

O₂ replenishment to fish nests: males adjust brood care to ambient conditions and brood development

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Parental care through nest defense and maintenance enhances offspring success. In nature, obligate anemone-dwelling fishes and their nests of benthic eggs are protected against most predators by their host anemone; thus, parental care generally consists of nest tending through fanning and mouthing. Tending in fishes is believed to oxygenate the eggs; however, a real-time link between fanning and oxygenation is tenuous. This study investigated whether tending modified the oxygen microenvironment of the embryos and, subsequently, whether tending was modified according to ambient dissolved oxygen (DO), increasing metabolic demands of developing embryos, and water temperature. There was a time lag of approximately 1 s between tending and increases in the amount of oxygen within the nest, demonstrating that DO is directly affected by parental tending. While there was evidence of biparental care, males invested more time tending embryos (40% initially) than did females (20–30%), and male investment increased to 70% as embryo development progressed and embryonic metabolic demands increased. Additionally, male fish adjusted tending effort on a diel cycle as ambient DO fluctuated: time spent tending was lowest between 1000 and 1400 h (35%), when ambient DO was highest, and increased throughout the day, reaching a peak of 70% between 2200 and 0200 h, when ambient DO was lowest. Increased water temperature reduced the number of tending bouts per minute throughout the day but did not influence any other aspect of tending behavior. These results suggest that fish adjust tending behavior coincident to changing conditions in the nest, both on a daily basis and throughout development of the embryos. *Key words:* dissolved oxygen, metabolism, parental care, temperature. [*Behav Ecol* 16:389–397 (2005)]

Parental fitness is measured by reproductive success, which in part depends on offspring survival. In oviparous species, females can enhance reproductive success by investing in the size, quality, and number of eggs. Males, on the other hand, make very little contribution to the embryonic material. One life-history mechanism that increases parental fitness is actively investing in some form of parental care. Parental care enhances offspring development (Sargent, 1988, 1997), can affect offspring phenotype (Shine et al., 1997), and increases offspring survival (Sabat, 1994). Brooding eggs on the substratum is the most common form of parental care in fishes (Sargent, 1997), and the male is generally the primary tender (Gross and Sargent, 1985; Mazzoldi et al., 2002). Thus, parental tending is a mechanism by which males can invest in their offspring (Bernardo, 1996).

Brooded eggs of many ectotherms remain in the parentally chosen environment, and developing embryos have minimal physiological capacity to adjust to environmental change (Pelster, 1999). Variables such as temperature and oxygen availability will influence embryo metabolism or physiology and therefore their survival (Hale et al., 2003). In organisms with parental care, adults can compensate for environmental conditions by changing the microclimate around the eggs. Snakes employ shivering thermogenesis, which acts to warm their eggs (Shine et al., 1997), amphipods actively ventilate the brood pouch (Dick et al., 1998), and fishes fan to increase water circulation (Coleman, 1992). Compensating for natural

environmental variation is rarely considered to be a cost in empirical studies of parental care (St Mary et al., 2001), even though environmental variation is ubiquitous. Theoretical models assume that the rate and degree of parental investment are dependent on environmental factors (Perrin, 1995; van Iersal, 1953); however, empirical evidence of this in fishes is limited (but see Jones and Reynolds, 1999a,b).

Temperature and dissolved oxygen (DO) are the most important influences on fish metabolism in early development (Rombough, 1988), and oxygen is more likely to be limiting in aquatic than terrestrial habitats (Kramer, 1987). DO levels fluctuate in coral reef habitats due to both biotic processes (e.g., respiration and photosynthesis of algae and coral) and abiotic processes (e.g., currents, tides, and small-scale water circulation) (Kraimes et al., 1996). In particular, the cryptic habitats where demersal eggs are often spawned may, by their nature, have poor water circulation. Because oxygen supply to the eggs can be a critical determinant of developmental success (Chaffee and Strathmann, 1984; Fernández et al., 2003), some species of fishes choose nest sites with reference to the oxygen environment (Jones and Reynolds, 1999b; Lukas and Orth, 1995; Takegaki, 2001), while other species tend the nest to compensate for sub-optimal oxygen conditions (Takegaki and Nakazono, 1999). Parental care of benthic fish eggs consists of several distinct behaviors: fanning the clutch using the fins serves to move water over the eggs, thereby removing debris and disturbing the boundary layer (St Mary et al., 2001) and replacing deoxygenated water with oxygenated water (Takegaki and Nakazono, 1999); mouthing removes dead embryos and cleans live ones (Keenleyside, 1991); and nest guarding protects the nest from predators (Keenleyside, 1979; Sargent, 1997). Some fishes have specialized tissues that secrete antimicrobial compounds that clean the eggs (Knouft et al., 2003).

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If nest tending compensates for environmental oxygen availability, then in order to maximize parental investment in brood survival without reducing their own future reproductive potential, parents should adjust nest tending to the needs and requirements of their brood and to the response of the brood to environmental variation. Fanning in fishes is generally assumed to increase the oxygen supply to the eggs (e.g., Hale et al., 2003); however, to our knowledge, this relationship has not been directly demonstrated.

