Breathing with a mouth full of eggs: respiratory consequences of mouthbrooding in cardinalfish

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Mouthbrooding occurs among several groups of fishes. Although a mouth full of eggs can be expected to pose a considerable respiratory problem, to our knowledge no study has examined respiratory consequences of mouthbrooding in fishes, or how hypoxia or strenuous swimming may affect the success of this reproductive strategy. In two species of cardinalfish (*Apogon fragilis* and *Apogon leptacanthus*), from the reef at Lizard Island (Great Barrier Reef), we found that mouthbrooding significantly reduced the ability to take up oxygen at low ambient oxygen levels. While the direct energetic cost of mouthbrooding appeared insignificant at rest in well-oxygenated water, mouthbrooding significantly reduced the respiratory scope of the fishes and their capacity for sustained aerobic swimming. The males spat out their eggs in hypoxia. Interestingly, the species with the larger brood, *A. fragilis*, spat out the brood at a higher water [O₂] than did *A. leptacanthus*, which had a smaller mean brood mass. Moreover, in contrast to mouthbrooding *A. leptacanthus*, mouthbrooding *A. fragilis* was unable to increase its ventilatory frequency in response to hypoxia. This suggests a trade-off situation between hypoxia tolerance and brood size. Apparently, *A. fragilis* has sacrificed hypoxia tolerance in favour of a large brood size to a greater extent than has *A. leptacanthus*.

Keywords: Apogonidae; coral reef; hypoxia; oxygen; parental care; swimming

1. INTRODUCTION

Mouthbrooding, the bearing of the offspring in the mouth, is known to occur in at least eight families of fishes (Oppenheimer 1970). Mouthbrooding is common in cichlids (Cichlidae), and all species of cardinalfish (Apogonidae) are mouthbrooders. Among cardinalfish, which are particularly abundant on tropical coral reefs, it is the males who take care of the brood. The cardinalfish males keep the eggs in the mouth at least until they hatch, which takes 1–2 weeks (Thresher 1984; Okuda 1999). Since the egg clutch virtually fills the oral cavity of the brooding males, the males do not feed, although they may eat some of the eggs (filial cannibalism) (Okuda & Yanagisawa 1996; Okuda 2001). The consequence of mouthbrooding on the nutritional status has been examined in several studies (e.g. Smith & Wootton 1994; Okuda 2001), and in most cases the starvation is not so severe that it leads to a higher mortality of the mouthbrooding sex (Okuda 2001).

However, the mouth of a fish also constitutes an important part of the respiratory pump, forcing water over the gills. Nevertheless, although there is a relatively large literature on mouthbrooding in fishes, to our knowledge no study has examined the possible respiratory consequences of having a mouth full of eggs. The size of the clutch that is fitted into the mouth can indeed be impressive. In the present study, the male apogonids were found to carry broods weighing up to 26% of their body weight. One would expect that such an egg mass would severely limit the irrigation of the gills, which may pose a particular problem when the demand for oxygen uptake is increased, such as during physical activity or environmental hypoxia.

Interestingly, it has been suggested that the reason for the filial cannibalism in cardinalfish may be to adjust the clutch size to the volume of the oral cavity rather than a need for energy and nutrients (Okuda 2001). Thus, filial cannibalism may be needed to avoid suffocation.

There has been one study examining the direct energetic cost of mouthbrooding, by measuring oxygen consumption (VO₂) in a mouthbrooding cichlid, *Pseudocrenilabrus multicolor* (Mrowka & Schierwater 1988). That study examined the cost of mouthbrooding only in resting fishes held in normoxic water. Under such conditions, it was found that the metabolisms of the mouthbrooding fishes were not significantly elevated when the VO₂ of the egg mass itself was subtracted.

Hypoxia regularly affects the habitats of many cichlids (Verheyen *et al.* 1994; Chapman *et al.* 1995, 2002), and recent studies indicate that hypoxia is also a common phenomenon on coral reefs—the habitat of many cardinalfish (Nilsson & Östlund-Nilsson 2004; Nilsson *et al.* 2004).

Thus, the aim of the present study was to examine the respiratory consequences of mouthbrooding in cardinalfish, particularly during hypoxia and swimming. Using closed respirometry, we studied how mouthbrooding affects metabolic rate and the ability to take up oxygen at low ambient oxygen levels in two species of cardinalfish that we found to differ significantly in the size of the brood. In addition, a swim tunnel was used to examine the effect of mouthbrooding on aerobic and anaerobic swimming performances in one of the species.

