MATE AVAILABILITY AND SOMATIC CONDITION AFFECT FILIAL CANNIBALISM IN A PATERNAL BROODING GOBY

by

NOBORU OKUDA[1], **SAYAKA ITO**[2] **and HAJIME IWAO**[2,3]
(Center for Marine Environmental Studies, Ehime University)

(Acc. 25-XI-2003)

Summary

We conducted a laboratory experiment to test two major predictions from a game-theoretical model for the evolution of filial cannibalism in species with paternal care — that parental males are more cannibalistic when their energy reserve is low and that filial cannibalism increases when mate availability is high. We used a freshwater goby, *Rhinogobius* sp. OR, in which males care for eggs from multiple females in a nest. For each breeding male, we manipulated food ration, sex ratio and nest space: the former to control its energy reserve and the latter two to control its mate availability. The ANOVA showed that all three factors had a significant effect on filial cannibalism, which was facilitated when male somatic condition was poor, the sex ratio was female-biased and the nest space was small. Furthermore, filial cannibalism became more intense with increasing brood size and with decreasing female body size. In conclusion, the parental energy reserve and the quality and availability of mates are important factors affecting filial cannibalism in *Rhinogobius* sp. OR.

---

[1] Correspondence: N. Okuda, Center for Marine Environmental Studies, Ehime University, 2-5 Bunkyo-cho, Matsuyama 790-8577, Japan, e-mail address: nokuda@mserv.sci.ehime-u.ac.jp.

[2] Department of Biology and Earth Sciences, Ehime University.

[3] We are very grateful to M.H.A. Keenleyside, M. Kondoh, A. Marlow and two anonymous referees for comments on this manuscript, to E. Forsgren, M. Kohda, K. Lindström, D. Takahashi and H. Tsujimoto for generously providing us with unpublished data, and to M. Inoue and H. Miyatake for their field assistance. This study was financially supported by Research Fellowships of the Japan Society for the Promotion of Science for Young Scientists.

Behaviour 141, 279-296

Introduction

Filial cannibalism is an interesting phenomenon from an evolutionary perspective because its occurrence is ubiquitous across a variety of taxa (Dominey & Blumer, 1984; Smith & Reay, 1991; FitzGerald & Whoriskey, 1992), in spite of the fact that parents necessarily decrease their current reproductive success through killing and eating their own offspring. Since Rohwer (1978) first gave an adaptive explanation of filial cannibalism, theoretical studies have been developed with increasing empirical evidence (Sargent, 1992; Sargent *et al.*, 1995; Lindström, 2000). Filial cannibalism can be generally viewed in terms of life history trade-offs between present and future reproduction. It could evolve when cannibals compensate present reproductive loss by future fitness benefit from eating the offspring. This hypothesis leads to some major predictions. For instance, parents in poor condition will be more cannibalistic because they can increase their chances of surviving to the next reproduction by improving their somatic condition through offspring consumption. This theoretical prediction accords well with results from field observations (DeMartini, 1987; Marconato *et al.*, 1993; Okuda & Yanagisawa, 1996a; Okuda *et al.*, 1997) and laboratory experiments (Belles-Isles & FitzGerald, 1991; Kvarnemo *et al.*, 1998; Lindström, 1998).

Based on their game-theoretical model for filial cannibalism in species with paternal care, Kondoh & Okuda (2002) proposed that two different mechanisms underly filial cannibalism; one is associated with intrinsic parental status (*i.e.* life history traits) and the other with extrinsic breeding system components. The latter has been hitherto neglected in studying filial cannibalism (Manica, 2002). The breeding system components, such as sex ratio, mating pattern and parental investment by both sexes, determine mate availability (Trivers, 1972; Emlen & Oring, 1977; Clutton-Brock & Parker, 1992; Parker & Simmons, 1996; Reynolds, 1996). When mate availability is high for parental males, they will have opportunities to obtain additional eggs which can be reallocated to future reproduction in the form of reserved energy. Thus mate availability is expected to influence filial cannibalism by males in paternal care species. Kondoh & Okuda's (2002) model predicts that parental males will cannibalize more eggs when the following conditions prevail: parental energy reserve is low; mate search efficiency is high; the population density is high; the sex ratio is female-biased; the male care period is long; and the female refractory period is short. These conditions, except the first, act to increase mate availability for males.

A series of studies by Okuda and his collaborator (Okuda & Yanagisawa, 1996a; Okuda, 1999, 2000) suggested the possibility that mate availability would affect filial cannibalism by male cardinalfishes in nature. However, this was inferred from interspecific differences on the basis of comparative methods, without controlling any factors other than mate availability. Here we report on an experimental study to test some predictions from Kondoh & Okuda's (2002) model, using a freshwater goby, *Rhinogobius* sp. OR (sensu Akihito *et al.*, 2002), in which males care for multiple clutches in a nest.

Model assumption

Prior to empirical testing, it is necessary to modify the assumption underlying Kondoh & Okuda's (2002) model. In its original version, the model assumes that cannibalistic behaviour is genetically determined, without phenotypic plasticity, for each individual. It also assumes that its fitness is expressed by reproductive rate (*i.e.* reproductive gain per breeding cycle). The reproductive rate depends on mate availability, which is an inverse function of the time to search for mates. Cannibalistic males remain sexually receptive but exploited females enter a refractory period to replenish their eggs. Hence, the decision by individual males to perform cannibalism determines mate availability at the population level. This should finally lead to an ESS, *i.e.*, a population consisting of individuals with the same cannibalism intensity under a given condition, not allowing invasion of individuals with different cannibalism intensity. In this paper, by contrast, we consider that each male can adapt its cannibalism intensity in response to local environmental conditions, resulting in a phenotype with optimal reaction norm which corresponds to a trajectory of the former ESS against environmental variables (see Fig. 1 in Kondoh & Okuda, 2002). This assumption seems to be theoretically plausible (Repka & Gross, 1995), as reported for a paternal brooding fish, in which filial cannibalism is regarded as a conditional strategy (Okuda *et al.*, 1997; Takeyama *et al.*, 2002).

