

Mate availability facilitates cannibalistic behaviour in a nest brooding fish: effects of timing during the brood cycle

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Summary

Filial cannibalism, eating one's own viable offspring, is accepted as an adaptive response to trade-offs between current and future reproduction. Theoretical models predict that high mate availability may induce more filial cannibalism, but this prediction is rarely tested. To examine this prediction, we performed laboratory experiments using the nest breeding goby *Rhinogobius flumineus*. Subject males were allowed to mate with a gravid female and care for the broods. A separate gravid female housed in a small cage (stimulus-female) was shown to the subject males at one of three different points during the brood cycle: prior to spawning, within 1 day after spawning and 1 week after spawning. Empty cages were shown as a control. Males that were shown the stimulus-female before spawning cannibalised more eggs than control males. In contrast, males that were shown the stimulus-females after spawning cannibalised as few eggs as control males did. Additionally, males that were shown the stimulus-female prior to spawning did not court females more intensively than other males. Thus, we suggest that the presence of an additional mate, rather than energy expenditure associated with courtship directed toward an additional mate, can facilitate males to cannibalise their eggs.

Keywords: Life history trade-off, mate availability, parental care, partial and whole clutch cannibalism, goby.

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1. Introduction

Filial cannibalism, i.e., consumption of one's own vital offspring, is found in various animal taxa (e.g., Thomas & Manica, 2003; Lewis et al., 2010) and is widely distributed throughout fishes (e.g., Rohwer, 1978; Dominey & Blumer, 1984; Sargent, 1992; Smith, 1992; Manica, 2002a, 2004). By consuming some or all of the offspring, parents can maximise their lifetime reproductive success through trade-offs between current offspring and future reproduction (e.g., Rohwer, 1978; Sargent, 1992). If the costs of cannibalism (i.e., reduced number of offspring) will be offset by benefits in the parent's life history traits (e.g., improvement of body condition, survival rates and attractiveness toward mates), parents should cannibalise their offspring. This life history-based hypothesis is strengthened by the fact that some ecological factors, e.g., brood size (Marconato & Bisazza, 1988; Sargent, 1988; Marconato et al., 1993; Lindström & Sargent, 1997), low paternity (Neff, 2003a,b; Green et al., 2008; Mehlis et al., 2010) and poor somatic condition (Takahashi & Kohda, 2001, 2004; Manica, 2002a), will facilitate filial cannibalism (Rohwer, 1978; Sargent, 1992).

Additional mating opportunities may also induce filial cannibalism (Kondoh & Okuda, 2002; Manica, 2002a). Okuda & Yanagisawa (1996a) first reported in the cardinalfish (*Apogon doederleini*) that parental males, which can receive a clutch of eggs from only one mate due to limitations of the buccal cavity, tend to cannibalise the entire clutch within the spawning day when another gravid mate is present. At this point, they immediately obtain another clutch from the mate. Based on these field studies it was proposed that high mate availability facilitates filial cannibalism in mouth-brooding cardinalfishes (Okuda & Yanagisawa, 1996a,b; Okuda et al., 1997).

Filial cannibalism is categorized into two types: a parent consumes the whole clutch (whole-clutch cannibalism) or a part of the clutch (partial-clutch cannibalism) (Rohwer, 1978). Whole clutch cannibalism represents investments in future reproduction, by terminating the current brood care, and is predicted to occur when clutches are small (e.g., Rohwer, 1978; Marconato et al., 1993; Manica, 2002a,b). In contrast, partial clutch cannibalism also functions to increase survival of remaining offspring, and the cannibals will recover their deteriorated body conditions (e.g., Manica, 2002a).