2. MATERIAL AND METHODS

(a) Animals

All experiments were carried out in February–March and October 2002 at Lizard Island Research Station (LIRS) on the

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Great Barrier Reef, Australia. *Apogon fragilis* and *A. leptacanthus* were caught by scuba diving in the lagoon at LIRS, where these two cardinalfish shoal together within or over branching coral at a depth of 1–5 m. They were caught with a hand net after lightly anaesthetizing them by spraying them with clove oil (50 ml of clove oil, 40 ml of ethanol and 400 ml of seawater). The fishes recovered within a minute of being moved to clove-oil-free water. Each species appeared to synchronize its spawning, so mouthbrooding males of a particular species were available for only limited periods. Therefore, while respirometry was carried out on both species, the subsequent swim-tunnel experiments were carried out only on *A. leptacanthus* as no mouthbrooding males of *A. fragilis* were available at that time.

The fishes were kept in shaded outdoor aquaria with a continuous supply of water pumped in directly from the ocean ($30 \pm 1^\circ\text{C}$). The water oxygen level varied between 80% and 105% of air saturation. The fishes were fed daily with brine shrimps (*Mysis*) until satiation, but were starved for 48 h before any experiments. All experiments were carried out in shaded daylight (10.00–18.00).

(b) Respirometry

Closed respirometry was performed essentially as described by Nilsson (1992, 1996). In closed respirometry, a fish is placed in a sealed container and the rate at which the water oxygen level falls is measured continuously using an oxygen electrode. The closed respirometer was custom made from a Perspex cylinder (inner diameter = 80 mm, length = 350 mm). The cylinder had a lid in one end and a plunger in the other to allow us to adjust the chamber volume, which was set between 150 ml and 250 ml so that each experiment lasted *ca.* 3–5 h. O-rings were used to seal the plunger, the lid and the opening for the oxygen electrode. The oxygen level in the respirometer was monitored using a galvanometric oxygen electrode (the microprocessor-controlled WTW Oximeter OXI 323A from Wissenschaftlich Technische Werkstätten in Weilheim, Germany) and recorded with a Powerlab 4/20 A/D converter (AD instruments) connected to a Macintosh Power Book G3, using the program CHART 4.0 (AD instruments). The chamber was submerged in a flow-through aquarium to maintain a stable temperature ($30 \pm 1^\circ\text{C}$). To ensure circulation in the chamber and over the electrode, a small magnetic propeller (also from WTW) was attached to the tip of the oxygen electrode. The propeller was driven by a magnetic stirrer placed outside the aquarium. Complete water mixing in the chamber was achieved within 1 min (tested by adding colour to the chamber). Before each experiment, we ran water through the respirometer to allow the fish to acclimatize for at least 2 h, or longer if it still showed any signs of agitation. The cardinalfish settled down rapidly and remained virtually motionless during respirometry. The experiments were terminated when the fishes showed problems maintaining equilibrium. They all recovered rapidly after the experiments.

The rate of ventilation was assessed visually at regular intervals during respirometry.

To measure the rate of oxygen consumption of egg clutches, we used the same oxygen electrode but fitted it to a 11 ml glass chamber, where a fine nylon mesh separated the egg clutch from the electrode and propeller.

The background microbial respiration in the water was measured daily and, if present, subtracted from the respiration measured in the presence of the fish or eggs.

VO_2 , in $\text{mg O}_2 \text{ h}^{-1} \text{ kg}^{-1}$ (fish wet weight), was plotted against water oxygen concentration ($[\text{O}_2]$), given as the percentage of air

Figure 1. VO_2 versus $[\text{O}_2]$ in (a,b) mouthbrooding males, (c) a non-mouthbrooding male, and (d) a female *Apogon fragilis*. The $[\text{O}_2]_{\text{crit}}$ is the intersection of the two regression lines. The figures show typical experiments, and the data from all individuals studied are summarized in table 1. Arrows indicate where the male spat out the brood.

saturation, and the critical oxygen concentration ($[\text{O}_2]_{\text{crit}}$) was determined. The $[\text{O}_2]_{\text{crit}}$ is the concentration below which the fish is unable to maintain a resting rate of oxygen consumption that is independent of the ambient $[\text{O}_2]$ (Beamish 1964). It was determined by fitting two linear regression lines to the curve, one for the normoxic oxygen-independent part of the curve, and one for the steeply falling hypoxic part (see figures 1 and 2). The point where these lines crossed was taken as the $[\text{O}_2]_{\text{crit}}$.