In this study we used the benthic-spawning, coral reef fish *Amphiprion melanopus* (Pomacentridae) to examine whether parental behavior directly modifies the oxygen environment of their offspring. We explored whether adults adjusted their nest tending to changes in the environment and needs of their offspring. Specifically, we addressed the following questions: (1) does tending change the quantity of oxygen at the nest and (2) do parents alter their tending according to changing environmental conditions and the increasing metabolic demands of their brood? We predicted that tending would increase the DO concentration available to the eggs, that tending would track changes in ambient O₂ concentrations, and that nest-directed tending would increase with increased embryonic development (and therefore the increasing metabolic requirements of their offspring).

MATERIALS AND METHODS

Study species and maintenance

In their natural environment, pairs of *A. melanopus* attach a monolayer of eggs in a circular clutch (diameter: 40–100 mm) to hard substrata under the margin of the stinging tentacles of their host anemone (Wilkerson, 1998). The embryos develop for 8–9 days before hatching at ambient temperature (28°C) and are tended by both parents, although males typically tend more than females (Allen, 1980).

Pairs of *A. melanopus* were collected from the northern section of the Great Barrier Reef, Australia, adjacent to Cairns (16° 8' S, 145° 7' E), where they would naturally experience annual temperature fluctuations from 25°C to 31°C. Pairs were housed in 70-l circular tanks with constant flowing saltwater at the James Cook University Research Aquarium. Pairs were randomly assigned to either hollow cement blocks (internal dimensions, 160 × 160 × 190 mm) or pieces of polyvinyl chloride (PVC) pipe (internal diameter, 100 mm) simulating a protected nest where they laid their eggs. Pairs were fed twice a day on a mixed diet of Wardley's marine flakes (Hartz, Secaucus, N.J.) and chopped pilchards (*Sardinops* spp.) and squid (*Loligo* spp.).

Experimental design and quantifying nest-tending behavior

For aquatic organisms, temperature and oxygen are linked as an increase in temperature causes a decrease in the amount of DO in water, as well as increases metabolic rate in exothermic animals. To determine whether variation in temperature affected parental behavior, nest tending was filmed on multiple clutches from different brood-stock pairs at three temperatures that spanned the range of temperatures that the species experiences during embryogenesis across its geographic range. Brood-stock pairs were assigned to their experimental temperature treatments at the commencement of the experiment; however, temperature was not manipulated until an egg clutch was laid. The saltwater supply to tanks containing breeding pairs was maintained at 28°C ± 0.5°C. Once eggs were laid, the temperature of water within the tank was maintained at 28°C ± 0.5°C ($n = 8$ brood-stock pairs), elevated to 31°C ± 0.5°C (by the addition of two 300-W

submersible water heaters per tank; Rena™) ($n = 8$ brood-stock pairs), or cooled to 25°C ± 0.5°C (using a chiller unit; Carrier's heat pump™) ($n = 5$ brood-stock pairs). Reducing the temperature to 25°C reduced or ceased egg production in the brood-stock assigned to this treatment, resulting in only two replicate clutches at 25°C.

Nest tending

After these temperature manipulations, nest-tending effort was recorded throughout embryonic development, from days 1 to 8 postfertilization, commencing 4 h after the temperature change. Tending behavior was recorded using waterproof color CCTV cameras with inbuilt infrared lights and automatic infrared triggers for filming under low illumination (AVC667, Jaycar, Townsville, Australia). As fish cannot see in the infrared spectrum (Batty, 1983; Higgs and Fuiman, 1996), we assumed that the infrared lighting did not affect tending behavior. Nest tending for 23 experimental clutches was recorded simultaneously from four nests, using four cameras connected to a four-channel quad processor and a real-time (long play) video recorder, which compresses more image fields onto a given length of tape, to obtain longer recording durations (Xpose, model QV3053, Electus Distribution, Silverwater, Australia), and viewed on a Sony 54-cm monitor. Fish behavior was recorded at 2.95 fields s⁻¹ on the real-time video recorder for this experiment, compared to domestic VCR recording speed of 25 fields s⁻¹.

To sample nest tending on a diel cycle, footage of the nest was recorded at eight separate intervals, each of 20 min, within each 24-h time period. Intervals were spaced 3 h apart, totaling 160 min of footage per pair per day. Each interval was randomly subsampled, and nest-tending video records were sampled for 1 min of high-speed playback time (corresponding to 2.5-min periods of real time), five times, every 3 h, for the 8 intervals day⁻¹. All video observations were recorded by an observer using a purpose-designed manually operated computer data logger program (written in Access 2000, Microsoft).

Quantifying nest tending

Nest tending was defined as any of two distinct types of behavior directed at eggs within the nest: (1) mouthing, when fish tended the eggs with their mouths, and (2) fanning, fanning eggs with either left or right pectoral fins or caudal fin. Nest tending was quantified by recording the start and end of each tending approach to the eggs, commencing when the parent fish was within one body length of the eggs. Each nest-directed activity within an approach was then recorded by type and frequency, that is, the number of times the mouth or the fins were directed at the eggs per minute. In order to detect subtle differences in behavior or trade-offs between length and regularity of tending event (Reebs et al., 1984), three measures of nest tending were recorded: proportion of time spent tending, frequency of each behavioral type per minute, and duration of each tending approach.

