and the eggs are deposited in a single layer on the inner surface of the nest. After spawning, the males guard and aerate the eggs until they hatch 6–7 days later. In contrast, the females exhibit no parental care. Multiple females successively visit a male's nest for spawning. Consequently, at any single time, males generally care for multiple clutches of eggs at different stages of development – a system known as male-territory-visiting polygamy (for terminology, see Kuwamura, 1996, 1997).

**Field investigation**

This study was conducted from June to September 2006 in three adjacent rocky intertidal pools (dimensions at low tide: area 1.3 m², depth 33 cm; 1.8 m², 29 cm; and 5.0 m², 36 cm) on the Koe coast, Nagasaki Prefecture, western Kyushu, Japan (32°45'N, 129°47'E). The investigation was conducted at low tide during the daytime every day, except for the days around neap tides and stormy days when the study area became inaccessible.

Almost all adult males found in the study pools were captured using a hand net and were individually identified [mean standard length (SL)±se=55.5±0.78 mm, range=36.4–71.1 mm, n=83]. The collected fish were anesthetized by immersion in MS-222, and their SL was measured with a slide calliper. The sex of the fish was determined by the shape of genital papillae: the male genital papilla is small whereas the female genital papilla is large and rounded (see Miyano et al., 2006). The fish were then marked by hypodermically injecting acrylic paint on each side of the body – individuals being identified by the color and position of the paint. After marking, the fish were returned to their respective collection sites. Whenever unidentified males were observed in the study area, they were captured and marked.

Because the males use small rock holes and vacant shells of vermetid gastropods as spawning sites, the eggs deposited on the inner surface of the nests cannot be observed and counted. In this study, to examine the within-nest egg-laying and egg mortality patterns, acrylic pipes with one end closed to form artificial nests were set in holes drilled into the inner wall of the pool, c. 1 month before the investigation commenced. To avoid disturbing the natural pattern of nest abundance and distribution in the tide-pool, the artificial nests were set adjacent to natural nests, and the entrances of the natural nests were then closed with stones to prevent males from using these nests. *Rhabdoblennius ellipes* males prefer to use the nest that tightly fits their body size (Takegaki et al., 2008);
therefore, artificial nests of three different diameters were used (large: 17 mm, n=9; medium: 14 mm, n=11; small: 11 mm, n=11; nest length nests for all: 70 mm) to minimize the influence of size-mismatched nest use on the egg mortality rate. An attachable translucent plastic sheet was set on the inner surface of the nest side wall to enable us to monitor the number of eggs deposited in the nest by withdrawing and repositioning the sheet. To avoid egg deposition at the end of the artificial nest, a fishing line was crossed 5 mm from the end of the nest. In addition, to prevent the plastic sheet from falling off from the nest, a tiny projection (width: 2 mm, height: 0.5 mm) was made using an adhesive bond on the inner surface of the nest entrance. Consequently, the potential spawning space of each nest was 7 mm shorter than the actual pipe length (63 mm).

In the field survey, all males occupying artificial nests were identified, and the plastic sheet taken from each nest was photographed using a digital camera with a macro-lighting system (EOS Kiss Digital N, EF 50 mm Macro Lens and Macro Twin Lite MT-24EX; Canon, Tokyo, Japan). When the plastic sheets were re-set into the nests, males readily returned to their nests and began to care for the eggs, if present. The digital images of the plastic sheets supporting eggs were uploaded onto a personal computer, and the number of eggs on the sheet was counted using counting software (Kachikachi counter 2.6; GT).

To examine the female spawning-site preference within a nest and the site-specific egg mortality rate, the nest inner surface area was divided into three sites ('entrance,' 'center' and 'deep'), and the number of eggs deposited at each site was counted separately. The eggs deposited on the borderlines were assorted by the position of the center of the eggs. The differences in the egg age between 0 and 2 days old, between 3 and 5 days old and between 6 and 7 days old could not be judged on the basis of the digital images due to subtle changes in egg characteristics and color; therefore, the number of eggs added by additional spawnings and lost by mortality could not be counted accurately. Thus, to estimate the number of added and lost eggs, the egg age was classified into three stages: stage 1: non-eyed eggs with an orange-yellow color (0–2 days old); stage 2: eyed eggs with a transparent color (3–5 days old); and stage 3: eggs with silvered eyes (6–7 days old). The increase in the number of stage 1 eggs compared with the previous day was regarded as the number of acquired eggs, and the decrease in the number of stages 1 and 2 eggs compared with the previous day was regarded as the number of lost eggs; both
counts, however, may have been underestimated. The decrease in stage 3 eggs from the previous day was regarded as egg hatch. The number of eggs acquired and lost was compared among the three sites within a nest and expressed as the mean daily number of acquired eggs and the mean daily mortality rate of eggs, respectively. The female spawning-site preference within a nest was estimated from the developmental stage of eggs deposited at each nest site when eggs of different stages coexisted in the nest. For example, stage 2 eggs were deposited before and after the deposition of stages 1 and 3 eggs, respectively. In this study, to enhance the accuracy of the data, 39 of the 83 marked males (SL±se=57.75±1.19 mm, range=41.40–71.10 mm) that had tended eggs for more than 8 days were used for analysis.