In the present study, we manipulated three factors: parental somatic condition, sex ratio and nest space. Reduced nest space results in increased mate availability for males because their reproductive potential, the maximum number of mates received in a breeding cycle, decreases and consequently available mates are in excess. We predict that males will cannibalize more eggs when their somatic condition lowers, the sex ratio is female-biased and the nest capacity is small.

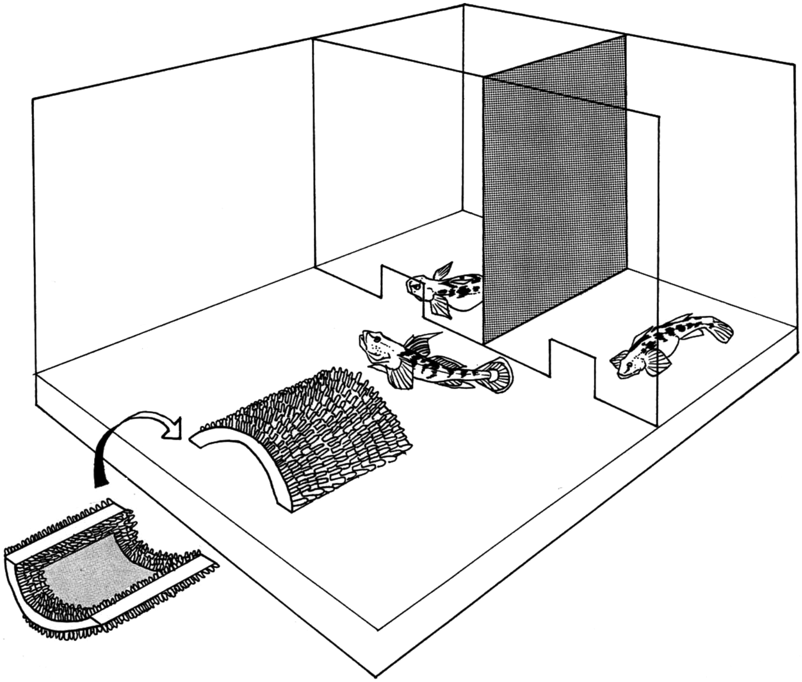

Fig. 1. Profile of an experimental tank as used in trials with sex ratio 1 : 2. A compartment housing a male was equipped with a nest and gravel. The nest is covered with a spinous mat to control oviposition space (gray area). Male (300 × 300 mm) and female (150 × 150 mm) compartments were separated by a transparent PVC wall, so that visual contacts were always allowed between the sexes. This wall had a small gate through which females could enter the male compartment only when the gate is opened. A black PVC wall partitioned the female compartment, so that visual contacts were not allowed between females until the onset of experiment. For behavioral observations, a video camera was set beside the male compartment in a position where the inside of the nest could be viewed.

Methods

Subject species

The subject fish, *Rhinogobius* sp. OR, is a freshwater goby. Since the pioneer research by Mizuno (1960), the *Rhinogobius brunneus* species' group is considered to consist of several undescribed species which are morphologically and genetically distinct (Masuda *et al.*, 1989). *Rhinogobius* sp. OR originally inhabited the lacustrine environment but has recently extended its distribution over many river systems in Japan, due mainly to human introduction from Lake Biwa (Koshikawa, 1989). At present, this goby is commonly found in the study area, Kamo River, Shikoku Island.

The breeding season is from May to July in this study population. Most individuals reach maturity when they are one year old, and continue to breed for several years. Females deposit their all eggs together in a male nest and have iteroparous spawnings in a season. Males also complete multiple breeding cycles in a season and increase their reproductive success as much as fivefold, with increasing body size (Okuda *et al.*, 2002b).

Breeding condition

We collected *Rhinogobius* sp. OR at Kamo River prior to and during the breeding season of 2001, using a dipnet and an electric shocker (Model 12 Backpack Electrofisher, Smith-Root Inc. Vancouver, USA). We chose small adults, mainly one-year-old fish, the predominating cohort in this population. This was done to minimize effects of age or body size on the experimental results, as it has been reported that filial cannibalism is related to age in another fish species (Okuda *et al.*, 1997; Takeyama *et al.*, 2002). In the laboratory, the fish were sexed and assigned separately to stock tanks (see Okuda *et al.*, 2002a for details of captive condition). We fed females with frozen chironomid larvae ad libitum twice a day. Males were assigned at random to two dietary treatments (hereafter, well-fed and poorly-fed males). Well-fed males had the same ration as females, while poorly-fed males were fed once every three days (*i.e.* on one-sixth of the ration of well-fed males). The poorly-fed males were subjected to this treatment for at least half a month prior to the experiment.