We conducted an experimental study to examine whether mate availability will facilitate filial cannibalism in caring males using the small lizard

goby (*Rhinogobius flumineus*), a nest brooder with exclusive paternal care (Mizuno, 1960; Matsumoto, 1996; Seki et al., 2000). Males caring for a clutch were provided with the stimulus of a reproductively receptive female, but were not allowed to mate. In studies that suggest mate availability will induce filial cannibalism, additional females were likely to appear prior to spawning of the males (e.g., Okuda & Yanagisawa, 1996a,b; Okuda et al., 1997, 2004). However, this effect of mate availability was not found when the stimulus was shown after mating (e.g., Bjelvenmark & Forsgren, 2003). Thus, it will be assumed that the timing of mate availability for paternal males during a brood cycle (i.e., before mating or after mating) will be important for the induction of filial cannibalism. We tested whether mate availability affects filial cannibalism by providing a gravid female (i.e., stimulus-female) to the males at one of three different points during the brood cycle: before spawning, within 1 day after spawning and 1 week after spawning (i.e., 1 week before hatching of young). We compared these data with that of control males that were not shown a stimulus-female. We predicted that the pre-spawning stimulus of mate availability would be effective in facilitating filial cannibalism by males of *R. flumineus*, and that post-spawning stimuli would not have an effect. However, if males that are provided a stimulus-female prior to spawning spend more energy in courtship activities, this additional energy expenditure may induce males to cannibalise eggs more often (e.g., Hoelzer, 1992; Manica, 2002a; Neff, 2003b; Pampoulie et al., 2004). Thus, we also examined if the energetic costs of pre-spawning courtship can affect filial cannibalism. We discuss the importance of mate availability and how specific timing during the brood cycle facilitates cannibalism and affects the parental males' behavioural decision about whether to care for or eat their eggs.

2. Materials and methods

2.1. Subject fish

The lizard goby is common in mountain streams of Japan (Mizuno, 1960). It reaches up to 7 cm in total length (TL) and has marked sexual dimorphism (e.g., male's first dorsal fin is longer than females). The breeding season is from June to early August, with two brood cycles (Matsumoto, 1996). Males build a breeding nest under a stone and females lay the eggs on the ceiling

of the nest. The female deposits all of her eggs in one spawning event, and males exclusively take care of the eggs until hatching (ca. 16 days) (Mizuno, 1960; Matsumoto, 1996). During this guarding period, males often close the nest entrance attend to the eggs inside the nests and consume no food (Matsumoto, 1996). Under such starvation conditions, males of *R. flumineus* often cannibalise their own eggs inside the nest (Myint et al., 2011). Unlike other *Rhinogobius* gobies, this fish spawns small numbers (usually ca. 150 eggs) of large eggs (ca. 6 mm in long diameter) in a single layer on the ceiling of nest stones (Mizuno, 1960; Seki et al., 2000). Most males obtain one clutch, but large males (ca. 10% of nesting males) have multiple clutches during one brood cycle (Matsumoto & Nagoshi, 1996). Both sexes mature at 1 year of age and can survive up to 3 years (Hayashi, 1997).

2.2. *Captive conditions*

We collected subject fish using small dip-nets in the Shigo River, Nara Prefecture, Japan, during late May in 2006 and 2007. We transported the captured fish to the laboratory using carrier boxes, which were aerated with small portable pumps. All fish survived the transportation. About 150 fish of the same sex were assigned to 10 different stock tanks (30 × 60 × 45 cm), which were kept at 20°C water temperature and in 14L:10D light regimes, which corresponds to natural conditions. We fed the fish 4.5 g of frozen bloodworms once a day per tank. We did not provide food for 1 day prior to weighing them to evacuate their gut contents.

2.3. *Experimental procedure*

We set up an experimental tank (17 × 30 × 22 cm) with a 2-cm gravel bottom, on which a cement tile (10 × 10 cm) with four legs was placed as the nest (Figure 1). The ceiling of tile nest, the spawning space, was much larger than the average area of an egg clutch (ca. 2–3 cm across). Water temperature and light regimes were the same as in the stock tanks. Standard length (SL to 0.1 mm), TL and body weight (BW to 0.01 g) of all fish used in the experiment were measured before and at the end of the experiment. As an index of fish somatic conditions, we used a condition factor ($CF = 10^6 \times BW \text{ (g)} / SL^3 \text{ (mm)}$) (e.g., Bolger & Connolly, 1989).