Oxygen consumption measurements

Oxygen concentration within the egg clutch was measured using a micro-optode (140-µm tip, PreSens, Regensburg, Germany), with a positioning accuracy of 5 µm. Oxygen measurements by this method are highly accurate because the optode does not consume oxygen and measurements are independent of changes in flow velocity (Klimant et al., 1995). The micro-optode was calibrated daily at 100% oxygen saturation (air saturated with water) and 0% oxygen saturation (water supersaturated with sodium sulfite).

In order to measure the real-time effect of parental tending on the oxygen available to benthic embryos, the quantity of oxygen (% air saturation) was measured within the egg clutch using a micro-optode while simultaneously recording parental nest tending with the CCTV camera equipment. Oxygen measurements were made on two clutches of eggs from different brood-stock pairs, referred to as clutch 1 and clutch 2. Clutch 1, laid on an acetate sheet attached to a cement block, was filmed on day 4 of development at 31°C. Clutch 2, laid inside PVC pipe, was filmed on days 1–3 of development at 28°C. Oxygen was measured in clutch 1 by pushing the micro-optode needle through the acetate from underneath the eggs so that the fiber protruded, sitting flush with the tops of the eggs. Oxygen was measured in clutch 2 by drilling a small hole through the pipe and inserting the needle probe. In both cases the tending parents damaged the fiber as they cleaned their nests of foreign objects, and so further measurements were aborted. Forty minutes of oxygen readings were collected from clutch 1 before the fiber was damaged, and 30-, 59-, and 27-min readings were collected on days 1, 2, and 3, respectively, for clutch 2. Presence or absence of tending was compared to the oxygen percent air saturation within the eggs per second.

Rates of embryonic oxygen consumption throughout development were collected as described in Green (2004) and have been included here for comparison with tending regime throughout development. Embryonic development was observed at 6-h intervals and related to oxygen consumption of embryos within clutches throughout development, measured using the vertical profiling method (Kuhl et al., 1995).

Ambient water oxygen content

Oxygen content of the aquarium water was measured over five nights to determine the natural variability within the breeding tank system, using a Clark-type microelectrode (YSI Inc., Yellow Springs, Ohio) attached to a data logger (WP 82, TPS Pty Ltd., Springwood, Queensland, Australia). The probe was placed in high-flow water stream at the outlet of a brood-stock tank within the flow-through system, where all breeding pairs were housed. Oxygen (% air saturation) was recorded every 20 min for five 24-h periods.

Data analysis

The real-time relationship between oxygen and parental tending was examined using autocorrelation function plots and partial autocorrelation function plots to indicate the degree of time lag that explained most of the variation in the data set (StatSoft, 2002). Once the appropriate time lag was identified, a polynomial distributed-lags ANOVA and regression were examined to identify whether the proposed time lags were significant. Distributed-lags analysis examines the relationship between an independent or explanatory variable that affects the dependent variables with some time lag. For example, it is common for one variable to be related to the occurrence of another with a time delay or a time (lagged) correlation between the variables of interest (StatSoft, 2002).

We determined the effects of time of day, egg age, and temperature on the quantity, frequency, and duration of parental tending behavior using repeated-measures multivariate analysis of variance (MANOVA) and ANOVA. Some data were missing from the original design due to anemones obscuring the camera's view of the eggs or the adult fish bumping the camera out of alignment, so it was not possible to conduct a repeated-measures MANOVA including all factors. Therefore, separate analyses were performed addressing nest tending over two time frames for this study: diel

Table 1

Real-time comparison of parental fanning behavior and quantity of oxygen within egg clutches

		df	MS	<i>F</i>	<i>p</i>
a	Regression	3	19.86	92.57	<.001
	Residual	603	0.21		
b	Regression	2	23.84	112.79	<.001
	Residual	484	0.21		
c	Regression	2	44.69	199.35	<.001
	Residual	1622	0.22		
d	Regression	2	6.76	52.07	<.001
	Residual	550	0.13		

Summarized by polynomial distributed-lags ANOVA for a 1-s time lag. a: clutch 1, day 4, 31°C; b: clutch 2, day 1, 28°C; c: clutch 2, day 2; d: clutch 2, day 3. $R = .56$, $R^2 = .31$, $N = 606$. Significant results are in bold type.

(different times of day) and between days (i.e., embryo age). First, we examined the influence of egg age (repeated measure) and temperature (fixed factor) on time spent nest tending, as well as on the duration and frequency of tending episodes. Second, we examined the influence of egg age (repeated measure) on the type of nest-directed tending. Third, we tested time of day (repeated measure) and temperature (fixed factor) on the proportion of time spent tending and the frequency and length of tending bouts. Finally, the influences of time of day (repeated measure), temperature, and developmental stage (divided into early, middle, and late development) on total time spent tending the nest were examined. Where ANOVA results did not conform to the assumption of sphericity, Greenhouse-Geisser approximations were used (StatSoft, 2002). Tukey's post hoc comparisons were used to determine the source of significant differences.