Experimental design

We designed a three-factor layout experiment, manipulating the following factors: male somatic condition, sex ratio and nest space. Male somatic condition was controlled by food ration, as previously described. The sex ratio (male : female) was set to 1 : 1 or 1 : 2. We prepared two sets of artificial nests (a 100 mm-gauge × 110 mm-length PVC pipe longitudinally cut into thirds), which did not differ from each other in overall nest size, only in nest space. This manipulation enabled us to eliminate the effects of nest size on female mating preference, as known from some nest brooders, in which females prefer males with a larger nest (Bisazza *et al.*, 1989; Takahashi & Kohda, 2002). To regulate nest space, we covered the entire convex outer surface and some of the concave inner surface with a vinyl spinous mat, on which females cannot deposit their eggs (Fig. 1). The small space nest (hereafter, small nest) had 36.0 cm^2 (6.0×6.0 cm) of concave surface available for oviposition. This space is nearly equal to the average area of a single clutch produced by wild females of similar size to the subject females (Okuda *et al.*, 2002b). For the large space nest (hereafter, large nest), spawning space was 72.3 cm^2 (8.5×8.5 cm), which was about twice that of the small nest. For each nest, a translucent sheet was attached to its oviposition space. Soon after the last spawning, we detached this sheet with eggs from the nest to photograph it using a digital camera. From the digital image of attached egg batches, we counted the number of eggs (Okuda *et al.*, 2002b).

With this experimental design, we expect that males mating with two females in a small nest cannot receive all their eggs, while males mating with one female in a large nest can afford to receive additional eggs. Mate availability is therefore high in the former situation and low in the latter. When a male mates with one female in a small nest or with two females in a large nest, the nest capacity matches the egg supply. Mate availability is moderate in this situation.

Experimental procedure

We conducted experiments from 31 May to 17 July, 2001. In the evening before each experiment, subject males selected randomly from stock tanks were introduced into an experimental tank (300 mm W × 450 mm L × 300 mm H; Fig. 1). At the same time, mature females were introduced at random into one (sex ratio 1 : 1) or both (sex ratio 1 : 2) female compartments. Tanks were kept in darkness until the observations started next morning. The experiment consisted of two stages (1st and 2nd), starting from dawn (at 0400-0500 hours). In the 1st stage, experimental males were allowed to mate with a first female for 6 h: this duration was long enough for females to complete spawning (Okuda *et al.*, 2002a). At the end of this stage, we checked for the presence of eggs in the nest. If the female did not spawn, the trial was considered a failure and discarded. If the female spawned, she was removed from the experimental tank. Subsequently, the focal male was allowed to mate with a second female for another 6h (2nd stage) in the case where the sex ratio is 1 : 2. Where the sex ratio is 1 : 1, a focal male was left with his eggs in the experimental tank for 6h after removal of his first mate. At the end of the 2nd stage, the male and his second mate if any, were removed from the tank.

After measuring total length (TL) of experimental fish to the nearest 0.01 mm, we carried out dissections to separate liver, gonad and eviscerated body. For males, eggs were sorted out from their stomach and gut contents. We dried and weighed these organs and eggs to the nearest 0.1 mg. For each fish, we calculated the hepatosomatic index (HSI $= 10^2 \times$ liver weight / total body weight) and the condition factor ($K = 10^3 \times$ total body weight (mg) / TL (mm)) as indicators of somatic condition, and gonadosomatic index (GSI $= 10^2 \times$ gonad weight / total body weight) as an indicator of gamete depletion. As an indicator of cannibalism intensity, we used the dry weight of eggs eaten by males rather than their number because eggs had been often semi-digested and thus could not be counted accurately.

To approximately estimate the number of eggs eaten by the males, we examined the assimilation rate for eggs. We fed a male on a known number of eggs and weighed the dry mass of these eggs in his stomach and gut after 10 h, nearly equal to the time from acquisition of eggs in the 1st stage to the male removal in the 2nd stage. We defined the assimilation rate as the weight ratio of digested to non-digested eggs of the same number: the latter was calculated by a regression of the dry weight against the number of eggs (3.245×10^{-2} mg per egg). With 13.3% an estimated value of the assimilation rate, the number of eaten eggs (y) was calculated from the equation, $y = (x/0.133)/3.245 \times 10^{-2}$; x: the dry egg weight in the stomach and gut.

In the treatment with sex ratio 1 : 2, we were unable to ascertain on the basis of final brood characteristics whether the second females actually spawned, because males could have cannibalized a large proportion of the brood. Thus, we judged the occurrence of second spawning from the GSI of females. They were considered to be non-spawners when their GSI was more than 20% (Okuda *et al.*, 2002b). The number of cases in which the second females did not spawn were two for poorly-fed males with a large nest, seven for poorly-fed males with a small nest, eight for well-fed males with a large nest and one for well-fed males with a small nest. The second spawning success was independent of nest space, male somatic condition and male body size (Okuda *et al.*, 2002b). Besides the cases (that were not counted) where the second female did not spawn, 12 replications were carried out for each experimental group of a given combination of treatments. In three cases where males were found to have a small number of eggs at the end of the 1st stage, their first females had nearly full, ripe ovaries (GSI = 22.2, 23.0 and 25.4%). We considered that these males failed to receive a large number of eggs from their mate. We excluded these three cases from the analysis because such males might perform filial cannibalism in a different context; *i.e.* males may cannibalize an entire brood when its size is small (Sargent, 1992; Hoelzer, 1995). In one of the successful cases, we missed data on brood characteristics due to failure to attach a translucent sheet to the oviposition space.

The females did not interfere with each other in a bigamous situation since the two matings were sequential. In addition, the presence of a dominant female did not suppress spawning by a subordinate female as they were separated by a transparent wall (Okuda et al., 2002b).

Marking eggs

In 41 bigamous matings we used a marking technique for live eggs to see whose eggs were eaten by parental males. We abdominally injected either of the two females with 0.05 ml of brilliant blue FCF or β-carotene salinity solution: the former stained eggs greenish blue and the latter orangish yellow (see Okuda *et al.*, 2002a for details of marking technique). In 26 cases in which marked eggs could be identified by eye, we separately dried and weighed marked and unmarked eggs eaten by males. This treatment was assigned randomly to either case in which eggs were marked for the first ($N = 14$) or second ($N = 12$) females (χ^2 test for goodness of fit: $\chi^2 = 0.15$, $p = 0.69$).