Experiments were performed in June (i.e., the first brood cycle in nature) 2006 and 2007. First, we introduced males into the experimental tank where

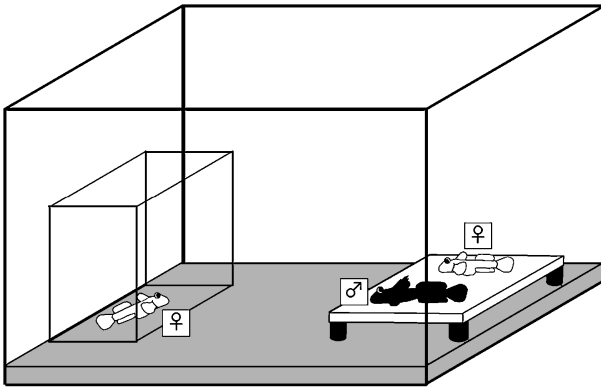


Figure 1. Diagram of the experimental tank ($17 \times 30 \times 22$ cm). A stimulus-female with a large abdomen housed in a small plastic cage was shown to the male. A nest of a 10×10 cm tile was on a 2-cm-thick sand bottom.

they constructed the nest under the tile. After completion of the nest, which usually occurred within 1 day, we added a gravid spawning female into the experimental tank. If spawning did not occur within 6 days, we cancelled the trial. Spawning occurred within 2.93 ± 2.45 days (mean \pm SD, $N = 64$) on average. After spawning occurred, we removed the spawning female from the experimental tank and allowed the males to take care of the eggs for 2 weeks. Fish were not fed during the experiment. We used males of 5.5–6.5 cm TL and females of 5.0–6.0 cm TL (i.e., 1-year-old fish) in all experiments. Fish were not used repeatedly.

To examine whether mate availability facilitates male filial cannibalism, a gravid female (stimulus-female) housed in a small plastic cage ($8.5 \times 8.5 \times 10$ cm) was introduced into the caring male's tank for 3 days (Figure 1). The cage had some slits in it to let freshwater through. To measure courtship frequency, we recorded male courtship behaviours using a video camera (Sony Video Hi8 CCD-TRV80) between 11:00 and 15:00 for the first 3 days (15 min/day) after the introduction of experiment-females and/or stimulus-females. Courtship behaviours consisted of (1) approaching females, (2) lateral display and quiver dancing with fin spreading around females and (3) leading females into nests, against either spawning-female or stimulus-female (refer to illustration of similar behavioural patterns in *R. sp. DA*; Takahashi, 2000). The mean of the total duration (15 min^{-1}) of these three behaviours towards females was used as a measure of male courtship activities. If spawning occurred on the next day after introduction of spawn-

ing females into the tanks, the observation on day one was used. Some of video images could not be used due to poor light conditions. Thus, additional observations on courtship behaviours were conducted with the same experimental procedure in 2008, and were also used in analyses of courtship behaviours.

The timing of presentation of stimulus-female to the male was different according to the experimental procedure. We presented the stimulus-female to a male at one of three different time points during the brood cycle: before spawning (Group A, $N = 14$), within 1 day after spawning (Group B, $N = 15$) and 1 week after spawning (Group C, $N = 15$). The stimulus-females were presented to the males for 3 days. All males displayed courtship behaviours toward the stimulus-female in the cage, indicating that they recognised the stimulus-female as a potential mate. If females spawned inside the nest within the day of introduction, male courtship frequency toward them was not used in the data analysis ($N = 1$ from Group A and 1 from Group B). Empty cages were provided as controls ($N = 20$). The four types of experiments were done in, 2006 and 2007.