RESULTS

Patterns of DO concurrent to parental fanning

Parental fanning affected the quantity of oxygen in the water adjacent to the eggs. There was an increase in the amount of oxygen (up to 95–100% air saturation) available to the eggs in response to parental fanning and a decrease to low levels (minimum 67% air saturation) when parents were away from the nest ($p < .001$, Table 1). A time lag of 1 s best described the relationship between parental fanning activity and an increase in the quantity of oxygen in the vicinity of the eggs for the four clutch/age combinations measured (Table 1).

Sex-related tending behavior

A 96-h subsample of parental egg care was recorded out of a total of 156 days of embryonic development available. Females fanned the clutch an average of 20–30% over the 8-day developmental period. Overall, male fish spent significantly more time (two to four times) tending the nest than did females (Figure 1a–c, Table 2). The amount of tending time by males and females did not change significantly with incubation temperature (Figure 1a–c; nonsignificant third-order interaction, Table 2b). Further, the female contribution was relatively constant throughout development with the exception of a small increase on the last day of development, corresponding to hatching time for the embryos (Figure 1a–c). Given the relatively small, unvarying contribution to overall nest tending by females, the rest of the results focus on the male contribution to tending the egg clutch.

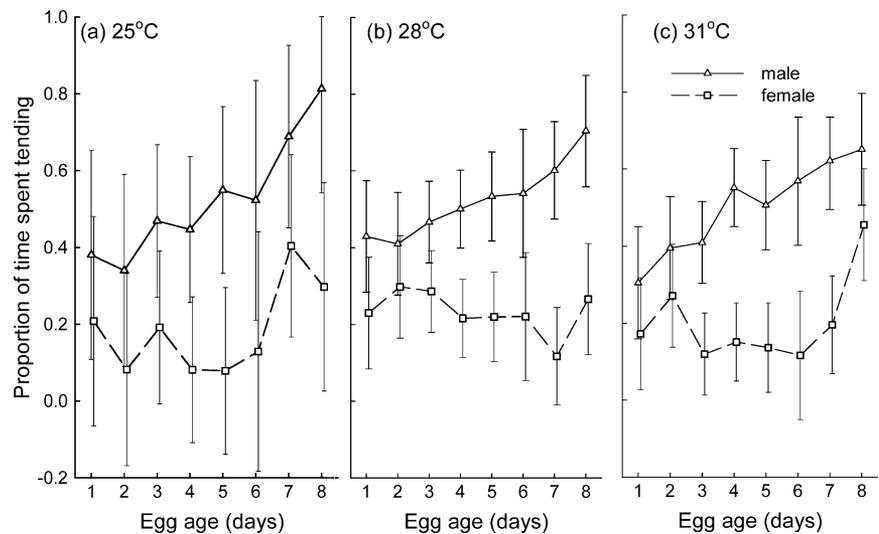


Figure 1

The average proportions of time females (squares) and males (triangles) spent tending their benthic egg clutches at three water temperatures, (a) 25°C, (b) 28°C, and (c) 31°C, for each day throughout embryonic development. Error bars are standard errors.

Nest-tending behavior

Egg fanning occurred more frequently than mouthing (Figure 2). The average frequency of fanning episodes per minute increased linearly from day 1 (7 min^{-1}) to day 7 (26 min^{-1}) of development, when it reached an asymptote until hatching at day 8 or 9 (Figure 2, Table 3). Egg fanning occurred considerably more frequently than mouthing on days 4–9 of development (Figure 2, significant egg age \times behavior interaction, Table 3). The observed frequency of egg mouthing was relatively constant throughout development, occurring approximately seven times per minute from days 1 to 9 of development.

Changes in nest-directed tending throughout embryogenesis

Time spent tending

The proportion of time the male fish spent nest tending the egg clutch increased gradually with development of the

embryos from days 1 to 8 (Figure 3a, Table 2ai); however, water temperature had no effect on the proportion of time spent tending throughout development (Table 2ai,bi). The overall increase in the proportion of time spent tending throughout development followed the general change in embryonic oxygen consumption throughout embryo development, except during the peak in oxygen consumption on day 4 of embryo development (Figure 3a).

Frequency of tending

Egg-tending activity was least frequent on the first day of development and increased sharply from approximately 7 tending episodes min^{-1} on day 1 of development to 12 min^{-1} on day 2 and then remained around 12 min^{-1} (Figure 3b); this was averaged over temperature treatments as there was no temperature effect on frequency of tending intervals (Table 2ai,bii).