The marking reagents had no harmful effects on female fecundity and egg mortality (Okuda *et al.*, 2002a).

Behavioural observation

In each of 34 replicates (four monogamous and 30 bigamous), we video-recorded male behaviours for 12 h, *i.e.* from the beginning of the experiment to its end. The behaviours were categorized as follows: courtship — courtship displays toward a mate, such as tail flip, mount and leading to the nest; extrapair courtship — courtship displays toward a second female through the transparent wall while mating with a first female; fanning — aerating eggs by fanning fins in an upside-down position; and biting — orally scraping eggs off the ceiling of the nest in a vertical position. We counted the number of observed behaviours for these four categories every 30 min. For each of two stages, 12 sub-samples from the 30 min observations were averaged. We regarded the frequency of courtship and extrapair courtship as an index of male mating effort, fanning frequency as parental effort, and biting frequency as cannibalism intensity.

In 17 cases, nesting males concealed their nest opening with sand in the course of video recording and thereafter we had difficulty in observing male behaviours inside the nest. In such cases, we averaged the 30 min observations which were made before the nest concealment by males. In three cases, the nest concealment occurred within 30 min of video recording; these records were excluded from the analysis of male behaviours inside the nest.

Ethical note

Permission to collect *Rhinogobius* sp. OR with an electric shocker was obtained from Ehime Prefecture (Inland Waters No. 4). The electric shocker was aimed at nesting males that were prone to hide themselves under a stone and thus were hard to catch using only a dipnet. Because of low water conductivity and low voltage (300 V), the electric shocker could pinpoint these males without harming other fish species and they recovered from the electric shock within a few minutes.

Food restriction entailed by the dietary treatment did not produce any ill effects on poorly-fed males (mortality rate $= 3.95\%$), compared to well-fed males (4.76%; Fisher's exact test, $p > 0.99$). Prior to dissecting fish, we euthanized them in 1% FA100 anaesthetic solution. All individuals that were not used for this experiment were also killed with 1% FA100 anaesthetic without releasing them at Kamo River because they are an alien species from Lake Biwa.

Data analysis

For data analysis, we used parametric tests when data fitted a normal distribution; otherwise we used non-parametric tests. The dry weight of eggs eaten by males was log-transformed to fit a normal distribution. We performed a three-factor ANOVA to examine the factors affecting cannibalism intensity, incorporating male somatic condition, sex ratio and nest space as independent variables. Moreover, we incorporated male size (TL mm), average female size (TL mm) and initial brood size as covariates, some of which might account for variance in cannibalism intensity within a treatment. We regarded the initial brood size as the number of eggs in the nest plus the estimated number of eggs eaten by males.

All behavioural data were subjected to non-parametric tests. Behavioural frequency is presented as the counts of each behaviour per 30 min. For descriptive purposes, means ± SE are given if parametric tests were performed and, if not, medians with 25% and 75% percentiles are given. All p values are two tailed.

Results

Somatic condition

Among experimental groups, body size of males ($\bar{x} \pm \text{SE} = 57.4 \pm 0.5$ mm) was not significantly different (ANOVA: $F_{7,85} = 0.62$, $p = 0.74$), and neither was that of females ($\bar{x} \pm \text{SE} = 51.8 \pm 0.4$ mm, $F_{7,85} = 1.72$, $p = 0.11$). The dietary treatment was successful in differentiating somatic condition between well-fed and poorly-fed males (three-way ANOVA: HSI: condition, $F_{1,85} = 144.81$, $p < 0.001$, nest, $F_{1,85} = 0.28$, $p = 0.60$, sex ratio, $F_{1,85} = 0.01$, $p = 0.94$; K: condition, $F_{1,85} = 102.39$, $p < 0.001$, nest, $F_{1,85} = 0.04$, $p = 0.85$, sex ratio, $F_{1,85} = 1.55$, $p = 0.22$; Fig. 2). The somatic condition of poorly-fed males was as low as that of wild males late in the breeding season (Ito S, unpublished data).

Courtship and parental behaviours

All males started to vigorously court a first female as soon as the lights were switched on in the experimental tanks to be videotaped. Following

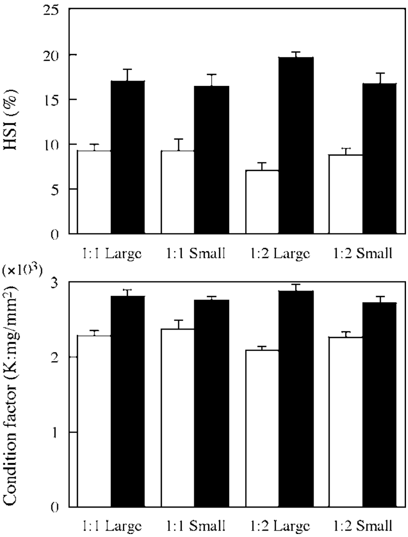

Fig. 2. The hepatosomatic index (HSI) and condition factor (K) of males in each experimental group. Open and closed bars are poorly-fed and well-fed males, respectively. The sex ratio is 1 : 1 or 1 : 2 and nest space large or small. Sample size is 12 for each group, except for three groups (monogamous poorly-fed males with a large nest, monogamous poorly-fed males with a small nest and bigamous well-fed males with a large nest), each of which misses one sample (see methods). Vertical lines are SE.

the courting males, the first female usually entered the nest within 9 min (median, 25% = 3 min, 75% = 15 min, $N = 34$) after the onset of the experiment, subsequently displaying spawning actions, defined as rubbing her abdomen against the ceiling of the nest. During the 1st stage, courtship frequency was significantly lower when the sex ratio was 1 : 2 (median = 0.6 per 30 min, 25% = 0.3, 75% = 2.5, $N = 27$) than 1 : 1 (median = 7.9, 25% = 3.5, 75% = 11.8, $N = 4$; Mann-Whitney U test: $z = -2.18$, $p < 0.03$). This was partly because bigamous males invested much time in extrapair courtship toward a second female (median = 2.0 per 30 min, 25% = 0.0, 75% = 7.3, $N = 27$).