The normoxic VO_2 values given in tables 1 and 2 and in the text were measured as the mean rate of oxygen decline at $[\text{O}_2]$ above 70% of air saturation.

$[\text{O}_2]$ is given as the percentage of air saturation, which can easily be converted to oxygen partial pressure (100% = 151 mmHg) or weight-based concentration (100% = $6.0 \text{ mg O}_2 \text{ l}^{-1}$ at 30°C in seawater).

(c) Swimming experiments

The swim-tunnel apparatus was constructed essentially as described by Stobutzki & Bellwood (1994). The swimming chamber holding the fish consisted of a 200 mm long cylindrical Perspex tube (inner diameter = 52 mm). The water was fed into the chamber using a submersible pump (Davey Sump Pump DC10A-2) placed in a 300 l reservoir below the chamber. The reservoir was continuously supplied with seawater at $30 \pm 1^\circ\text{C}$. Maximum water flow was 1200 ml s^{-1} , which corresponded to

Figure 2. VO_2 versus $[\text{O}_2]$ in (a,b) mouthbrooding males, (c) a non-mouthbrooding male, and (d) a female *Apogon leptacanthus*. The $[\text{O}_2]_{\text{crit}}$ is the intersection of the two regression lines. The figures show typical experiments, and the data from all individuals studied are summarized in table 2. Arrows indicate where the male spat out the brood.

a linear speed of 565 mm s^{-1} . The speed was regulated with a gate valve and the water was forced through a flow straightener containing densely packed plastic drinking straws (diameter = 5 mm, length = 45 mm) to create a laminar flow in the chamber. The fish was retained in the chamber by the flow straightener at the front end and by a 2.5 mm plastic mesh at the rear end. The water speed was determined by measuring the volume of water leaving the chamber per unit of time. This swim tunnel is constructed for field use where relative measures are of prime interest. It may not be optimal from a theoretical perspective as the diameter of the chamber is not large enough to eliminate hydrodynamic drag.

The swimming experiments were done on *A. leptacanthus*. In common with the other cardinalfish that we found on the reef, this species displays a labrid swimming mode (pectoral-fin swimming) and uses the caudal fin only for very rapid swimming bouts. Two measures of swimming performance were taken. The first measure was the maximum water speed at which the fish used only its pectoral fins and did not engage the caudal fin. This was considered to be a fully aerobic swimming mode since an initial experiment on four fishes showed that they were able to maintain this speed for at least 4 h, during which time no caudal-fin beating occurred. The measure was taken by slowly increasing the water speed until the first bouts of caudal-fin beating were seen. The water speed was then lowered until no

caudal-fin beating occurred, and the water flow at this point was recorded.

The second measure, taken 15 min later, was the swimming endurance (in seconds) at the maximum water speed of the swimming apparatus (565 mm s^{-1}), which in this case corresponded to 14 standard body lengths per second, since all individuals of *A. leptacanthus* used in this experiment had a standard body length of $40 \pm 1 \text{ mm}$ (no significant difference in length between the mouthbrooding males and non-mouthbrooding males groups). This water speed was approximately three to four times faster than the maximum pectoral swimming speed, and forced the fishes to use their caudal fin continuously, and thus, to engage the white (anaerobic) lateral muscles. The fish was considered exhausted when it came to rest against the rear plastic mesh in the chamber, at which point the experiment was terminated.

Dissection showed that the pectoral-fin muscles in *A. leptacanthus* are red, whereas the lateral swimming muscles are white and virtually translucent, with no apparent section of red muscle tissue even along the lateral line. This confirms the swimming observations, clearly indicating that the pectoral muscles are primarily aerobic whereas the lateral muscles driving the caudal fin are anaerobic.

(d) Statistics

All values presented are means $\pm$ s.e.m., except lengths and temperatures, which are given as mean $\pm$ range. Comparisons between two groups were made with Student's *t*-test, while multiple comparisons were made with one-way ANOVA and the Tukey–Kramer test as post-test. A value of $p < 0.05$ (two-tailed) was considered significant.

