RESPONSES OF THE EGG-TENDING GOBIID FISH VALENCIENNEA LONGIPINNIS TO THE FLUCTUATION OF DISSOLVED OXYGEN IN THE BURROW

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ABSTRACT

Responses of the egg-tending male Valenciennae longipinnis (Gobiidae), a species inhabiting the near-shore moat on coral reefs, to the fluctuation of dissolved oxygen (D.O.) concentration in its enclosed burrow, were studied. D.O. concentration in egg-tending burrows decreased from high tide to low tide during the daytime. D.O. concentrations in 12 egg-tending burrows at low tide ranged from 2.17–4.50 ml D.O. L⁻¹ (43.8–91.9% D.O. saturation) on the day after spawning. In the laboratory, an egg-tending male increased the frequency and duration of fanning as D.O. concentration decreased. Below about 1.2 ml D.O. L⁻¹ (about 25% D.O. saturation), the frequency of fanning began to decrease. The frequency and duration of fanning increased with developmental stage of the eggs, perhaps in response to an increase in oxygen consumption of eggs with developmental stage.

The rapid adjustments of fanning behavior to D.O. concentration in the burrow and developmental stage of the eggs showed indirectly that the primary role of fanning is a supply of oxygenated sea water to the eggs.

Dissolved oxygen (D.O.) availability can affect the ecology of fish not only through its direct effects on survival but also through the availability of energy for locomotion, growth and even reproduction (Kramer, 1987). Tidepools and heavily vegetated swamps and marshes are often subject to hypoxic conditions at night because of respiration and lack of photosynthesis by algae or aquatic plants. In such situations, fishes show locomotory behavior to avoid low oxygen, or engage in aquatic surface respiration to acquire D.O. effectively (e.g., Congleton, 1980; Saint-Paul and Soares, 1987). The effects of low oxygen availability on parental behavior in fishes have not been widely studied (Kramer, 1987).

Two distinct functions are served by the parental care of eggs among fishes: protection against predation, and promotion of normal growth and development (Keenleyside, 1979). Various parental styles have been reported among fishes (Thresher, 1984; Barlow, 1991; Turner, 1993; Kuwamura, 1997). In some species, eggs are carried on the surface of the parent’s body or inside the buccal cavity. Egg-carrying is a highly efficient parental strategy for avoiding predation and unsuitable water conditions. However, the most common form of parental care is brooding eggs on the substrate (Blumer, 1982; Gross and Sargent, 1985; Kuwamura, 1987). Gobiid fish are typically substrate brooders, constructing a burrow, attaching their adhesive eggs to its surface and caring for them (Breder and Rosen, 1966; Thresher, 1984). The egg-tending males often display fanning behavior to create a current of water which removes metabolic waste products and increases oxygen concentration near the eggs (see Keenleyside, 1978, 1979, 1991; Potts, 1984). Although the burrow may be effective for guarding against predation of the eggs, the supply of oxygenated water may be reduced. Moreover, on coral reefs, where gobiid fishes reach their peak diversity and abundance (Thresher, 1984; Nelson, 1994), D.O. concentration in the water fluctuates considerably during the day because of the influence of biological factors (photosynthesis or respiration by corals and algae) and physical factors (tidal levels or cur-
rents) (e.g., Kraines et al., 1996). Such fluctuations may affect D.O. concentration in the goby’s burrow, and therefore male parental behavior. Some stickleback fishes, which live in tidepools, increase the frequency of fanning and time spent fanning under hypoxic conditions (van Iersel, 1953; Reebs et al., 1984) and the cichlid fish *Heronilapia multispinosa*, which lives in shallow, heavily vegetated, flood plain pools, carries the non-swimming wrigglers from the substrate pits to leaves of submerged aquatic plants to avoid lower oxygen concentration (Courtenay and Keenleyside, 1983). But D.O. concentrations in the burrows of egg-tending gobies have not been reported, and little is known about relationships between egg-tending behavior and D.O. concentration in the burrow (Torricelli, 1985).

*Valenciennnea longipinnis* is a goby that lives in the near-shore moat on coral reefs. The species is distributed in the west Pacific Ocean, including the Ryukyu Islands (Yoshino, 1984), being found in pairs in shallow sandy areas (Hoese and Larson, 1994; Takegaki and Nakazono, 1999a,b). Takegaki and Nakazono (1999a,b) have reported the reproductive ecology of *V. longipinnis* in detail. A paired male and female excavate several burrows under coral pavement and rubble within their home range. Each burrow has several openings, but all entrance usually being covered with substratum-derived materials: dead-coral fragments, pebbles, shells, sand and algae. The pair spawns in one of the burrows monogamously. Eggs are connected to each other with adhesive filaments, forming an egg mass. The egg mass on the ceiling of the burrow is tended continuously by the male for 3–5 d until hatching. The male remains beneath the eggs in a normal posture intermittently fanning the eggs with his pectoral fins while undulating his body. The paired female closes the entrance with substratum-derived materials from outside and constructs a conspicuous mound by carrying and piling up the same materials onto one of the openings, except for the entrance. The remaining openings are thinly covered with the same materials. Although the mound is constructed mainly by the male before spawning, only the female continues in its construction following spawning until hatching.