In the treatment with sex ratio 1 : 2, second females entered the nest 11 min (median, 25% = 4 min, 75% = 16 min, $N = 28$) after the onset of the 2nd stage. In four of six cases in which the second mating resulted in a failure, the second females took more time to enter the nest (successful: median = 9 min, 25% = 3 min, 75% = 13 min, $N = 24$, unsuccessful: median = 18 min, 25% = 16 min, 75% = 19 min, $N = 4$; Mann-Whitney U test: $z = -2.13$,

$p < 0.04$) and in the other two cases, second females did not enter the nest until the end of the experiment though they sometimes passed through the nest to inspect its inside. In the unsuccessful cases where females did enter the nest, they often displayed spawning actions in the nest, like females that actually spawned. The failure to spawn could not be ascribed to rejection by the male because unsuccessful males displayed courtship more frequently (median = 1.5 per 30 min, 25% = 1.4, 75% = 2.3, $N = 5$) than successful males (median = 0.3, 25% = 0.0, 75% = 0.8, $N = 22$; Mann-Whitney U test: $z = -2.40$, $p < 0.02$).

Males usually displayed fanning without eggs before their first mate entered the nest, suggesting that they used this behavior to attract mates as well as to aerate eggs. Males that obtained a second clutch showed a higher fanning frequency in the 2nd stage (median = 7.1 per 30 min, 25% = 5.3, 75% = 9.7) than in the 1st stage (median = 6.0, 25% = 3.9, 75% = 7.9; Wilcoxon signed-ranks test: $z = -2.18$, $N = 22$, $p < 0.03$), while they decreased their courtship frequency in the 2nd stage (median = 0.3, 25% = 0.0, 75% = 0.8) compared to the 1st stage (median = 6.6, 25% = 5.2, 75% = 9.2; Wilcoxon signed-ranks test: $z = -3.19$, $N = 22$, $p < 0.002$).

Cannibalism

The proportion of eaten eggs to the initial brood was estimated at 0.0-94.0% ($\bar{x} \pm \mathrm{SE} = 29.2 \pm 2.3\%$). Cannibalism intensity increased when male somatic condition was poor, the sex ratio was female-biased and the nest space was small (Fig. 3; Table 1). Somatic condition and sex ratio influenced filial cannibalism more strongly than nest space. Males were also more cannibalistic with the increasing number of eggs gained from females (Table 1). Although a positive correlation was expected between female body size and fecundity, the female size per se had a negative effect on filial cannibalism (Table 1).

When males mated bigamously, they showed a tendency to cannibalize more eggs from the first female than the second female (paired t test: $t_{25} = 1.96$, $p = 0.06$). In cases in which behavioural observations were made, the frequency of biting was higher in the 2nd stage (median = 1.7, 25% = 0.5, 75% = 3.2) than in the 1st stage (median = 0.2, 25% = 0.0, 75% = 0.8; Wilcoxon signed-ranks test: $z = -2.12$, $N = 22$, $p < 0.04$). The average frequency of biting positively correlated with the quantity of eggs cannibalized ($r = 0.40$, $F = 5.67$, $N = 31$, $p < 0.03$).

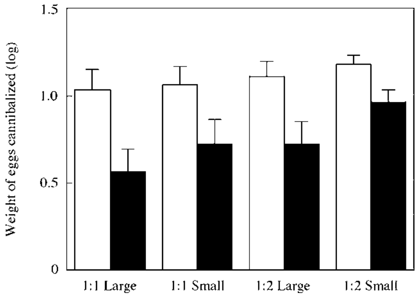

Fig. 3. The dry weight of eggs eaten by poorly-fed (open bars) and well-fed (closed bars) males. Abbreviation for each group and its sample size are the same as in Fig. 2. Vertical lines are SE.

TABLE 1. *The effects of three factors and their interactions on filial cannibalism by males after adjustment for three covariates*

Factor	Mean Square	df	F	p	Power*
Somatic condition	0.94	1	11.55	0.001	0.92
Sex ratio	1.46	1	17.97	0.001	0.99
Nest space	0.38	1	4.68	0.03	0.57
Somatic condition × Sex ratio	0.02	1	0.20	0.66	0.05
Somatic condition × Nest space	0.26	1	3.22	0.08	0.43
Sex ratio × Nest space	0.01	1	0.11	0.74	0.05
Somatic condition × Sex ratio × Nest space	0.01	1	0.12	0.73	0.05

Covariate	β	t	p	Power*
Male size	0.12	1.45	0.15	0.30
Average female size	−0.30	−3.42	0.001	0.92
Initial brood size	0.86	7.21	0.001	1.00

* Power of test at $p = 0.05$.