3. RESULTS

(a) Oxygen consumption (VO_2)

Mouthbrooding males of *A. fragilis* were found to have a normoxic VO_2 of $465 \pm 22 \text{ mg kg}^{-1} \text{ h}^{-1}$. This was 75% higher than the VO_2 of males without eggs, which in turn did not differ from the VO_2 of females (table 1, exemplified in figure 1). The mouthbrooding males of *A. leptacanthus* had a normoxic VO_2 of $356 \pm 23 \text{ mg kg}^{-1} \text{ h}^{-1}$. This was significantly lower than that of mouthbrooding *A. fragilis* ($p = 0.0062$), and only 27% higher than the VO_2 of *A. leptacanthus* males without eggs, which in turn was significantly higher than the VO_2 of their females (table 2, exemplified in figure 2).

Of course, the VO_2 measured in the mouthbrooding males also included the VO_2 of their brood. We therefore calculated the contribution of the brood VO_2 (based on the measured VO_2 given in table 3 and discussed later). When this was subtracted from the VO_2 values of the mouthbrooding males, the VO_2 values obtained for the males themselves (values within parentheses in tables 1 and 2) were no longer significantly different from those of males without eggs. This indicates that the direct energetic cost of mouthbrooding under normoxic resting conditions is insignificant, which was also found to be the case in the mouthbrooding cichlid *Pseudocrenilabrus multicolor* (Mrowka & Schierwater 1988). Studying more material may of course reveal a small energetic cost of mouthbrooding under resting conditions, and a reduced VO_2 caused by the starvation induced by mouthbrooding could mask an energetic cost of mouthbrooding.

Table 1. Effect of mouthbrooding on respiration and ventilation in *Apogon fragilis*.

(All mouthbrooding males had eye-pointed eggs. Values are means $\pm$ s.e.m. The number of individuals was six (mouthbrooding) or eight (others). The value within parentheses is the calculated VO_2 of the fish itself obtained by subtracting the VO_2 of the egg clutch (see § 3a). In each column, values marked with different superscript letters are significantly different ($p < 0.01$ (same letters indicate $p < 0.05$); ANOVA and Tukey–Kramer test); fv: ventilation frequency. Normoxic fv was measured at 80% of air saturation.)

	body weight (g)	VO_2 ($\text{mg kg}^{-1} \text{h}^{-1}$)	$[\text{O}_2]_{\text{crit}}$ (% of air saturation)	fv in normoxia (min^{-1})	fv at $[\text{O}_2]_{\text{crit}}$ (min^{-1})
males mouthbrooding	1.65 ± 0.11^a	465 ± 22^a (289 ± 21) ^b	32.7 ± 1.3^a	91.8 ± 5.8^a	89.7 ± 2.1^a
males without eggs	1.81 ± 0.06^a	266 ± 12^b	17.1 ± 0.5^b	55.4 ± 2.4^b	109.8 ± 2.9^b
females	1.88 ± 0.09^a	244 ± 15^b	16.7 ± 0.7^b	57.0 ± 3.1^b	110.0 ± 3.6^b

Table 2. Effect of mouthbrooding on respiration and ventilation in *Apogon leptacanthus*.

(All mouthbrooding males had eye-pointed eggs. Values are means $\pm$ s.e.m.; n : number of individuals. The VO_2 value within parentheses is the calculated VO_2 of the fish itself obtained by subtracting the VO_2 of the egg clutch (see § 3a). ANOVA followed by a Tukey–Kramer test was used to test for significant differences within the columns. In each column, values with no superscript letters in common are significantly different ($p < 0.01$ (same letters indicate $p < 0.05$); fv: ventilation frequency. Normoxic fv was measured at 80% of air saturation.)

	body weight (g)	VO_2 ($\text{mg kg}^{-1} \text{h}^{-1}$)	$[\text{O}_2]_{\text{crit}}$ (% of air saturation)	fv in normoxia (min^{-1})	fv at $[\text{O}_2]_{\text{crit}}$ (min^{-1})
males mouthbrooding ($n = 8$)	1.63 ± 0.11^a	356 ± 23^a (228 ± 21) ^{b,c}	32.2 ± 1.7^a	70.6 ± 3.6^a	96.1 ± 1.8^a
males without eggs ($n = 9$)	1.70 ± 0.05^a	281 ± 9^b	19.1 ± 0.9^b	68.7 ± 3.5^a	108.1 ± 2.6^b
females ($n = 7$)	1.17 ± 0.05^b	186 ± 9^c	18.1 ± 0.6^b	53.3 ± 2.6^b	101.9 ± 4.3^b

Table 3. Weight of egg clutches and spitting out of egg clutches during hypoxia in *Apogon fragilis* and *A. leptacanthus*.