Table 2

Test for effects of temperature, egg age, and sex on parental nest tending

a	Source	G-G epsilon	G-G adjusted dfl	G-G adjusted df2	G-G adjusted <i>p</i>
i	Egg age	0.63	4.42	115.09	<.001
	Egg age \times sex	0.63	4.42	115.09	.019
	Egg age \times temp	0.63	8.85	115.09	.205
	Egg age \times sex \times temp	0.63	8.85	115.09	.457
ii	Egg age	0.54	3.76	52.63	.033
	Egg age \times temp	0.54	7.51	52.63	.866
iii	Egg age	0.39	2.76	35.85	.041
	Egg age \times temp	0.39	5.51	35.85	.599
b		df	MS	<i>F</i>	<i>p</i>
i	Sex	1	4.43	48.94	<.001
	Temp	2	0.02	0.22	.802
	Sex \times temp	2	0.01	0.09	.916
	Error	26	0.09		
ii	Temp	2	102.44	2.59	.110
	Error	14	39.53		
iii	Temp	2	751.21	0.96	.409
	Error	13	783.57		

Repeated-measures ANOVA testing for the effect of temperature (temp) and egg age on (i) sex and the proportion of time spent tending, (ii) frequency of male tending events, and (iii) average length of male tending sequence. (a) Assumption of sphericity and compound symmetry was violated, so Greenhouse and Geisser (G-G) approximations were used. (b) Between subjects summary. Significant results are in bold type.

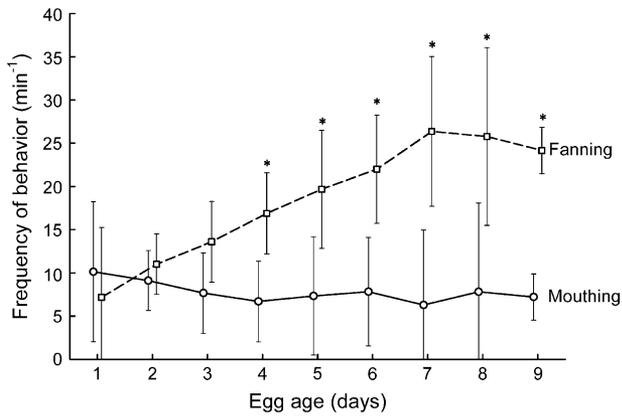


Figure 2 Frequency of different nest-tending behaviors of males throughout embryology. Squares represent fanning, circles represent mouthing. * denotes significant differences from Tukey’s honest significant difference means comparisons ($\alpha = .05$). Error bars are 95% confidence intervals.

Duration of tending event

Average duration of each egg-tending event increased nearly two-fold throughout development (Figure 3c) from approximately 15 s sequence⁻¹ (day 1 of egg development) to 30 s sequence⁻¹ (day 8) and was not affected by the experimental temperatures (Table 2aiii,biii).

Diel changes in nest tending

Time spent tending

Time of day influenced the proportion of time spent tending egg clutches (Table 4ai) with distinct patterns of tending after a diel cycle and patterns in ambient DO concentration (Figure 4a). Time spent tending was lowest during the daylight hours when DO was highest, increased at dusk (1800 h) as DO was decreasing, and peaked in the hours around midnight (2200–0200 h) when DO was lowest (Figure 4a). Experimental manipulation of water temperature did not affect the proportion of time spent tending throughout the day (Table 4bi).

Frequency of tending

The number of tending events per minute showed a general decrease throughout the day, and at all temperatures was lowest between 2200 and 0200 h (Figure 4b; Table 4aii,bii). Water temperature affected the frequency of tending events throughout the day (Table 4bii). Fish tending eggs in 25°C water had the highest average number of tending events for each time period, with approximately 10 more approaches to the eggs per minute than fish in 31°C water, although due to the small sample size at 25°C, this should be interpreted

Table 3 Repeated-measures ANOVA testing the effect of egg age on frequency of type of tending

Source	df	MS	F	p
Behavior	1	1553.88	18.07	.013
Error	4	85.97		
Egg age	8	53.19	6.29	<.001
Egg age × behavior	8	90.36	10.68	<.001
Error	32	8.46		

Significant results are in bold type.