The quantity of eggs cannibalized was also compared between males that did and did not succeed in mating with a second female, provided that everything else was equal. For well-fed males with a large nest and poorly-fed males with a small nest, there were enough unsuccessful cases for a comparison with successful cases. These unsuccessful males cannibalized as many eggs as successful bigamous males did (Student's t test: well-fed males with a large nest, $t_{18} = 0.31$, $p = 0.76$; poorly-fed males with a small nest, $t_{17} = -1.20$, $p = 0.25$), showing a similar biting frequency to that of the latter males (Mann-Whitney U test: 1st, $z = -0.78$, $N_1 = 5$, $N_2 = 22$, $p = 0.44$; 2nd, $z = -0.91$, $N_1 = 5$, $N_2 = 22$, $p = 0.37$).

Although we expected that bigamous males with a small nest would not be able to receive all eggs from both females, in all but one case, second females deposited all their eggs in the nest. As a result, such a mixed brood showed an egg density twice as high as that from a single female in a small nest (see Okuda *et al.*, 2002b).

Discussion

Our experiment showed that male somatic condition, sex ratio and nest space had significant effects on filial cannibalism by male *Rhinogobius* sp. OR, supporting theoretical predictions by Kondoh & Okuda (2002). Among these factors, the relative importance of somatic condition was high (Table 1). This is often the case in paternal brooding fishes, in which parental males are more cannibalistic when their somatic condition lowers (DeMartini, 1987; Belles-Isles & FitzGerald, 1991; Marconato *et al.*, 1993; Kvarnemo *et al.,* 1998; Lindström, 1998). In *Rhinogobius* males, somatic condition deteriorates due to restricted feeding opportunities during the parental phase (Takahashi & Yanagisawa, 1999; Ito, 2002). Although *Rhinogobius* males are perennial, low energy reserve may decrease the probability of surviving a severe winter in mountain streams. If one-year-old males, which were used for the present study, survive to the following breeding season, it is expected that they will enjoy disproportionately high mating success because mating success augments as much as fivefold with age and thus body size (Okuda *et al.*, 2002b). For young males, therefore, somatic condition will be one of the most critical factors determining whether to cannibalize eggs or not.

As well as the somatic condition, the sex ratio accounted for a great part of the variation in cannibalism intensity between experimental treatments (Table 1). This study is the first to experimentally demonstrate that high mate availability facilitates filial cannibalism in paternal brooders. When given a chance to mate bigamously, male *Rhinogobius* sp. OR showed a nonrandom cannibalistic pattern: more eggs from the first female were cannibalized than from the second female. The situation is similar to that of the mouthbrooding cardinalfish, *Apogon doederleini*, in which bigamous males have been found to cannibalize an entire egg mass from a first mate to mouthbrood another egg mass from a second mate (Okuda & Yanagisawa, 1996b). In *A. doederleini*, the selective cannibalism is associated with a spatial constraint where males can mouthbrood only one egg mass at a time. We supposed that such a spatial constraint would be simulated by our experimental condition in which two females were available to a male with a small nest. Contrary to expectations, the bigamous male could, however, receive eggs from both females. Thus, the reason why male *Rhinogobius* sp. OR cannibalized more eggs from the first female than the second is not merely due to spatial limitation.

One may expect that bigamous males adjusted their brood size through filial cannibalism to improve hatching success. Okuda *et al.* (2002b) reported that for *Rhinogobius* sp. OR nest crowdedness increased egg mortality in the absence of parental males. In nature, however, parental males are able to care for very dense broods from more than two females and to keep the egg mortality low (Okuda *et al.*, 2002b). Our behavioral analysis showed that bigamous males invested more in fanning eggs during their second than their first mating. They may increase parental effort with increasing brood size to meet the embryonic demand for parental resources such as aeration, as reported for some nest brooders (Coleman *et al.*, 1985; Sargent, 1988). It is possible that bigamous males cannibalized eggs to compensate for the energy cost of this increased fanning rather than to thin out their brood in the nest.

In *Rhinogobius* sp. OR, the bigamous situation altered male mating behaviours. During the first mating, the bigamous males devoted time to extrapair courtship towards a second female at the cost of current mating effort towards a first female. Such males would be able to assess the possibility of mating with the second female during their first mating. They more often took pieces of their brood during the second than during the first mating, though they cannibalized more eggs from the first female. For *Rhinogobius* sp. OR, the optimal timing of filial cannibalism may lie between the first female leaving the nest and the second female depositing eggs. If they perform cannibalism in this period, they can prevent the first female from discerning this behaviour and prompt the second female to spawn at a low risk of cannibalism for her eggs. In our experiment, even when the bigamous males did not receive eggs from the second female, they cannibalized as many eggs as successful bigamous males. Since rejecting females usually acted as spawning females did in the nest, the unsuccessful bigamous males might have committed cannibalism in the expectation of getting the second female.

Bjelvenmark & Forsgren (2003) and Pampoulie *et al.* (in press) conducted sex ratio manipulation with gobies, *Gobiusculus flavescens* and *Pomatoschistus minutus*, respectively, in order to examine the effect of mate availability on filial cannibalism. Although their breeding ecology is similar to that of *Rhinogobius* sp. OR, the experimental results were negative: males that were provided with additional mature females were not more cannibalistic than monogamous males (Bjelvenmark & Forsgren, 2003; Pampoulie *et al.*, in press). Under these experimental conditions, focal males were not allowed to contact their potential mates physically, only visually. In the strict sense, mate availability is defined as the possibility of mate acquisition but not as the presence and number of potential mates. For instance, in the mouthbrooding cardinalfish *A. doederleini*, it has been suggested that filial cannibalism is affected by mate availability, but yet it was found to be independent of the number of females with which males made premarital contact (Okuda & Yanagisawa, 1996b). In cases of *G. flavescens* and *P. minutus*, the visual stimuli of potential mates might not be enough to induce filial cannibalism.