(Values are means $\pm$ s.e.m.; $n = 6$ (*A. fragilis*) or $n = 8$ (*A. leptacanthus*). Eggs were eye-pointed. Comparisons between species were done with Student's t -test (p -values are given within parentheses).)

	<i>Apogon fragilis</i>	<i>Apogon leptacanthus</i>
weight of egg clutch (g)	0.329 ± 0.046	0.235 ± 0.021 ($p = 0.067$)
weight of male (g)	1.65 ± 0.11	1.63 ± 0.11 ($p = 0.91$)
weight of egg clutch (% of male weight)	20.0 ± 2.3	14.4 ± 0.7 ($p = 0.023$)
water $[\text{O}_2]$ at time of clutch spitting (% of air saturation)	21.7 ± 1.5	13.0 ± 0.7 ($p < 0.0001$)
VO_2 of eggs ($\text{mg kg}^{-1} \text{h}^{-1}$)	800 ± 61	918 ± 113 ($p = 0.42$)

The masses of eggs carried in the mouths of these cardinalfish were impressive (table 3). The average mass of the clutches of the males tended to be higher in *A. fragilis* than in *A. leptacanthus*, and this difference was significant when the masses of the eggs were related to the male masses. Thus, the average weight of the eggs in *A. fragilis* was 20.0% of the male mass, whereas it was 14.4% of the male mass in *A. leptacanthus*. In a few instances, the number of eggs in a clutch was counted and was found to be *ca.* 1000–2000 in both species.

All mouthbrooding males in these experiments had eye-pointed eggs in their mouths. The VO_2 values of eye-pointed eggs did not differ significantly between the species (table 3). The eggs used for the VO_2 measurements were from males that had been kept in normoxic water, and not from those participating in the respirometry measurements, since hypoxia could have affected the viability of the eggs. We also measured the VO_2 of young clutches (yellow not eye-pointed eggs) of *A. leptacanthus* that had been spat out by males kept in normoxic water.

These clutches had a normoxic VO_2 of $289 \pm 3 \text{ mg kg}^{-1} \text{h}^{-1}$ ($n = 4$), which was significantly lower than that ($918 \pm 113 \text{ mg kg}^{-1} \text{h}^{-1}$; table 3) displayed by the eye-pointed eggs ($p < 0.01$). An increase in fish-egg VO_2 with age is primarily explained by the increasing mass of the growing embryo (Kamler 1992), and, if the oxygen consumption of the eggs *per se* has a negative effect on the oxygen uptake of the brooding male, mouthbrooding may be more challenging to the male at the end of the incubation period.

(b) Critical oxygen concentration ($[\text{O}_2]_{\text{crit}}$) and spitting of eggs

The $[\text{O}_2]_{\text{crit}}$ values of the mouthbrooding males of both species were *ca.* 32% of air saturation, which was almost twice those of males without eggs and of females, which had $[\text{O}_2]_{\text{crit}}$ values of 17–19% of air saturation in both species (tables 1 and 2, exemplified in figures 1 and 2). All mouthbrooding males spat out their eggs after the

Figure 3. Ventilatory frequencies displayed by (a) mouthbrooding male, (b) non-mouthbrooding male and (c) female *Apogon fragilis*. Values are means $\pm$ s.e.m. The number of individuals was (a) six or (b,c) eight; ** $p < 0.01$ and *** $p < 0.001$ compared with 80% air saturation (ANOVA with Tukey–Kramer post-test).