Male TL (mm) was not different between the 2 years (2006: 61.43 ± 5.67 , $N = 24$; 2007: 62.78 ± 4.06 , $N = 40$; ANOVA, $F_{1,62} = 1.22$, $p = 0.27$). Similarly, no differences were observed in female body sizes between the 2 years (2006: 54.71 ± 3.28 , $N = 24$; 2007: 55.76 ± 3.51 , $N = 40$; $F_{1,62} = 1.43$, $p = 0.24$). No differences in CF were observed for either sex (males, 2006: 18.07 ± 0.97 , $N = 24$; 2007: 18.39 ± 1.05 , $N = 40$; $F_{1,62} = 1.47$, $p = 0.23$; females, 2006: 21.99 ± 1.41 , $N = 24$; 2007: 22.79 ± 2.71 , $N = 40$; $F_{1,62} = 1.83$, $p = 0.18$). Thus, samples from the 2 years were pooled. Body sizes and CF were not different amongst the three experiment groups and the controls (TL, males: ANOVA, $F_{3,60} = 0.57$, $p = 0.64$; females: $F_{3,60} = 1.59$, $p = 0.20$; CF, males: $F_{3,60} = 1.26$, $p = 0.29$; females: $F_{3,60} = 0.34$, $p = 0.79$), respectively.

We picked up the nests and took pictures of the egg masses twice, one day after spawning and 2 weeks after spawning (i.e., just before hatching), with a digital camera (Canon, IXY Digital 800IS) and put the nest tiles back into the tank. When the eggs were photographed, we gently captured and weighed the caring male. After releasing the males into the tanks, all males continued fanning eggs with similar frequencies as previously did (Myint, unpublished data). Hence this manipulation seems unlikely to cause any behavioural changes in parental care. The digital photo images were used

in counting egg numbers. Similar to previous studies on *Rhinogobius* gobies and other brood-caring fishes, the difference in egg number was regarded as the number of eggs cannibalised by the parental males (e.g., Okuda et al., 2004; Takahashi & Kohda, 2004; Gomagano & Kohda, 2008).

In fish with paternal brood care, filial cannibalism often takes place during spawning (e.g., FitzGerald, 1992; Okuda & Yanagisawa, 1996a,b). Body weight of male *R. flumineus* that were not fed increased just after spawning (Myint et al., unpublished data), indicating that males cannibalise eggs during spawning. Thus, we may underestimate the gross number of spawned eggs (i.e., initial clutch size) even if we count egg numbers just after spawning. To estimate the number of eggs cannibalised by the male during spawning, we calculated the number (y) from the following formula:

$$y = 356.0x + 4.1,$$

where x (g) = the differential of male BW before and just after spawning. This formula was obtained by examining the relationship between change in caring male BW and the reduced number of eggs between after spawning ($R^2 = 0.789$, $p < 0.0001$; $N = 38$; Myint et al., unpublished data). We added this value to the initial clutch size.

When whole clutch cannibalism occurred, all eggs in the nest disappeared and males always appeared outside of the nests. If males performed whole clutch cannibalism, we stopped the experiment and weighed the males. Since whole-clutch and partial-clutch cannibalism are regarded as being functionally different (e.g., Manica, 2002a; Bjelvenmark & Forsgren, 2003; Pam-poulie et al., 2004; Gomagano & Kohda, 2008), we made sure to distinguish the two cases and analysed them separately.

2.4. Ethical notes

No fish were killed in this study. After the experiment, all fish and newly hatching young were released at the collection site of the river. All the experiments were performed at the laboratory of Osaka City University (OCU) in compliance with the guidelines of the Animal Care and Use Committee of OCU and the Japan Ethological Society.

2.5. Statistic analyses

Whether the presence of stimulus-females affected filial cannibalism, we compared the number of cannibalised eggs between the experimental and

control groups using an analysis of covariance (ANCOVA). To assess the factors that might affect the amount of cannibalised eggs in each group (initial clutch size, male CF, male TL), we used a general linear model (GLM univariate test) analysis. Nonparametric statistics were used for data that were not normally distributed. All tests were two-tailed. For descriptive purposes, means and standard deviations (SD) are provided. Analyses were performed with statistical computer software (Statistica, StatSoft).