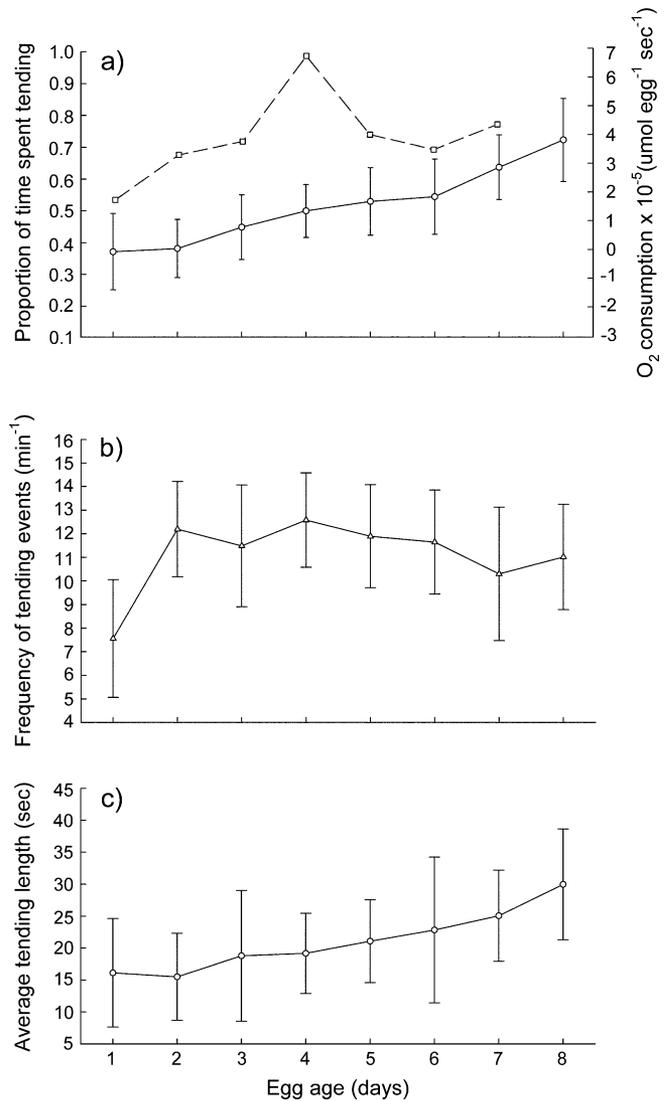


Figure 3 Nest-tending activity of males throughout development: (a) proportion of time spent tending (broken line), including mean rate of embryo oxygen consumption (solid line); (b) mean frequency of tending events per minute; and (c) average length of tending events. Error bars are 95% confidence intervals.

cautiously. Fish at 28°C or ambient temperature had the lowest frequency of tending episodes (Figure 4b).

Duration of tending event

There was a diel pattern in the average length of egg-tending event that was not affected by water temperature (Figure 4c; Table 4aiii,biii). The average tending duration was lowest during the day (approximately 15 s sequence⁻¹) and increased at dusk (1800–2200 h) to peak around midnight and early morning (2200–0200 h) (Figure 4c).

Trade-offs between frequency and duration

The length of tending sequence showed an inverse relationship to the number of tending events in relation to time of day (Figure 4b cf. 4c). When the number of tending events was high, the length of tending sequence was low (Figure 4b cf. 4c). Conversely, average length of tending sequence increased similarly to the proportion of time spent tending (Figure 4a cf. 4c) such that as the proportion of time spent tending

Table 4
Test of temperature and time of day on parental nest tending

a	Source	Effect df	Error df	Pillai's trace	<i>F</i>	<i>P</i>
i	Time of day	4	13	0.79	12.28	<.001
	Time of day × temp	8	28	0.45	1.03	.437
ii	Time of day	4	16	0.57	5.35	.006
	Time of day × temp	8	34	0.32	0.80	.604
iii	Time of day	4	14	0.57	4.71	.013
	Time of day × temp	8	30	0.24	0.51	.842
b		df		MS	<i>F</i>	<i>p</i>
i	Temp	2		0.03	0.38	.684
	Error	16		0.07		
ii	Temp	2		573.38	3.86	.039
	Error	19		148.41		
iii	Temp	2		492.95	1.18	.329
	Error	17		415.71		

Repeated-measures MANOVA (a) and ANOVA (b) results summary table for the effect of temperature (temp) and time of day on (i) the proportion of time males spent tending their eggs, (ii) frequency of male tending events (per minute), and (iii) average length of a tending event. Pillai's trace statistic is used for MANOVA. Significant results are in bold type.

increases, males spend more time in each tending bout, reducing the frequency of events, thereby leaving the clutch for other activities less often.

Temperature, developmental, and diel fluctuations in time spent tending

When the effects of time of day, developmental stage, and water temperature on time spent tending were considered simultaneously, the diel allocation of time spent actively tending the eggs changed in response to the developmental stage of the embryos but not in response to experimental water temperature (Table 5). The overall time spent tending late-stage embryos was approximately 10% higher than that for early-stage embryos for all times throughout the day.

DISCUSSION

Parental fanning increases oxygen to eggs

A comparison of parental tending behavior and the simultaneous measurement of the quantity of DO at the embryos' surfaces showed a significant link between these two events, suggesting that tending does indeed replenish oxygen to the eggs. We found an increase in the amount of oxygen available to the eggs in response to parental tending and a decrease to low levels when parents were away from the nest, so increasing O₂ to the eggs is the likely objective of fanning. Similarly, abdominal flapping in a marine invertebrate, *Cancer setosus*, was demonstrated to directly replenish oxygen to developing embryos (Baeza and Fernandez, 2002). In *A. melanopus*, such behavior is probably necessary because the semicryptic areas where many demersal eggs are laid have poor water circulation and boundary layers rapidly form around eggs, reducing the transfer of oxygen from the surrounding water to the developing embryos (Rombough, 1988). Males spent more time than females in active egg tending, which is typical of the genus *Amphiprion* (Allen, 1980; Wilkerson, 1998) and 58% of other fish families with parental care (Clutton-Brock, 1991).