ambient $[O_2]$ fell below their $[O_2]_{crit}$. However, as shown in table 3 (and exemplified in figures 1 and 2) the spitting out of the brood occurred at a higher ambient oxygen concentration in *A. fragilis* (at 21.7% of air saturation) than in *A. leptacanthus* (at 13.0% of air saturation). After the eggs were spat out, the VO_2 typically rose (figures 1a,b and 2a,b). In *A. leptacanthus*, VO_2 rose from 115 ± 8 to 180 ± 16 $mg\ kg^{-1}\ h^{-1}$ ($p < 0.001$) immediately after egg spitting, while the VO_2 values before and after spitting in *A. fragilis* were 227 ± 21 and 288 ± 45 $mg\ kg^{-1}\ h^{-1}$, respectively (marginally significant, $p = 0.062$, owing to a higher variance). This clearly indicates that the egg clutch reduces the ability of the male to take up oxygen. There were also significant correlations between clutch weight and $[O_2]$ at the spitting of eggs in *A. leptacanthus* males (Spearman's $r = 0.929$, $p = 0.0022$, $n = 8$) and in both species taken together (Spearman's $r = 0.582$, $p = 0.029$, $n = 14$), but not in *A. fragilis* alone.

Figure 4. Ventilatory frequencies displayed by (a) mouthbrooding male, (b) non-mouthbrooding male and (c) female *Apogon leptacanthus*. Values are means $\pm$ s.e.m. The number of individuals was (a) eight; (b) nine or (c) seven; ** $p < 0.01$ and *** $p < 0.001$ compared with 80% air saturation (ANOVA with Tukey–Kramer post-test).

(c) Ventilatory frequencies in normoxia and hypoxia

The ventilatory frequencies of *A. fragilis* (figure 3) and *A. leptacanthus* (figure 4) were recorded during the respirometry experiment. Statistical comparisons between the three different groups (mouthbrooding males, non-mouthbrooding males and females) are summarized in tables 1 and 2.

While the mouthbrooding males of *A. fragilis* appeared to be totally unable to increase their ventilatory frequencies in response to falling ambient O_2 levels (figure 3a), the males without eggs (figure 3b) and the females (figure 3c) doubled ($p < 0.001$) their ventilatory frequencies when faced with hypoxia. The ventilatory frequency of the mouthbrooding *A. fragilis* males appeared to be already maximized in normoxia (table 1), where the frequency was $91.8 \pm 5.8\ min^{-1}$ (at 80% air saturation), a value significantly higher ($p < 0.001$) than that of

Table 4. Effect of mouthbrooding on swimming performance in *Apogon leptacanthus*.

(Values are means $\pm$ s.e.m. from five (mouthbrooding) or 13 (males without eggs) fishes. Their standard body lengths were 39–41 mm, and their body weight was 1.87 ± 0.07 g (no difference between groups). The weights of the egg clutches were 198 ± 24 mg. In each column, values marked with different superscript letters are significantly different ($p < 0.05$; in this case $p = 0.0011$; Student's *t*-test).)

	maximum pectoral swimming speed (body lengths per second)	swimming endurance at 14 body lengths per second (s)
males mouthbrooding	3.49 ± 0.33^a	55.2 ± 5.7^a
males without eggs	5.13 ± 0.23^b	46.0 ± 10.0^a

non-mouthbrooding males ($55.4 \pm 2.4 \text{ min}^{-1}$) and that of females ($57.0 \pm 3.1 \text{ min}^{-1}$). At the $[\text{O}_2]_{\text{crit}}$, where ventilation could be expected to be maximized, the ventilatory frequency of the mouthbrooding males ($89.7 \pm 2.1 \text{ min}^{-1}$) was significantly lower ($p < 0.001$) than those of both the non-mouthbrooding males ($109.8 \pm 2.9 \text{ min}^{-1}$) and the females ($110.0 \pm 3.6 \text{ min}^{-1}$) (table 1). With regard to ventilatory frequency, there were no significant differences between non-mouthbrooding males and females (figure 3*b,c*).

In contrast to those of *A. fragilis*, the mouthbrooding males of *A. leptacanthus* did not display a higher ventilatory frequency in normoxia ($70.6 \pm 3.6 \text{ min}^{-1}$) than males without eggs ($68.7 \pm 3.5 \text{ min}^{-1}$), and the mouthbrooding *A. leptacanthus* males were able to increase their ventilatory frequency significantly in response to the progressive hypoxia encountered in the respirometer (figure 4*a*). However, this rise in frequency from normoxia to $[\text{O}_2]_{\text{crit}}$ was only 36% in the mouthbrooding males, which was significantly less than the 57% and 91% rises displayed by the males without eggs and the females, respectively (table 2; figure 4*b,c*). Moreover, at the $[\text{O}_2]_{\text{crit}}$, the ventilatory frequency of the mouthbrooding males ($96.1 \pm 1.8 \text{ min}^{-1}$) was significantly lower than those of both the non-mouthbrooding males ($108.1 \pm 2.6 \text{ min}^{-1}$) and the females ($101.9 \pm 4.3 \text{ min}^{-1}$; table 2). In normoxia, the females had a slightly lower ventilatory frequency than the non-mouthbrooding males (figure 4*b,c*, table 2).