To examine the effect of female within-nest spawning-site preference on the female mate choice, the relationship between female selectivity for the entrance site and the total vacant space at the center and deep sites in all study nests was analyzed. An index of selective spawning at the nest entrance site was represented by the ratio of observed and expected daily total number of eggs added at the nest entrance site in all study nests. The expected number of eggs was calculated by the following equation: the daily total number of acquired eggs in all study nests × (vacant space at the nest entrance site/vacant space at the nest center and deep sites). If females avoid the nests where only the entrance site is available for spawning and spawn in the nests where the center and deep sites are available, the index of selective spawning for the nest entrance site is assumed to decrease with an increase in the total vacant space at the center and deep sites among all nests in the study area.

**Data analysis**

Differences among spawning sites with respect to the mean daily number of acquired eggs, the mean daily number of eyed (stages 2 and 3) and non-eyed eggs (stage 1) and the mean daily mortality rate of eggs were tested using the Friedman test. Subsequently, multiple comparisons were performed using the Wilcoxon signed-ranks test with Bonferroni correction because the homogeneity of variance assumptions was not satisfied. The correlation between female selectivity for the entrance site and the total vacant space at the center and deep sites in all study nests was analyzed using the Kendall rank correlation test. Probabilities of <5% were accepted as significant. All statistical tests were conducted using StatView 5.0 (SAS Institute Inc., Cary, NC, USA).
Results

The mean daily number of eggs spawned at the nest entrance site was significantly less than that spawned in either the center or the deep site (Friedman test: $\chi^2 = 45.0$, $n=39$, $P<0.0001$; Wilcoxon signed-ranks test with Bonferroni correction: both $P<0.01$; Fig. 1), and the mean daily number of acquired eggs was significantly less at the deep site than at the center site (Bonferroni correction: $P<0.01$; Fig. 1). In the nests containing multiple stages of eggs, the number of stage 1 eggs deposited at the deep site was significantly less than those deposited at the entrance and center sites ($\chi^2 = 21.1$, $n=33$, $P<0.0001$; Bonferroni correction: $P<0.01$; Fig. 2a); however, there was no significant difference in the number of stage 1 eggs deposited at the entrance and center sites (Bonferroni correction: $P>0.05$; Fig. 2a). On the other hand, the number of eyed eggs at the entrance site was significantly less than those at the other two sites ($\chi^2 = 53.9$, $n=33$, $P<0.0001$; Bonferroni correction: both $P<0.01$; Fig. 2b), and the number of eyed eggs was significantly less at the deep site than at the center site (Bonferroni correction: $P<0.05$; Fig. 2b). The mean daily mortality rate of eggs at the entrance site was higher than that at the other two sites ($\chi^2 = 7.4$, $n=39$, $P<0.05$; Bonferroni correction: $P<0.05$; Fig. 3). In our study area, the eggs were predated by other fishes such as Bathygobius fuscus and Omobranchus loxozonus and by other *R. ellipes* individuals in the vicinity of the nests when the egg-tending males vacated their nests to feed.

![Figure 1](image_url)

Figure 1 Comparison of the mean daily number of acquired eggs ($\pm$se) among different sites within a nest ($n=33$). Different letters at the top of the vertical lines indicate significant differences (all $P<0.01$).
Figure 2  Comparison of the mean daily number of eggs (±se) of (a) stage 1 and (b) stages 2 and 3 among different sites within a nest when the eggs at different stages of development coexisted in the nests (n=33). Different letters indicate significant differences (P<0.01 for all, except for the comparison of stages 2 and 3 eggs between the center and deep sites, P<0.05).

Figure 3  Comparison of the mean daily egg mortality rate (±se) among different sites within a nest (n=39). Different letters indicate significant differences (both P<0.05).

The index values of selective spawning at the nest entrance site were below 1.0 on 40 of 42 days (Fig. 4), and these values decreased with an increase in the total vacant space at the center and deep sites among all nests in the study area (Kendall's rank correlation: \( \tau =0.55 \), n=42 days, P<0.001; Fig. 4); this suggests that among the nests in the study area, females preferred the nests with a vacant space at the center and deep sites.
Discussion

This study demonstrated that female *R. ellipes* exhibit spawning-site preferences within a nest probably because of the difference in the egg mortality rate among the sites; this finding strongly suggests that the within-nest site preference affects the female nest choice (i.e. mate choice).