3. Results

3.1. Whole- and partial clutch cannibalism

Filial cannibalism occurred in all of the 64 trials: 7 males performed whole-clutch cannibalism and 57 males performed partial clutch cannibalism. Initial clutch size in males that performed whole-clutch cannibalism (119.9 ± 24.1 eggs) was significantly smaller than in partial clutch cannibalism (152.1 ± 33.7) (Mann–Whitney U -test: $U = 85.0$, $N_1 = 7$, $N_2 = 57$, $p = 0.013$). In partial-clutch cannibalism, males cannibalised on average 43.6 ± 32.8 eggs, which is equivalent to $28.1 \pm 21.7\%$ (mean \pm SD) of their initial clutch size. Whole clutch cannibalism occurred at the early stages of the brood cycle (on average 2.1 ± 1.5 days after spawning, range = 0–4 days), once in Group A and B and five times in the control treatment.

3.2. Effect of mate availability on filial cannibalism

The GLM revealed that the number of eggs cannibalised by parental males significantly differed among the timing of mate availability and correlated with initial clutch size; however, these values did not correlate with male CF and TL (Table 1).

Comparisons of partial-clutch cannibalism between the three experimental groups and the controls are shown in Figure 2. Males in Group A cannibalised more eggs than control males (Figure 2; ANCOVA: $F_{1,25} = 8.81$, $p = 0.006$), but males in Groups B and C did not cannibalise more than control males (Group B: $F_{1,26} = 0.04$, $p = 0.84$, Group C: $F_{1,27} = 1.4$, $p = 0.25$).

Table 1. Results of GLM analysis on the factors affecting egg numbers partially cannibalised by *Rhinogobius flumineus* males.

Variable	df	<i>F</i>	<i>p</i>
Timing of visual stimulus	3	3.239	0.029
Total length	1	0.683	0.412
Condition factor	1	0.792	0.378
Clutch size	1	4.422	0.040

Intercept: $F = 1.336$, $p = 0.253$, $N = 57$, only partial-clutch cannibalism is used.

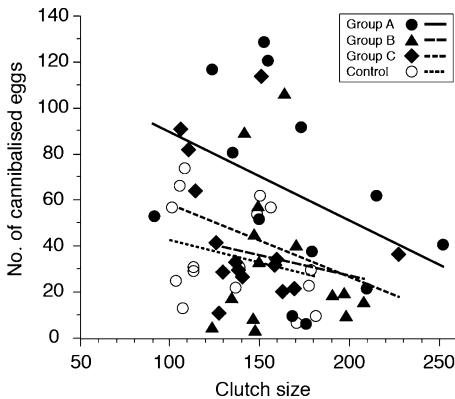


Figure 2. Relationship between the initial clutch size and the number of eggs cannibalised by males of Group A (stimulus-females prior to spawning), Group B (1 day after spawning), Group C (1 week after spawning) and control. Whole-clutch cannibalism is not included. Linear regression lines are shown.