It should be emphasized that, at a steady ventilatory frequency, there may still be an increase in the flow of water through the mouth and over the gills since the ventilatory volume can be increased. Indeed, we observed visually that the ventilations became deeper as $[\text{O}_2]$ fell in all groups of fishes. Thus, the mouthbrooding *A. fragilis* can probably increase the ventilation of the gills (and the brood) to some extent when faced with hypoxia.

(d) *Swimming performance in Apogon leptacanthus*

Apart from affecting the ability to take up oxygen during hypoxic conditions, the ventilatory restriction imposed by a large egg clutch in the oral cavity could also limit the scope of physical activity. We tested this by swimming mouthbrooding and non-mouthbrooding cardinalfish in a swim tunnel. As mentioned in § 2*a*, owing to the limited access to mouthbrooding males, we were able to do this only in *A. leptacanthus*.

Table 4 shows that the maximum water speed at which only aerobic pectoral swimming muscles were engaged was 30% lower in mouthbrooding males than in

non-mouthbrooding males. By contrast, there was no difference in the maximum swimming endurance at a very high water speed (14 body lengths per second), where the swimming must have been highly anaerobic since it was endured for less than 1 min and involved a continuous forceful beating of the caudal fin. Thus, although the scope for aerobic (pectoral) swimming was clearly limited by mouthbrooding, the capacity for rapid bouts of anaerobic swimming did not appear to be inhibited. Egg spitting was not induced by the exhaustive swimming at the high water speed.

4. DISCUSSION

The results show that mouthbrooding is not without problems. It has significant effects on the ability of cardinalfish to cope with hypoxia, and it can also reduce the capacity for aerobic swimming. This suggests that the mouthbrooding male is forced into a trade-off situation between parental care and the capacity for oxygen uptake. When exposed to progressive hypoxia in the respirometer, the mouthbrooding males of both species spat out their clutches. The most obvious explanation for the egg-spitting response to hypoxia is that it improves the male's ability to take up oxygen, thereby serving to save the male from hypoxia-induced energy deficiency that may endanger his survival. Cardinalfish males have multiple broods in a season (Okuda 2000, 2001) and can therefore be expected to sacrifice a clutch if their own survival is at risk. Indeed, in another species of cardinalfish (*Apogon notatus*), a 4–8% incidence of brood loss (non-cannibalistic) has been observed in nature (Okuda 1999).

Interestingly, the results indicate that the two species studied have adopted different strategies in the trade-off between clutch size and hypoxia tolerance. Thus, *A. fragilis*, which had a significantly larger brood size (20% of the body weight), spat out its eggs when the water oxygen level had fallen to 22% of air saturation. By contrast, *A. leptacanthus*, where brood made up only 14% of the body weight, did not spit out the eggs until water $[\text{O}_2]$ had fallen to 13% of air saturation.

Obviously, a larger brood will constitute a larger obstacle for gill ventilation. Consequently, while *A. fragilis* was at its maximal ventilation rate in normoxic water and was unable to increase it when faced with hypoxia, *A. leptacanthus*, with its smaller brood, had the capacity to increase its ventilation rate in response to a falling oxygen level. The high ventilatory frequency of mouthbrooding *A. fragilis* in normoxic water could be explained by a necessity to supply enough oxygen to the large and highly

oxygen-demanding brood, or it could reflect a need for the male itself to compensate for the reduced water flow over the gills, or a combination of both.