The nest space also influenced filial cannibalism significantly but not as strongly as the sex ratio did (Table 1). There is one possible reason for this. Experimental males could afford to care for two clutches even in a small nest. This is because the second female used the minute interspaces among eggs preexisting in the small nest to deposit their eggs, resulting in a mixed brood twice as dense as that from a single female (Okuda *et al.*, 2002b). This result means that the nest space reduction was not so effective in producing a surplus of available mates. The logic will also be applicable to the sex ratio manipulation that has the same effect on mate availability as the nest space manipulation. There is a critical difference, however, between these two factors in relation to their impact on male fitness. In our experiment in which the mating system is controlled, male reproductive success was determined by the sex ratio but not by nest space (Okuda *et al.*, 2002b). In other words, the nest space only represents the reproductive potential of subject males. As assumed by Kondoh & Okuda's (2002) model, the optimal decision by parental males should be based on maximization of their reproductive rate (*i.e.* reproductive gain per unit time). This may be the reason why the sex ratio was more influential in causing filial cannibalism, at least under our experimental conditions.

Our experiment furthermore revealed that filial cannibalism became more intense with increasing brood size and with decreasing female body size (Table 1). The former result accords with the theoretical prediction from Sargent's (1992) model; *i.e.*, parental males will increase the proportion of eggs to be cannibalized as the brood size increases. Sargent (1992) and Hoelzer (1995) also predict that parental males will cannibalize their brood entirely when the brood size is reduced below the threshold where its reproductive return will outweigh the cost of parental care. This prediction has been supported by many empirical studies on paternal brooding fishes (Sargent, 1988; Petersen & Marchetti, 1989; Petersen, 1990; Forsgren *et al.*, 1996; Lindström & Sargent, 1997; Bjelvenmark & Forsgren, 2003; Pampoulie *et al.* in press). Okuda & Yanagisawa (1996b) also reported that for the mouthbrooding cardinalfish, *A. doederleini*, entire brood cannibalism frequently occurred when males mated with small females expected to produce a small number of eggs. In our study species, by contrast, there was no entire brood cannibalism even if males mated with small females. This is ascribable to the short experimental duration: a brood was exposed to its parental male for only half a day. Since some males ate most of the eggs during this time, entire brood cannibalism, rather than partial brood cannibalism, might take place during the four-day brooding period.

In conclusion, our experimental results with a nest brooding goby, *Rhinogobius* sp. OR, supported several predictions from theoretical models by Okuda & Yanagisawa (1996b) and others (Sargent, 1992; Hoelzer, 1995) to explain the evolution of filial cannibalism in fishes with paternal care. Supported by considerable empirical evidence from a variety of taxa, the present study suggests the possibility that mechanisms underlying filial cannibalism are common and ubiquitous among paternal fish.

References

Akihito, Sakamoto, K., Ikeda, Y. & Sugiyama, K. (2002). Gobioidei. — In: Fishes of Japan with pictorial keys to the spcies, English edition (T. Nakabo, ed.). Tokai University Press, Tokyo, p. 1139-1310.

Belles-Isles, J.C. & FitzGerald, G.J. (1991). Filial cannibalism in sticklebacks: a reproductive management strategy? — Ethol. Ecol. Evol. 3, p. 49-62.

Bisazza, A., Marconato, A. & Marin, G. (1989). Male competition and female choice in *Padogobius martensi* (Pisces, Gobiidae). — Anim. Behav. 38, p. 406-413.

Bjelvenmark, J. & Forsgren, E. (2003). Effects of mate attraction and male-male competition on parental care in a goby. — Behaviour 140, p. 55-69.

Clutton-Brock, T.H. & Parker, G.A. (1992). Potential reproductive rates and the operation of sexual selection. — Q. Rev. Biol. 67, p. 437-456.

Coleman, R.M., Gross, M.R. & Sargent, R.C. (1985). Parental investment decision rules: a test in bluegill sunfish. — Behav. Ecol. Sociobiol. 18, p. 59-66.

DeMartini, E.E. (1987). Paternal defence, cannibalism and polygamy: factors influencing the reproductive success of painted greenling (Pisces, Hexagrammidae). — Anim. Behav. 35, p. 1145-1158.

Dominey, W.J. & Blumer, L.S. (1984). Cannibalism of early life stages in fishes. — In: Infanticide: Comparative and evolutionary perspectives (G. Hausfater & S.B. Hrdy, eds). Aldine Publishing Company, New York, p. 43-64.

Emlen, S.T. & Oring, L.W. (1977). Ecology, sexual selection, and the evolution of mating systems. — Science 197, p. 215-223.

FitzGerald, G.J. & Whoriskey, F.G. (1992). Empirical studies of cannibalism in fish. — In: Cannibalism: Ecology and evolution among diverse taxa (M.A. Elgar & B.J. Crespi, eds). Oxford University Press, Oxford, p. 238-255.

Forsgren, E., Karlsson, A. & Kvarnemo, C. (1996). Female sand gobies gain direct benefits by choosing males with eggs in their nest. — Behav. Ecol. Sociobiol. 39, p. 91-96.

Hoelzer, G.A. (1995). Filial cannibalism and male parental care in damselfishes. — Bull. Mar. Sci. 57, p. 663-671.

Ito, S. (2002). Reproductive tactics and mating system in stream gobies of the genus *Rhinogobius*. — Doctoral thesis, Ehime University, pp. 96. (in Japanese)

Kondoh, M. & Okuda, N. (2002). Mate availability influences filial cannibalism in fish with paternal care. — Anim. Behav. 63, p. 227-233.