3.3. Courtship activities

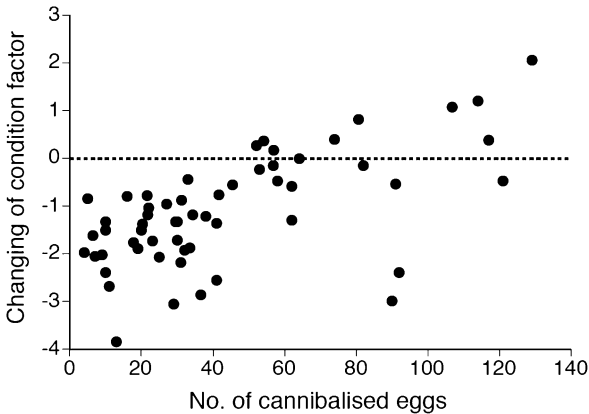
Courtship frequency before spawning was different amongst the four groups (Kruskal–Wallis test: $H_3 = 8.23$, $p = 0.042$, Table 2). Courtship frequency before spawning in Group A (toward both experiment- and stimulus-females) was not different from Group B (Mann–Whitney U -test, $z = -0.54$, $p = 0.59$) and Group C ($U = 67.5$, $p = 0.43$) but lower than the control ($z = -2.58$, $p = 0.01$). Numbers of days from female introduction into the experiment tank until spawning (during which males courted females) were not significantly different amongst the four groups (Kruskal–Wallis test: $H_3 = 2.58$, $p = 0.46$; Group A: 2.92 ± 2.25 , $N = 13$; Group B: 2.07 ± 1.59 , $N = 14$; Group C: 4.20 ± 3.45 , $N = 15$; Control: 2.46 ± 1.64 , $N = 15$). Thus, males of Group A would not court females more than other

Table 2. Duration (min) of courtship display (15 min^{-1}) before and after spawning in Group A, B, C and Control.

Males	Timing of courtship display		N
	Before	After	
Group A	6.76 ± 4.63 (4.88 ± 4.50 and $1.88 \pm 1.80^*$)	–	24
Group B	7.36 ± 4.70	$2.22 \pm 2.35^*$	23
Group C	4.91 ± 4.73	$6.52 \pm 4.10^*$	7
Control	10.09 ± 3.64	–	25

Stimulus-females shown prior to spawning (Group A), 1 day after spawning (Group B) and 1 week after spawning (Group C).

* Courtship display towards stimulus-females.

**Figure 3.** Relationship between the numbers of cannibalised eggs (only partial clutch cannibalism) during care and changes in male body weight before and after parental care.

males. Moreover, males of Group B and C performed courtship display to stimulus-females for 3 days after spawning additionally (Table 2).

3.4. Outcome of cannibalism

The total number of eggs partially cannibalised by parental males positively correlated with CF changes (Figure 3; regression line: $y = 0.021x - 2.075$, $R^2 = 0.39$, $p < 0.0001$, $N = 57$). The CF of males that performed whole clutch cannibalism increased (CF: 2.85 ± 0.36 SD, $N = 7$), whereas CF of males that conducted partial clutch cannibalism decreased when they

finished brood care (CF: -1.11 ± 1.15 ; Mann–Whitney U -test: $U = 0.0$, $p < 0.0001$, $N = 57$). The decrease in CF of Group A males that performed partial clutch cannibalism (CF: -0.50 ± 1.17 , $N = 13$) was not significantly different from that of control males (CF: -1.26 ± 1.14 , $N = 15$), but was significantly smaller when other males that did partial clutch cannibalism were pooled (CF: -1.29 ± 1.09 ; $U = 179.0$, $p = 0.042$, $N = 44$).

4. Discussion

Our data provide evidence that mate availability facilitates parental males of *R. flumineus* to perform filial cannibalism more frequently. That is, males that have seen another potential mate prior to spawning might expect to be able to get more clutches, so they are more prone to cannibalise under the assumption that cannibalism increases their attractiveness. In contrast, the cues of mate availability shown after spawning will not be effective, suggesting that the period sensitive to the cue may exist before spawning. In addition, our study confirms differences in the causes and effects of whole- and partial clutch cannibalism.

