Determinants of male mating success in a natural population of a stream goby of the genus Rhinogobius

S. Ito* † and Y. Yanagisawa‡

*Center for Marine Environmental Studies, Ehime University, 2-5 Bunkyo-cho, Matsuyama 790-8577, Japan and ‡Department of Biology, Ehime University, 2-5 Bunkyo-cho, Matsuyama 790-8577, Japan

(Received 17 July 2004, Accepted 27 June 2005)

The mating success of individually identified males of a stream goby, Rhinogobius sp. CB (cross-band type), was recorded over one breeding season. One to 3 year-old males were active in mating, but 3 year-old males, which accounted for 36% of the population, amounted to c. 70% of the total males guarding eggs in the nest. Three quarters of the breeding males had only one brooding cycle, but the others had two or three. All the latter males changed their nest sites between cycles within a riffle. In 1 and 2 year-old males, the number of brooding cycles contributed more to the mating success than egg mass size in one brooding cycle. For mating success of 3 year-old males, the egg mass size in one brooding cycle, which can be enhanced by spawning with a large female or multiple females, was as important as the number of brooding cycles. These male reproductive tactics could be attributed to the age-related ability of nest construction and mate acquisition.

Key words: brooding cycle; goby; mating success; mating tactics; Rhinogobius.

INTRODUCTION

In many animals, large males have the advantage in acquiring mates and nests and therefore male mating success varies greatly with the size and age (Brown & Downhower, 1982; Goto, 1987; Bisazza et al., 1989; Forsgren et al., 1996; Lindström & Seppä, 1996; Natsumeda, 1998). Small or young males may gain mating success by using different tactics from those of large ones, sometimes adopting alternative forms of reproductive behaviour such as female mimicry and sneaking behaviour (Clutton-Brock et al., 1979; Howard, 1984; Andersson, 1994). Environmental circumstances are also important for males to decide mating tactics (Arak, 1983; Kitano, 1996). In some animals, males change optimal tactics flexibly with ecological and demographic factors such as the quality and quantity of available nests, the operational sex ratio, and population structure (Perrill et al., 1982; de Fraipont et al., 1993; Magnhagen, 1998).

In natural populations of fishes with paternal care, male mating success has usually been estimated from the number of eggs received at a time (Marconato

†Author to whom correspondence should be addressed. Tel. and fax: +81 927 9635; email: akasaya@sci.ehime-u.ac.jp

© 2006 The Fisheries Society of the British Isles
et al., 1989; Lindström, 1992), and many studies have documented that large males receive a large egg mass (Lindström, 1988; Lugli et al., 1992). The total eggs one male receives in one breeding season, which can be an approximate estimate of his reproductive success of the year, however, has rarely been quantified (Katano, 1990; Kitano, 1996). The total egg size is determined by two elements, the egg mass size in a brooding cycle and the number of brooding cycles. Males of different sizes or ages may adopt different tactics, attaching more importance to either of these elements, and may maximize the lifetime reproductive success by changing the tactics according to the size or age.

Males of a stream goby, *Rhinogobius* sp. CB (cross-band type), often gather multiple females at one time in their nests and then spawn with one to three of them within a short span of time, causing the deposited eggs to be at the same developmental stage (Ito & Yanagisawa, 2003). Eggs are deposited in a single layer on the undersurface of a stone. Large males of this goby usually receive more eggs in a brooding cycle through size-assortative mating and by providing a larger nest, which facilitates spawning with multiple females (Ito & Yanagisawa, 2003).

This study examined male mating success of this goby over one breeding season and analysed factors influencing the mating success. The primary aim of this study was to understand how the relative importance of the number of brooding cycles and the brood size differs among males of different sizes or ages.