In *A. melanopus*, parental care consisted of fanning and mouthing the eggs, initially in equal amounts, but as development progressed, fanning increased to 83% of tending activity

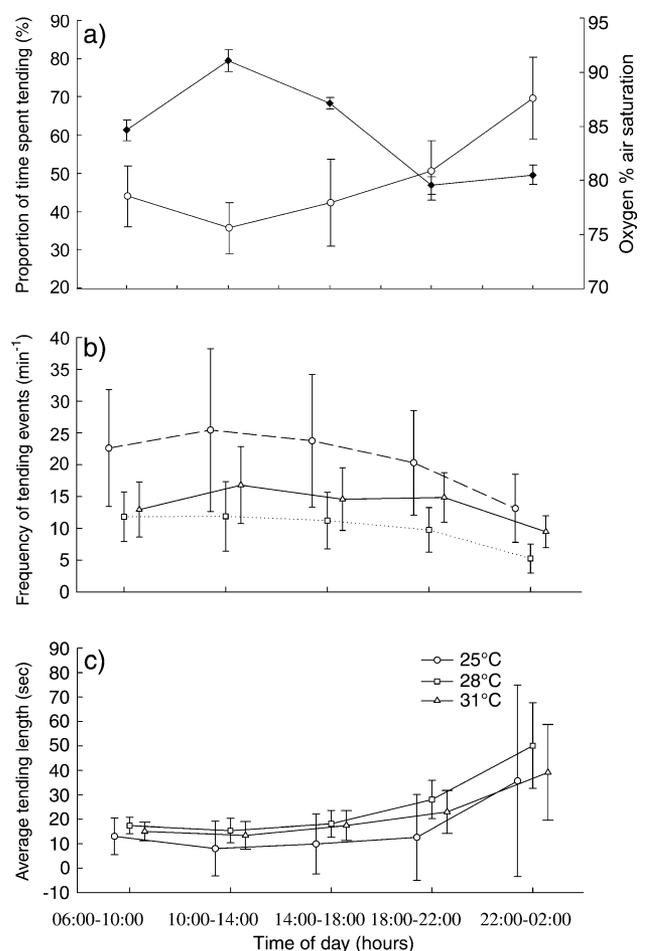


Figure 4
 Diel patterns in nest-tending activity of males summarized for five time periods throughout the day at three experimental water temperatures: (a) proportion of time spent tending (○) and ambient DO (□); (b) average number of tending events per minute; and (c) average length of tending event. No significant temperature effect in (a), so temperature is not given. Symbols for (b) and (c): ○, 25°C; □, 28°C; and △, 31°C. Error bars are 95% confidence intervals.

Table 5

Test of the combined effect of temperature, time of day, and developmental stage of the proportion of time males spent tending their eggs

a. Source	Effect df	Error df	Pillai's trace	F	p
Time of day	4	15	0.91	40.13	<.001
Time of day × stage	8	32	0.53	1.46	.211
Time of day × temp	8	32	0.80	2.67	.023
Time of day × stage × temp	16	72	0.77	1.08	.392
b. Source	df	MS	F	p	
Stage	2	0.32	21.03	<.001	
Temp	2	0.04	2.46	.114	
Stage × temp	4	0.01	0.71	.593	
Error	18	0.01			

(a) Repeated-measures MANOVA, using Pillai's trace, and (b) ANOVA results. Significant results are denoted in bold type. Temp, temperature.

while mouthing remained at a constant level. In addition to oxygenation, fanning is thought to remove metabolic wastes (Keenleyside, 1991), while mouthing removes dead larvae and cleans live ones (Keenleyside, 1991). Parental care is vital in maintaining the health of a brood in many organisms (e.g., crabs, Baeza and Fernandez, 2002; amphipods, Dick et al., 1998; fish, Knouft et al., 2003), through oxygen replenishment and waste and bacteria removal, and antimicrobial egg cleaning, which in turn increases the reproductive success by increasing the chances of offspring survival.

Diel patterns in ambient oxygen and tending

Parental tending changed with time of day and ambient DO levels. Although oxygen levels were not directly manipulated, there was a clear relationship between ambient oxygen availability and tending. As ambient DO decreased after dark, when algal photosynthesis ceases and respiration dominates, parental tending activity increased. Parental tending was reduced from dawn throughout the daylight hours, corresponding to increasing DO. We conclude that fish are adjusting their nest-tending behavior on a diel basis according to the availability of DO and consequently minimizing the costs of parental care.

Time invested in nest tending is the product of frequency and duration of tending events. Subtle differences in the composition of tending behavior were apparent in *A. melanopus* over a 24-h cycle, whereby night tending bouts were longer and less frequent than day bouts. Similar trade-offs compensating for a DO decrease have been described in *Gasterosteus aculeatus*, the three-spined stickleback (Reebs et al., 1984), where despite the increase in nocturnal bout length, an overall difference in time spent tending was not detected. Diel patterns of tending are rarely considered in fishes, despite evidence that differences in day and night exist (Hinch and Collins, 1991; Reebs et al., 1984). Typically, tending is observed and quantified once a day for a short period (10 min) (Bjelvenmark and Forsgren, 2003). Subtle adjustments in behavior to environmental changes may be overlooked without multiple measures of tending over both day and night.