4.1. Effect of mate availability on cannibalism

In the present paper, we show that mate availability alone facilitates male filial cannibalism in a brood caring fish if the stimulus is presented prior to mating (Figure 2). The effects of the pre-mating stimulus are consistent with theoretical predictions from the mate availability hypotheses; i.e., cannibals benefit from offsetting the current reproductive loss due to cannibalism by subsequent mating (Kondoh & Okuda, 2002; Manica, 2002a; Okuda et al., 2004). In contrast, these effects were not observed, when the stimulus was presented after spawning. Older clutches were of higher value as males already invested considerable brood care into the eggs, and could be an explanation for lower cannibalism when stimulus-females were shown 7 days after spawning (Group C). However, this explanation cannot be applicable to males that encountered stimulus-females just after spawning (Group B). These results suggest that the timing of mate availability is crucial for male filial cannibalism. Effects of the mate availability are also observed in the cardinalfish (*Apogon doederleini*), a paternal mouth-brooder (Okuda & Yanagisawa, 1996a,b; Takeyama et al., 2007) and probably in a goby of

Rhinogobius sp. DA (Okuda et al., 2004), where potential mates are present before spawning. No effects of stimulus-females on cannibalism were observed in gobies where potential mates were presented after spawning (e.g., Bjelvenmark & Forsgren, 2003; Pampoulie et al., 2004).

When mate availability facilitates filial cannibalism, the possibility should be examined that cannibalism may be indirectly induced to compensate for the higher energy consumption associated with extra courtship (Manica, 2002a; Okuda et al., 2002, 2004; Pampoulie et al., 2004). Males that had two females at the same time (Group A) might invest more energy in courtship efforts as compared to other males. Before spawning, however, courtship frequencies and the number of days during which males courted were not different amongst the four groups. These results suggest that males in Group A did not invest more in courtship than other males. Additionally, males in Groups B and C courted the stimulus-females for 3 days after spawning. Thus, higher energy expenditure associated with courtship does not explain the induced filial cannibalism in males with high mate availability before spawning. Furthermore, males in Group A showed a lower decrease of CF than other males, suggesting that their frequent cannibalism is beyond the necessity of energy costs associated with reproductive activities.

Increases in body condition through cannibalism that were induced by the stimulus of mate availability could possibly be advantageous for males in attracting females (e.g., Hoelzer, 1990, 1992). Females of *R. flumineus* prefer males in good somatic condition (Kohda, unpublished data), which is similar to what is observed in other *Rhinogobious* gobies (Ito & Yanagisawa, 2000; Takahashi & Kohda, 2001, 2004; Okuda et al., 2004) and other fishes (e.g., Knapp & Kovach, 1991; Karino, 1995; Manica, 2002a). Frequent filial cannibalism may improve parents' body condition, which could possibly be advantageous for males in attracting females. Thus, this type of cannibalism might be consistent with the hypothesis that males may trade reproductive investment between mate attraction or male body condition (future investment) and paternal care (current investment). Cannibalism induced by mate availability has been hypothesised to function in creating spawning space for further mating (e.g., Okuda & Yanagisawa, 1996a; Okuda et al., 2004). In our study, however, males had nests large enough for receiving several clutches; thus, space was not a limiting factor.

Filial cannibalism induced by the cue of stimulus-females will also be consistent with theoretical predictions that males should cannibalise their clutch when risk of predation on it is high (e.g., Lindström, 1998; Manica, 2002a; Huang, 2008). If males regard the stimulus-females as potential brood predators, they will cannibalise eggs more frequently. However, males of *R. flumineus* were observed to court stimulus-females frequently but never attack them that were always smaller (subordinate) than males in all cases of experiments, suggesting that males might not regard the stimulus-females as potential predators. In another experiment using females with slender abdomen without nuptial coloration (females having no eggs to spawn) as stimulus-females, males did not court and ignored these stimulus-females, and these stimuli did not facilitate male filial cannibalism at all (Takeyama, pers. observ.). These results suggest that males do not regard stimulus-females housed in the cage as having potential predatory risk to the eggs.

Almost all examples of filial cannibalism induced by mate availability have been observed in a cardinalfish with paternal mouth brooding, where males perform whole clutch cannibalism. Based on these observations it is suggested that if mate availability facilitates filial cannibalism, males will do whole clutch cannibalism (e.g., Bjelvenmark & Forsgren, 2003). However, the present study clearly indicates that the cue of mate availability facilitates partial clutch cannibalism in a nest brooding fish.