By contrast, being successful in hatching a large clutch means a larger reproductive success. The species differences could be interpreted as *A. fragilis* gambling on a brooding period without any severely hypoxic episodes, while *A. leptacanthus* does not take this chance. However, although the two species shoal together in the daytime, it is possible that they spend the night in slightly different habitats. If hypoxia occurs in a coral-reef habitat, this is most likely to happen at night, when photosynthesis has stopped and the entire coral community is consuming oxygen. On Heron Island, further south on the Great Barrier Reef, nocturnal hypoxia has been shown to occur when shallow parts of the reef become cut off from the surrounding ocean at very low tides (Kinsey & Kinsey 1966; Routley *et al.* 2002). Another environment where hypoxia may occur at night is between coral branches in coral colonies. Many coral-reef fishes, including cardinalfish, have been observed to move deep into colonies of branching coral at night, possibly hiding from predators. In colonies of *Acropora nasuta* kept in outdoor tanks, we have found that water $[O_2]$ between the branches may fall below 20% of air saturation at night (Nilsson *et al.* 2004). Thus, it is tempting to speculate that mouthbrooding males of *A. fragilis* have to avoid such hypoxic habitats at night more than do *A. leptacanthus*, and may therefore have to face a higher risk of predation. In any event, the egg-spitting response shows that hypoxia could be an important environmental factor that negatively influences the breeding success of cardinalfish in some habitats.

The low $[O_2]_{crit}$ values of the two cardinalfish studied (17–19% of air saturation) indicate a considerable degree of hypoxia tolerance. However, a recent survey of $[O_2]_{crit}$ values for fishes at the Lizard Island reef suggests that hypoxia tolerance is widespread in this coral-reef habitat (Nilsson & Östlund-Nilsson 2004), and that many species, including, for example, damselfish, have $[O_2]_{crit}$ values in the same range as those of cardinalfish. Thus, the low $[O_2]_{crit}$ of the cardinalfish is probably related more to habitat than to an adaptation to mouthbrooding.

The swim-tunnel experiments on *A. leptacanthus* showed that the scope for aerobic (pectoral) swimming was reduced by 30% during mouthbrooding, probably because of an impaired ability to oxygenate the blood. However, the mouthbrooding individual's capacity for rapid anaerobic-swimming bouts did not appear to be reduced. This was not unexpected, since the anaerobic performance should be limited by factors such as glycolytic ATP generating capacity in the muscle and lactic acid tolerance, and these are unlikely to be compromised by mouthbrooding. Thus, the results indicate that cardinalfish maintain their capacity for rapid-flight swimming during mouthbrooding but have a reduced ability to perform sustained swimming. It is likely that mouthbrooding reduces the aerobic-swimming capacity of *A. fragilis* even more than that of *A. leptacanthus*, owing to the larger brood size and the more limited ventilatory scope of mouthbrooding *A. fragilis*, but, unfortunately, mouthbrooding males of *A. fragilis* were not available on the reef at the time of the swim-tunnel experiments.

Since water currents around Lizard Island are typically 100–300 mm s⁻¹ (Frith *et al.* 1986), which for these small cardinalfish correspond to 2.5–7.5 body lengths per second, the reduction seen in sustained pectoral-swimming ability could influence the habitat choice of these fishes. Indeed, we observed *A. fragilis* and *A. leptacanthus* to be rather stationary on relatively sheltered areas of the reef, staying close to the substrate, within or immediately above colonies of branching coral. Interestingly, on the reef, we found mouthbrooding males of these species to be more stationary when disturbed and less willing to move than non-mouthbrooding individuals (making them easier to catch).

In contrast to the hypoxia exposure, even exhaustive swimming at the high water speed did not induce egg spitting. This makes sense since the performance of the male in this situation was not negatively affected by mouthbrooding.

5. CONCLUSIONS

Mouthbrooding males of both species had elevated rates of oxygen consumption. However, this was a result of the high specific metabolic rate of the egg clutch itself, and the direct energetic cost of the mouthbrooding at rest in normoxic water appeared to be insignificant.

By contrast, mouthbrooding significantly reduced the ability of the males to take up oxygen at a low ambient $[O_2]$ and impaired the capacity for sustained aerobic swimming. In hypoxia, interesting species differences were revealed. The males spat out their eggs when faced with hypoxia, and the species with the larger brood, *A. fragilis*, spat out the eggs at a higher $[O_2]$ than *A. leptacanthus*, which had a significantly smaller brood. Moreover, in contrast to *A. leptacanthus*, *A. fragilis* was unable to increase its ventilatory frequency in response to hypoxia. This indicates that there is a trade-off situation between hypoxia tolerance and brood size, and that *A. fragilis* has sacrificed hypoxia tolerance in favour of a large brood size to a greater extent than has *A. leptacanthus*.

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