Nest-directed tending throughout embryonic development

In addition to diel variation in tending, *A. melanopus* demonstrated a change in tending over the course of embryonic development. Time spent tending the nest was low in the early embryonic stages and then increased as embryogenesis progressed and embryonic oxygen consump-

tion increased (Green, 2004), aside from a peak in O₂ consumption midway through development, which coincided with the appearance of hemoglobin within the circulatory system (Green, 2004).

Such changes in time spent tending over development, coupled with the real-time correlation between tending and oxygen level, suggest that males increased their nest tending to compensate for the increased requirements of their developing offspring. However, the present study is the first demonstration in fishes, or to our knowledge, any aquatic vertebrate, of real-time changes in oxygen relative to parental care and changes in embryonic metabolism and parental care. Similar adjustment of parental tending to increasing metabolic needs of offspring throughout development has been demonstrated in a marine crab, *C. setosus* (Baeza and Fernandez, 2002), demonstrating an increased cost to the parent (and therefore investment) as the brood develops. Quantity of nest fanning has been negatively correlated to oxygen concentration within fish nests throughout development (Jones and Reynolds, 1999a; Reebs et al., 1984; Takegaki and Nakazono, 1999; Torricelli et al., 1985; van Iersal, 1953), and Baeza and Fernandez (2002) demonstrated a definite link between active brood care, oxygen provision, and embryonic development in the crab *C. setosus*.

Temperature effects on tending behavior

DO decreases with increasing temperature, while the metabolism of poikilotherms increases with increasing temperature. Considering this, we expected an increase in temperature to increase parental tending; however, *A. melanopus* did not modify their nest-directed tending in response to a temperature increase or decrease of 3°C (this range representing the boundaries of the temperature fluctuations they experience in the wild). The small sample size of nest tending in the 25°C temperature treatment may have reduced the likelihood of detecting a significant change in parental tending at a decreased temperature. However, this does not explain the lack of change in parental tending to an increase in temperature. As temperature increases, the relative reduction in DO decreases, that is, there is an exponential decrease (Broecker and Peng, 1974), such that the reduction in DO with increased temperature at tropical temperatures is small (approximate q10: O₂ solubility, 28°C–31°C = 0.15 cf. 10°C–13°C = 0.24). It is possible that the small difference in DO caused by the experimental 6°C range among temperature treatments was not enough to limit the oxygen available to the embryos. Further, the efficient oxygen uptake mechanisms of

fishes may preclude the small oxygen change resulting from increased temperature having much impact, except during times of high demand (Fry, 1971).

Nest-tending trade-offs

Fanning a nest to provide oxygen to the eggs can be the most demanding part of parental care (van Iersal, 1953). Through active nest tending, parents are resolving a behavioral conflict between present and future reproductive success. Theory predicts that parents should maximize lifetime reproductive success by allocating tending effort according to the costs and benefits derived from investing in current broods relative to those forfeited for future broods (Sargent and Gross, 1993). On one hand, active nest tending increases the survival of the current brood, while on the other hand, tending may reduce parental condition by the amount of energy allocated to tending or missed feeding opportunities and, therefore, can compromise future reproduction (Sargent, 1985; Zink, 2003). Adjusting parental care to the prevailing conditions and to propagule requirements can optimize this trade-off by reducing the costs to the parents while providing sufficient parental care for the offspring.

Relevance to field studies

This study examined tending in a laboratory, free from predators, and did not mimic a tending fish's natural environment, where they may be faced with trade-offs between nest fanning and defending their nests from predators and competitors. All fishes of the genus *Amphiprion* have obligate associations with anemones and lay their eggs on the substrata under the shelter of the anemone's stinging tentacles (Wilkerson, 1998). Therefore, they have a natural system of defenses against predators, and time allocated to predator defense probably does not differ markedly between the laboratory and field (Sargent, 1985). Further, our results are similar to observations on wild nests of a congeneric, where the tending parents were subject to multiple natural stimuli, for which tending was found to increase on the last 2 days of development from 30% to 87% of the males time (Allen, 1972). Monitoring behavior and oxygen concentration in the laboratory has allowed us to partition the importance of temperature, oxygen, and embryonic development to the level of parental tending, without the confounding influences of irregular defense and foraging excursions. This laboratory study suggests that embryonic developmental stage, time of day, and ambient oxygen concentrations are the most important factors in determining the amount of parental nest tending.

Conclusions

Adult brood care modifies the oxygen environment of the eggs as concurrent measures of oxygen and tending illustrated. Parental tending increased with decreasing ambient DO levels and with the developmental stage of the embryo, supporting our predictions. The trends in tending and DO suggest that parents are adjusting their tending rates in response to oxygen requirements and availability, minimizing their own costs in nest tending. Previous studies have documented that fish larvae (Breitbart, 1992), juveniles, and adults (Kramer, 1987) modify their behavior and avoid water with suboptimal quantities of DO. It appears that fish have some mechanism for detecting subtle DO concentration gradients and a feedback mechanism that directs them to modify their behavior. Our study suggests that parental

tending of benthic clutches actively replenishes oxygen supply around the eggs. Being able to detect and respond to subtle differences in oxygen concentration will have obvious fitness advantages to the parents if it leads to greater survival of offspring while optimizing parental input.

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