**MATERIALS AND METHODS**

**CENSUS OF MALE REPRODUCTION**

The field study was conducted in the Tani River, a tributary of the Kamo River, Ehime Prefecture, Japan (33°52′ N; 133°10′ E). The study site is a riffle in a 110 m long reach between two large pools (7 and 22 m wide and 3 m deep). All stones >c. 10 cm in the major axis were carefully turned over during the breeding season of 1998 (early May to late August) to find egg masses attached on the undersurface of stones. If an egg mass was found, the nesting male was captured and measured for total length (L_T) to the nearest 0.1 cm and injected with coloured silicon (Brand name Elastomer, Tanaka-Sanjiro Co. Ltd., Ogori City, Fukuoka, Japan) under the dorsal skin for individual identification. At the same time, the major and minor axes of the egg mass and those of the undersurface area not covered with an algal mat, which corresponded roughly with the area buried in the riverbed, were measured. Those areas approximated to an ellipse were regarded as the egg mass size and the potential spawning area (PSA), respectively (Ito & Yanagisawa, 2003). After dead eggs were counted, the nest stone was carefully put back and the male released underneath. The nesting male was usually insensitive to this treatment and never abandoned his nest. Water depth of the nest stone was measured with a ruler and water velocity measured just above the nest stone by a portable current meter (Model CR-7WP, Cosumo-Riken Inc., Kashihara, Japan). Finally, the position of the nest stone was plotted on the chart of the riverbed.

The guarding period ranged between 7 and 14 days (Ito, 2002), depending on the water temperature that varied from 16° C (early May) to 27° C (late August) in the breeding season. Developmental stages of embryos were classified into three: (1) early stage (the yolks are large and yellowish and the embryo do not yet have eyes); (2) middle stage (the eye and the tail of the embryo are clearly visible); (3) late stage (the embryo change position frequently, most of the yolk have been absorbed and sometimes the eggs hatch by the stimulation of daylight). A rough estimate for the dates of the start of guarding...
was obtained from one, three and five sixths of the guarding period subtracted, respectively, from the day when the eggs were found.

The first census was conducted in May, when egg masses were first found. The following censuses were planned 10 times at the intervals of 10–15 days in the breeding season, but three censuses were cancelled due to spates following a heavy rain. The high flow events overturned many stones of the riverbed, making nesting of gobies impossible for c. 10 days. The periodical observations of their courtship and nesting behaviours, however, ensured that all identified males guarding in the nest were found in each brooding cycle.

In order to confirm whether the identified males moved out of the study site, searches were made for them up to 50 m upstream and downstream from the study site by snorkelling at each census. They were never found, however, outside the study site.

After the breeding season, gobies were captured with an electrofishing unit in the study area and in a stretch 500 m upstream, and $L_T$ of males was measured to determine their size-frequency distribution.

**AGE ESTIMATION**

Studies on the age estimation of *Rhinogobius* gobies have demonstrated that the growth rings of the second pectoral radial are the most reliable indicator of age (Washio *et al.*, 1991; Hayashi, 1997; Kobe, 2001). In *Rhinogobius* sp. CB, the rings are formed twice a year, in early summer and late winter (Kobe, 2001). Therefore, gobies with one, three, five and seven rings are judged to be 0, 1, 2 and 3 years-old, respectively.

During the breeding season, 92 males were collected in stretches 50 m upstream and downstream of the study site and preserved in 10% formalin solution. In the laboratory, after their $L_T$ was measured, the second pectoral radial was extracted with a pair of forceps and the rings were counted under a microscope. These males were analysed into cohorts (Cassie, 1954), assuming the size-frequency distribution of the same age followed the normal distribution, and the $\chi^2$ test for goodness of fitting the calculated distribution to the observed one indicated that 5-9 and 7-3 cm $L_T$ were the ‘border’ sizes between 1 and 2 years and between 2 and 3 years, respectively (Fig. 1; $\chi^2$, d.f. = 36, $P = 0.15$).

**STATISTICAL ANALYSIS**

The annual mating success of each male is the product of the number of brooding cycles and the average egg mass size. The coefficients of variation ($CV = 100 \text{s.d.}/\bar{x}$) of the two variables were calculated to estimate the relative importance of these variables to male mating success. In order to reveal factors influencing the number of brooding cycles, seven variables (male $L_T$, days from the beginning of the breeding season to the start of the first guarding, egg mass size, PSA, number of dead eggs, current velocity and water depth in the first brooding cycle) were incorporated as explanatory variables into a model of stepwise multiple regression analysis. For the egg mass size, five variables (male $L_T$, days from the beginning of the guarding to the start of the guarding, PSA, current velocity and water depth) were incorporated as explanatory variables.