In this study, we measured diurnal changes of D.O. concentration in the closed egg-tending burrows of *V. longipinnis* in the field. Then, in an aquarium, we investigated the effects of D.O. concentration on male egg-tending behavior. Because parental behavior is usually affected by the developmental stage of eggs (van Iersel, 1953; Sevenster, 1961; Torricelli et al., 1985; Lindström and Wennström, 1994), we examined the relationship between egg-tending behavior and age of the eggs.

**Methods**

To investigate the diurnal change of D.O. concentration in the egg-tending burrow, a field study was carried out on the coral reefs along the western beach of Sesoko Island (26°39’N, 127°52’E), on 19 May and 29 June 1995 and Nagahama Beach on the northern coast of Motobu Peninsula (26°42’N, 127°57’E), Okinawa, Japan, on 6 May 1996. Three burrows were investigated on the day after spawning. They were situated about 10–35 m from shore and in water 2.0 m deep. Before spawning, a water sampling tube (Teflon: 30 cm long, 0.3 cm outside diameter, 0.2 cm inside diameter) and a digital thermistor (40 cm long, 0.2 cm diameter) were inserted into the each spawning burrow (about 42 cm long; Takegaki, unpubl. data) through an entrance. *V. longipinnis* usually deposited the eggs around the center of the burrow, therefore, the sampling tube was inserted into there (15–25 cm long from the entrance). Although, as mentioned above, the entrance is closed with substratum-derived materials after spawning by the paired female, the sampling tube allowed us to sample a water (about 120 ml) from the burrow without disturbing the burrow structure. Water sampling was
performed at 25–68 min intervals. Before sampling the water, three pretreatments were performed underwater to obtain proper sample for determinand of D.O. concentration in the burrow: at first, the air bubble in the syringe was exhausted completely; second, a stagnant water in the sampling tube was withdrawn and drained out; third, about 5 ml water was withdrawn from the burrow to rinse an inner wall of the syringe and drained out. Preliminary observations using polyester resin to displace the water in four spawning burrows indicated that burrows contained more than 3000 ml of water. Therefore, sampling about 120 ml about every 45 min should have little influence on D.O. content in the burrow. To examine a precision of D.O. value sampled with the syringe, the comparisons of D.O. data in the water between sampled with a syringe and a siphon were conducted in an aquarium. The differences were less than 3.7% (Wilcoxon signed ranks test, P = 0.39, n = 11), so that precise D.O. data could be obtained from the water sampled with a syringe.

To examine the factors influencing D.O. concentration in the burrow, D.O. concentration outside the burrow and current velocity on the sea bottom were also measured. Sea water samples were collected 30 cm from the burrow with a syringe. We brought the water sample to the beach, poured it into an oxygen-bottle (93–106 ml), and fixed it by adding MnCl, solution, Alkali-iodide solution and HCl. The D.O. content of the samples was determined by the Winkler method in the laboratory of Sesoko Station, Tropical Biosphere Research Center, University of the Ryukyus. To determine D.O. saturation, water temperature and salinity were also measured. Salinity was measured with a salinometer. Current velocity was measured as follows; first, a plastic ruler (100 cm) was put on the sea bottom, and then blue-black ink (mean density ± SD = 1.02574 ± 0.00404 g mm⁻³, n = 10) was ejected on to it with a syringe. The distance of the ink-flow and its required time were recorded, and the velocity was calculated from them. It was measured 3–5 times for every D.O. measurement.

To investigate the degree of hypoxia in the egg-tending burrow, D.O. concentrations in another nine egg-tending burrows were measured on the day after spawning at Nagahama Beach during 5 May–24 June 1996. Because D.O. levels in the burrow tend to decrease from high tide to low tide (see Results), D.O. concentrations in these burrows were measured 1 h before and after low tide. To determine D.O. saturation, water temperature and salinity were also measured by the above mentioned methods. To analyze the degree of hypoxia, the D.O. data in the three burrows, which were used for investigating diurnal change of D.O. in the egg-tending burrow, as well as that in the nine burrows.