4.2. *Partial- and whole clutch cannibalism*

It is often documented that filial cannibalism improves or maintains the body condition of cannibal fishes, but the relationship between the frequency of partial filial cannibalism and the degree of improvement in the cannibal's body condition is rarely documented (e.g., Manica, 2002a; Mehlis et al., 2009). Our results clearly show a positive relationship between the number of eggs cannibalised by males and their body condition (Figure 3). The same result has been found in a stickleback (Mehlis et al., 2009). Cannibalism of a larger number of eggs increased male body weight, but apparently brought about smaller outcomes with respect to reproduction. Therefore, partial clutch cannibalism in most cases cannot fully compensate for energetic losses during parental care, as suggested in other fishes (e.g., Sargent, 1992; Lindström & Sargent, 1997; Manica, 2002a,b; Mehlis et al., 2009). However, body condition of *R. flumineus* males that infrequently cannibalised

eggs largely decreased, which reduced the life history trait advantage (e.g., attractiveness in female choice, competition ability and survival rate) (e.g., Takahashi & Yanagisawa, 1999; Ito & Yanagisawa, 2000; Lindström, 2000; Manica, 2002a; Okuda et al., 2004; Gomagano & Kohda, 2008).

In contrast, when the current brood was terminated by whole clutch cannibalism, the body condition of the cannibal males of *R. flumineus* largely increased. Small sized clutches will produce smaller outcome but require rather constant cost of brood care in fish (e.g., egg fanning, defending against brood predators) (e.g., Sabat, 1994; Lindström, 1998; Bakker et al., 2006). Thus, benefits from small clutches will not be traded off with constant costs of care (e.g., Manica, 2002a,b) and, hence, a small clutch may induce males to cannibalise the entire clutch. Parents with small clutches are predicted to cannibalise the entire clutch during the early days of the brood cycle (Hoelzer, 1992; Manica, 2002a,b). *R. flumineus* males with small clutches cannibalised the entire clutch within the first few days of the long care period. Control males were likely to cannibalise all eggs ($5/20 = 25\%$) more frequently than males of Groups A and B ($2/29 = 7\%$), where stimulus-females were shown before or just after spawning. If this will be the case, presence of potential mates might reduce whole clutch cannibalistic behaviour, presumably because (1) males will expect to obtain additional clutches immediately (Manica, 2002b; Pampoulie et al., 2004), and/or (2) females might reject males that perform whole clutch cannibalism (Lindström & Kangas, 1996; Manica, 2010).

Several studies have suggested that partial clutch cannibalism will increase with brood size (see Manica, 2002a); however, we found that small-sized broods were more prone to be partially cannibalised (Table 1). Most other studies on fish have not found a relationship between brood size and partial cannibalism (e.g., Sargent, 1988; Lindström & Sargent, 1997; Neff, 2003b). Thus, further empirical data are necessary to explain this cannibalistic behaviour.

4.3. Parental care decision-making

Although the proximate cues of mate availability during the pre-spawning stage affect male decision-making on current brood care in *R. flumineus*, cues after spawning do not show such effects. This sensitive period, before the start of brood care, might be associated with the breeding habits of parental

males in this fish. In natural habitats, males of this goby often close the nest entrance during care period (Mizuno, 1960; our experiment males could not close the nest entrance). Closing the nest entrance would be advantageous for defending eggs from brood predators in the wild, but males may lose the chance of further mating. We propose a hypothesis that these males will largely decide to invest in current brood care before the start of parental care, if no additional access to females occurs before spawning. Thus, the proximate cue of additional mates after spawning (i.e., after the decision-making) will not be so effective to induce cannibalistic behaviour. We suggest that specific timing of the presentation of the cues will be crucial in decision making of parental care in the lizard goby *R. flumineus*.

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