Koshikawa, T. (1989). *Rhinogobius* sp. OR. — In: Freshwater fishes of Japan (H. Kawanabe & N. Mizuno, eds). Yama-to-Keikoku-sha, Tokyo, p. 594-597. (in Japanese)

Kvarnemo, C., Svensson, O. & Forsgren, E. (1998). Parental behaviour in relation to food availability in the common goby. — Anim. Behav. 56, p. 1285-1290.

Lindström, K. (1998). Effects of costs and benefits of brood care on filial cannibalism in the sand goby. — Behav. Ecol. Sociobiol. 42, p. 101-106.

— — (2000). The evolution of filial cannibalism and female mate choice strategies as resolutions to sexual conflict in fishes. — Evolution 54, p. 617-627.

— — & Sargent, R.C. (1997). Food access, brood size and filial cannibalism in the fantail darter, *Etheostoma flabellare*. — Behav. Ecol. Sociobiol. 40, p. 107-110.

Manica, A. (2002). Filial cannibalism in teleost fish. — Biol. Rev. 77, p. 261-277.

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, p. 229-237.

Masuda, Y., Ozawa, T. & Enami, S. (1989). Genetic differentiation among eight color types of the freshwater goby, *Rhinogobius brunneus*, from Western Japan. — Japan J. Ichthyol. 36, p. 30-41.

Mizuno, N. (1960). Study on a freshwater goby, *Rhinogobius similis* Gill, with a proposition on the relationships between land-locking and speciation of some freshwater gobies in Japan. — Mem. Coll. Sci. Univ. Kyoto Ser. B. 27, p. 97-115.

Okuda, N. (1999). Female mating strategy and male brood cannibalism in a sand-dwelling cardinalfish. — Anim. Behav. 58, p. 273-279.

— — (2000). Interspecific differences in male cannibalistic behaviour between two sympatric cardinalfishes (Pisces: Apogonidae). — J. Ethol. 18, p. 5-10.

— —, Ito, S. & Iwao, H. (2002a). A marking technique for live fish eggs and larvae. — Ichthyol. Res. 49, p. 367-370.

— —, — — & — — (2002b). Female spawning strategy in *Rhinogobius* sp. OR: how do females deposit their eggs in the nest? — Ichthyol. Res. 49, p. 371-379.

— —, Takeyama, T. & Yanagisawa, Y. (1997). Age-specific filial cannibalism in a paternal mouthbrooding fish. — Behav. Ecol. Sociobiol. 41, p. 363-369.

— — & Yanagisawa, Y. (1996a). Filial cannibalism by mouthbrooding males of the cardinal fish, *Apogon doederleini*, in relation to their physical condition. — Environ. Biol. Fish. 45, p. 397-404.

— — & — — (1996b). Filial cannibalism in a paternal mouthbrooding fish in relation to mate availability. — Anim. Behav. 52, p. 307-314.

Pampoulie, C., Lindström, K. & St. Mary, C.M. (in press). Have your cake and eat it too: male sand gobies show more parental care in the presence of female partners. — Behav. Ecol.

Parker, G.A. & Simmons, L.W. (1996). Parental investment and the control of sexual selection: predicting the direction of sexual competition. — Proc. R. Soc. Lond. B 263, p. 315-321.

Petersen, C.W. (1990). The occurrence and dynamics of clutch loss and filial cannibalism in two Caribbean damselfishes. — J. Exp. Mar. Biol. Ecol. 135, p. 117-134.

— — & Marchetti, K. (1989). Filial cannibalism in the Cortez damselfish, *Stegastes rectifraenum*. — Evolution 43, p. 158-168.

Repka, J. & Gross, M.R. (1995). The evolutionarily stable strategy under individual condition and tactic frequency. — J. theor. Biol. 176, p. 27-31.

Reynolds, J.D. (1996). Animal breeding systems. — Trends Ecol. Evol. 11, p. 68-72.

Rohwer, S. (1978). Parent cannibalism of offspring and egg raiding as a courtship strategy. — Am. Nat. 112, p. 429-440.

Sargent, R.C. (1988). Parental care and egg survival both increase with clutch size in the fathead minnow, *Pimephales promelas*. — Behav. Ecol. Sociobiol. 23, p. 33-37.

— — (1992). Ecology of filial cannibalism in fish: theoretical perspectives. — In: Cannibalism: Ecology and evolution among diverse taxa (M.A. Elgar & B.J. Crespi, eds). Oxford University Press, Oxford, p. 38-62.

— —, Crowley, P.H., Huang, C., Lauer, M., Neergaard, D. & Schmoetzer, L. (1995). A dynamic program for male parental care in fishes: brood cycling and filial cannibalism. — Behaviour 132, p. 1059-1078.

Smith, C. & Reay, P. (1991). Cannibalism in teleost fish. — Rev. Fish Biol. Fish. 1, p. 41-64.

Takahashi, D. & Kohda, M. (2002). Female preference for nest size in the stream goby *Rhinogobius* sp. DA. — Zool. Sci. 19, p. 1241-1244.

— — & Yanagisawa, Y. (1999). Breeding ecology of an amphidromous goby of the genus *Rhinogobius*. — Ichthyol. Res. 46, p. 185-191.

Takeyama, T., Okuda, N. & Yanagisawa, Y. (2002). Seasonal pattern of filial cannibalism by *Apogon doederleini* mouthbrooding males. — J. Fish Biol. 61, p. 633-644.

Trivers, R.L. (1972). Parental investment and sexual selection. — In: Sexual selection and the descent of man (B. Campbell, ed.). Aldine Publishing Company, Chicago, p. 136-179.