The size-frequency distribution of guarding males was compared with that of males collected randomly after the breeding season, using the Kolmogorov-Smirnov test. To compare the egg mass size among males of different ages and among brooding cycles, Mann–Whitney $U$-tests and paired $t$-tests were used, respectively. If a significant difference was detected among three brooding cycles, a significance level was set at 0.05 (n = the number of comparisons) to offset increased type I error. After the ratio of egg mass area to PSA was arcsin transformed, the relationship of male $L_T$ to four variables (total egg mass area in a season, average egg mass size, PSA and the ratio of egg mass area to PSA) was analysed by Pearson’s correlation analysis, and the relationship of male $L_T$ to two variables (number of brood cycles in a season and days from the beginning of the breeding season to the first incubation) by Kendall’s rank
correlation analysis. All of the analyses were performed using Stat-View Version 5.0 (SAS Institute, Inc., Cary, NC, U.S.A.).

RESULTS

MATING SUCCESS BY AGE AND SIZE

The size-frequency distribution of guarding males differed from that of males in the random sampling (Fig. 2: Kolmogorov-Smirnov, $P < 0.001$); the former males (mean ± s.d. 74 ± 7 mm, $n = 116$) were larger than the latter (70 ± 10 mm, $n = 159$). Of 116 guarding males, six (5.2%), 28 (24.1%) and 82 (70.7%) were 1, 2 and 3 year olds, respectively. Three quarters (74.1%) of them had only one brooding cycle, but the others two (20.7%) or three (5.2%) cycles. Males which had two or three brooding cycles always shifted their nesting sites between cycles within a riffle (mean ± s.d. 11.2 ± 18.5 m, range 0.8–81.2 m, $n = 36$).

Average egg mass size and the ratio of egg mass size to PSA correlated positively with male size (egg mass size: $r = 0.60$, $n = 116$, $P < 0.001$; ratio: $r = 0.39$, $n = 116$, $P < 0.001$). Days from the beginning of the breeding season to the start of the first guarding correlated negatively with male size (Kendall rank correlation analysis, $n = 116$, $P < 0.005$). The average PSA and the number of brooding cycles did not correlate with male size (PSA: $r = 0.06$, $n = 116$, $P = 0.50$; cycles: $\Gamma = -0.01$, $n = 116$, $P = 0.85$).
Three year-olds \((n = 82)\) gained a greater annual mating success than 2 year-olds \((n = 28)\) (Fig. 3; Mann–Whitney \(U\)-test, \(P < 0.005\)), and the latter gained a greater annual success than 1 year-olds \((n = 6)\) (Mann–Whitney \(U\)-test, \(P < 0.01\)). Among males with a single brooding cycle, 3 year-olds \((n = 62)\) had a larger egg mass size than 2 year-olds (Mann–Whitney \(U\)-test, \(P < 0.001\)), and the latter had a larger egg mass size than 1 year-olds \((n = 5)\) (Mann–Whitney \(U\)-test, \(P < 0.05\)). One and 2 year-olds with multiple brooding

Fig. 2. Size-frequency distributions of male *Rhinogobius* sp. CB captured (a) on the riverbed \((n = 159)\) and (b) in the nest \((n = 116)\).
cycles \((n = 10)\) gained a greater annual mating success than 3 year-olds with a single cycle \((n = 62)\) (Mann–Whitney \(U\)-test, \(P < 0.01\)).

In males with two brooding cycles \((n = 24)\), the first egg mass size was larger than the second one (paired \(t\)-test, \(P < 0.005\)). In males with three brooding cycles \((n = 6)\), the first egg mass size was larger than the second (\(t\)-test, \(P < 0.01\)) but there was no difference between the second and third (\(t\)-test, \(P = 0.49\)).

**FACTORS INFLUENCING MALE MATING SUCCESS**

In 1 and 2 year-olds, the CV of the number of brooding cycles was higher than that of the egg mass size, indicating that the former contributed more to the mating success than the latter (Table I). For mating success of 3 year-olds, the egg mass size in one brooding cycle was as important as the number of brooding cycles.