Spawning-site preference within a nest

The number of eggs deposited at the entrance site was less than those at the center and deep sites; notably, the number of eggs deposited at the entrance site was less by >40% than that at the center site (Fig. 1). Moreover, the within-nest difference in the egg developmental stage indicated that eggs at the nest entrance site were deposited after those at the center and deep sites (Fig. 2a and b). These results indicated that females prefer to spawn eggs at the center and deep sites rather than at the entrance site. Owing to the higher egg mortality rate at the entrance site (Fig. 3), females may avoid spawning at such sites. The smaller number of acquired eggs at the deep site than at the center site may be attributed to the difficulty in laying eggs on the deep edge.

The main causes of egg mortality were predation and filial cannibalism by the egg-tending males. In our study area, some predatory fishes such as gobies and blennies
were observed to eat the eggs by pecking from outside the nests (Y. Matsumoto, pers. obs.). Moreover, a large number of carnivorous snails were observed in our study tide-pools, and these snails can predate on the eggs of the hole-nesting blenny (A. Murase & T. Sunobe, pers. comm.). Because the extended radula of these snails is limited in length, they are able to eat only those eggs that are deposited near the nest entrance. Although male *R. ellipes* cannibalize the eggs tended in their nests (Y. Matsumoto, unpubl. data), it seems reasonable to assume that the higher mortality rate of eggs at the entrance site was caused by predation rather than by filial cannibalism. A similar female avoidance of the spawning site with a high predation risk within a nest has been observed in the ostrich (Bertram, 1979); furthermore, in the garibaldi damselfish, the position of predators in the vicinity of the nest may influence the female within-nest spawning-site selection (Sikkel, 1994). Even within a single clutch, to minimize the cost of egg mortality, female insects (Mappes, Mappes & Lappalainen, 1997; Kudo, 2001) and fish (Green, Anthony & McCormick, 2006) lay smaller eggs at a site with a higher egg mortality rate.

**Effect of within-nest spawning-site preference on mate choice**

The index of selective spawning at the entrance site increased with the decrease in the available space at the center and deep sites (Fig. 4). This result suggests that female *R. ellipes* tend to avoid those nests where only the entrance site is available for spawning and prefer to spawn in the nests where the center and deep sites are available. Because some females with swollen abdomens were observed to emerge from a male's nest without spawning (Y. Matsumoto, pers. obs.), these females might monitor the available spawning sites within the nests, although there are other possible reasons for the female nest avoidance, such as avoidance of nests without enough space for depositing a single egg batch (e.g. Lindström, 1992) and avoidance of nests with old eggs (e.g. Sikkel, 1994). Because searching for suitable mating partners and breeding sites generally incurs a cost to females in terms of time, energy and predation risk, theoretical models predict that females may become less choosy with the increase in the cost of searching (Real, 1990; Reynolds & Gross, 1990; Crowley et al., 1991). However, females may seek more suitable males and sites if there are sufficient differences in the quality (Reynolds & Côté, 1995) and/or if the costs are low (Milinski & Bakker, 1992; Hedrick & Dill, 1993; Karino et al., 2000). In *R. ellipes*, in addition to the difference in the egg mortality rate among
spawning sites, nest monitoring costs may be low because the spawning nests are densely located within the tide-pools. Therefore, females can monitor several nests and select the most suitable nest.

In many substrate-spawning fishes, it has been demonstrated that females prefer males with eggs in their nests on account of the potential dilution effect (Rohwer, 1978; Ridley & Rechten, 1981; Kraak & Groothuis, 1994; Forsgren et al., 1996) and enhanced parental investment (Coleman et al., 1985; Sargent, 1988); however, little is known about the effects of within-nest spawning-site preference on mate choice. Female nest avoidance and choice on the basis of the within-nest site preference have only been observed in two damselfish species (Sikkel, 1994, 1995; Afonso & Santos, 2005). In Hypsypops rubicundus, in particular, because females avoid the nests in which they are unable to deposit eggs adjacent to young clutches, the egg-tending males cannibalize the old eggs in the nests probably to readily acquire new additional eggs (Sikkel, 1994). A similar effect of the within-nest site preference on mate choice is likely to occur in the reproductive systems in which females try to spawn encounter already-laid eggs or other females spawning in the same nest. In such systems (e.g. male-territory-visiting polygamy), to examine the effect of the nest resource on mate choice, the difference in the quality not only between nests but also within a nest should be considered because between-nest differences in the quality could be reversed under certain circumstances.

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