In 1 and 2 year-olds, \(L_T\) and PSA were adopted as explanatory variables against the egg mass size in one brooding cycle but neither was adopted against the number of brooding cycles (Tables II and III). In 3 year-olds, the egg mass size was explained by \(L_T\), PSA, current velocity and days from the beginning of the breeding season to the start of the guarding, the relative importance of \(L_T\) and PSA being greater (Table II). The number of brooding cycles was best modelled by days from the beginning of the breeding season to the start of the first guarding (Table III).

![Graph showing annual mating success of males in relation to total length](image)


**DISCUSSION**

The relative contribution of the egg mass size in one brooding cycle and the number of brooding cycles to the annual mating success differed between males of different ages in *Rhinogobius* sp. CB. Two variables, PSA and male $L_T$, were chief determinants of egg mass size. In many gobiid fishes whose eggs are laid in a single layer like *Rhinogobius* species, male mating success is constrained by the area of spawning substratum, resulting in greater success for males with a larger nest (Lindström, 1988, 1992; Bisazza et al., 1989; Magnhagen & Kvarnemo, 1989; Kvarnemo, 1995). Males of *Rhinogobius* species secure a nest space by

Table I. Mean ± s.d. and coefficient of variation of number of brooding cycles and egg mass size by age

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Variable</th>
<th>Mean ± s.d.</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>Number of brooding cycles</td>
<td>1·3 ± 0·5</td>
<td>41·0</td>
</tr>
<tr>
<td></td>
<td>Egg mass size (cm$^2$)</td>
<td>49·9 ± 20·1</td>
<td>40·2</td>
</tr>
<tr>
<td>1 and 2</td>
<td>Number of brooding cycles</td>
<td>1·4 ± 0·6</td>
<td>46·5</td>
</tr>
<tr>
<td></td>
<td>Egg mass size (cm$^2$)</td>
<td>28·6 ± 7·2</td>
<td>25·3</td>
</tr>
<tr>
<td>Total</td>
<td>Number of brooding cycles</td>
<td>1·3 ± 0·6</td>
<td>43·0</td>
</tr>
<tr>
<td></td>
<td>Egg mass size (cm$^2$)</td>
<td>43·6 ± 19·8</td>
<td>45·4</td>
</tr>
</tbody>
</table>

Table II. Result of stepwise multiple regression analysis by age using the egg mass size per brooding cycle as a dependent variable and five biological and environmental characteristics as independent variables

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Age (years)</th>
<th>Independent variable</th>
<th>Slope regression coefficient</th>
<th>d.f.</th>
<th>$r^2$</th>
<th>$F$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg mass size</td>
<td>3</td>
<td>Male $L_T$ (cm)</td>
<td>0·44</td>
<td>4,100</td>
<td>0·49</td>
<td>23·6</td>
<td>&lt;0·001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PSA (cm$^2$)</td>
<td>0·35</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Current velocity at nesting stone (cm s$^{-1}$)</td>
<td>0·22</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Days to the start of guarding</td>
<td>−0·21</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 and 2</td>
<td>Male $L_T$ (cm)</td>
<td>0·36</td>
<td>2,44</td>
<td>0·20</td>
<td>5·7</td>
<td>&lt;0·01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PSA (cm$^2$)</td>
<td>0·28</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>Male $L_T$ (cm)</td>
<td>0·51</td>
<td>4,147</td>
<td>0·51</td>
<td>38·7</td>
<td>&lt;0·001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PSA (cm$^2$)</td>
<td>0·30</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Current velocity at nesting stone (cm s$^{-1}$)</td>
<td>0·19</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Days to the start of guarding</td>
<td>−0·17</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

PSA, undersurface area of the nesting stone.

excavating sand and gravel under a stone on the riverbed. Therefore, the available spawning area of the nest is difficult to measure. In the present study, the undersurface area of a nest stone buried in the riverbed was defined as PSA. A low ratio of egg mass size to PSA (median = 15%; Ito & Yanagisawa, 2003) indicates that an actually available spawning area is much smaller than PSA. Nevertheless, a positive correlation of PSA with egg mass size shows that PSA is a good indicator of the available spawning area.

In nests of *Rhinogobius* sp. CB, males often gather two or more mature females before spawning, but the number of eggs actually deposited in one nest is close to the fecundity of one female (Ito & Yanagisawa, 2003), suggesting that eggs in most nests are contributed only by one female. By contrast, in aquarium observations where a large piece of a clay pot was given as a spawning substratum, about half the males received two or more females simultaneously and spawned with all of them (Ito, 2002). These observations suggest that the low level of polygyny in the field is due to a limited ability of nest construction by males. In the present study, male *L* had a positive correlation with the ratio of egg mass size to PSA but did not with PSA. This may mean that larger males, which have a greater ability for nest construction, can create a wider available spawning space and consequently receive more eggs. Another factor causing a positive association between male *L* and egg mass is size-assortative mating of this fish, where large males mate with large females having greater fecundity (Ito & Yanagisawa, 2003).

In 3 year-olds, days from the beginning of the breeding season to the start of the guarding and current velocity at the nest site were also adopted as determinants of the egg mass size. Since female fecundity decreased with the progress of the breeding season, the variation of egg mass size may have reflected the seasonal change of female fecundity. *Rhinogobius* sp. CB males construct their nests in riffles. Fast water currents at the nests may have a positive effect on the egg mass size in two ways: first, water currents may facilitate the construction of large nests because the currents wash away fine particle such as silt, and second, fast water currents may decrease egg death during the guarding stage due to a continuous supply of oxygen (Ito & Yanagisawa, 2000; Vinyoles *et al*., 2002). A recent study showed that *Rhinogobius* sp. DA (dark colour type) females use male courtship in fast water currents as an indicator of parental quality.

### Table III. Result of stepwise multiple regression analysis by age using the number of brooding cycles as a dependent variable and seven biological and environment characteristics as independent variables

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Age (years)</th>
<th>Independent variable</th>
<th>Slope regression coefficient</th>
<th>Model</th>
<th>d.f.</th>
<th>$r^2$</th>
<th>$F$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of brooding cycles</td>
<td>3</td>
<td>Days to the start of the first guarding (None was adopted)</td>
<td>-0.28</td>
<td>1,80</td>
<td>0.08</td>
<td>6.9</td>
<td>&lt;0.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 and 2</td>
<td>Total (None was adopted)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

It would be of interest to examine if *Rhinogobius* sp. CB females also utilize water currents as an indicator of male quality in the process of mate choice.

The number of brooding cycles 3 year-olds had in a breeding season was affected by the timing of the first spawning. In gobiids, the physical condition of guarding males greatly declines, since guarding males usually fast within the nest (Magnhagen, 1986; Torricelli et al., 1985; Lindström & Hellström, 1993; Takahashi & Yanagisawa, 1999). A quick recovery of the male physical condition after guarding is a key factor for repeated spawnings. Naturally, males which start the first spawning earlier will have more opportunity of recovering their physical condition after guarding and consequently having another brooding cycle within the season. Breeding activities of stream gobies are greatly influenced by high flow events that may overturn stones of the riverbed. The frequency of such high flow events varies annually: at the study river, high flow events rarely occurred in 1997 but several times in 1998, the year of the present study (per. obs.). In both years, however, the maximum breeding cycles *Rhinogobius* sp. CB males had in a season was three (unpubl. data). This fact suggests that breeding cycles males can have in a season are severely limited by the deterioration in their physical condition caused by their guarding activities.

The relative importance of the egg mass size in one brooding cycle and the number of brooding cycles against mating success in a season varied according to age. In 1 and 2 year-old males, the number of brooding cycles contributed more to the mating success than the egg mass size. This is attributable to their limited ability of nest construction, which will restrict an opportunity of polygynous mating and will result in little variation of their egg mass size (Fig. 3). For 1 and 2 year-old males, those who succeed in having multiple brooding cycles can ensure a great mating success. For mating success of 3 year-old males, the egg mass size in one brooding cycle was as important as the number of brooding cycles. This is probably because those who have large nests can enhance their mating success by spawning with a large female or multiple females in each brooding cycle. The present study strongly suggests that the variation of male mating success is chiefly caused by the age-related ability of nest construction and mate acquisition.

We thanks K. Iguchi, National Research Institute of Fisheries Science, K. Omori, M. Inoue and N. Okuda for helpful advice and discussion, and K. Matsumoto and other members of the Kamogawa Fishermen’s Union for facilities and assistance during the fish collection.

References