To observe responses of the egg-tending male to D.O. fluctuation, we changed D.O. concentration in an aquarium in which a male tended eggs. A male (135 mm standard length) and a female (150 mm) were captured on the coral reefs of Nagahama Beach on 4 June 1996, and kept in an aquarium (60 × 30 × 35 cm) with running sea water (27.2–28.4°C). An artificial burrow (50 × 10 × 10 cm) was made using bricks along the front glass of the aquarium. A water sampling tube was inserted into the burrow through the crevice of the bricks. All sides of the aquarium were covered with black vinyl sheets so as not to disturb spawning behavior. After spawning, we removed the female from the aquarium. D.O. concentration in the aquarium was controlled by bubbling nitrogen gas (1.0–7.5 L min⁻¹), and was checked with an oxygen meter (Toko Model TOX-90). To avoid influence of the time spent experiment on result (i.e., physical and mental fatigue of fish), the concentration was set high and low levels alternately at 6–17 min intervals (mean interval ± SD = 9.99 ± 2.81 min, n = 32). From spawning to hatching (4 d), changes of frequency and duration of fanning with D.O. fluctuation were observed nine times every day. Three-minute observations were conducted just after D.O. adjustment. To avoid disturbing the egg care by light, we carried out the observations after sunset (18:00–22:00) through a small opening in the vinyl sheet using a flashlight covered with red cellophane. However, observations on the day of hatching were performed between 14:00 and 16:00, because hatching occurs after sunset (Takegaki and Nakazono, 1999a). One cycle of the pectoral fins was counted as one fanning movement. The duration of fanning was measured with a stopwatch. Immediately after every observation, a water sample from the burrow was siphoned into an oxygen-bottle (90–110 ml) and fixed. The D.O. content of the samples was determined by the Winkler method. Water temperature and salinity were measured to compute D.O. saturation.
To clarify the relationship between fanning activity and developmental stage of the eggs, the oxygen consumption of an egg (major axis, 1.1 mm; minor axis, 0.4 mm; Takegaki and Nakazono, 1999a) was measured every day from spawning to 3rd day (i.e., 1 d before hatching). An egg mass (about 120,000 eggs) which was spawned in an aquarium on 4 July 1996 was transformed to another aquarium. Running sea water (26.0–27.9°C) was supplied to the egg mass. We put a part of the egg mass (mean number ± SD = 1361 ± 967 eggs, range = 379–5001 eggs, n = 36) into each of a series of oxygen bottles filled with sea water (93–106 ml, 35‰), then sealed them completely. To remove an influence of the metabolism or photosynthesis by microorganisms, an artificial sea water was used. The 12 sample bottles and four control bottles without eggs were put into a homoiothermal aquarium (26.5–27.5°C) and shaken for 8 h by a shaker to prevent hypoxia in the boundary layer around the eggs. After shaking, we opened the bottles, removed the eggs with a stainless steel wire, and then fixed them in 3% formalin. The fixed eggs were classed as either alive or dead, and were counted under a binocular microscope (mean percent dead ± SD = 1.6 ± 2.4%, range = 0–10.5%, n = 36). Because dead eggs become clouded, they were easily distinguishable from live eggs. Most dead eggs had already died before sealing the bottles. We could not remove the dead eggs before sealing because the eggs were connected to each other with adhesive filaments. The number of dead eggs did not influence the result of the experiment (see Results). Sample water in the bottle was fixed chemically, and D.O. content was determined by the Winkler method. Because oxygen consumption of an egg and larvae is generally affected by the time of day (Rombough, 1988), the experiments were carried out during the same time period every day (12:00–20:00). Oxygen consumption of an egg in each bottle was calculated as: (average oxygen content in control bottles after experiment - oxygen content in each sample bottle after experiment) / number of live eggs in each sample bottle.

To clarify the factors affecting oxygen consumption of an egg, a stepwise multiple-regression analysis was performed. The stage of egg development, the number of live and dead eggs in each bottle, and the D.O. content at the start and end of incubation in each bottle were chosen as independent variables in regression analysis. Oxygen consumption of one egg, and the number of live and dead eggs in each bottle did not show a normal distribution (Kolmogorov-Smirnov one sample test, all P < 0.05). These data were therefore transformed into natural logarithms (Kolmogorov-Smirnov one sample test, all P > 0.05). Before taking logs, we added one to the number of dead eggs in each bottle because some bottles had no dead eggs. The decisions on whether to add or remove a variable were based on the F-value (F in: F to enter = 4, F to remove = 3.96). All variables with a statistically significant (5% level) regression coefficient were identified.

Figure 1. Diurnal changes in water temperature (triangles), D.O. concentration (large circles), oxygen saturation (small circles) inside (solid plots) and outside (open plots) three egg-tending burrows and current velocity on the adjacent sea bed (diamonds). Salinity in three burrows ranged from 34.4 to 35.2‰. Broken lines with H and L indicate the time of high and low tide, respectively.
RESULTS

D.O. FLUCTUATION IN THE EGG-TENDING BURROW.—D.O. concentration in egg-tending burrows was always lower than that outside the burrows during the daytime (Fig.1). D.O. concentration in the burrows and current velocity nearby showed minimum values near the lowest tide, while D.O. concentration outside of the burrow gradually increased from high tide to low tide. D.O. concentrations in 12 egg-tending burrows (including D.O. data from three burrows shown in Fig.1) at low tide ranged from 2.17 to 4.50 ml D.O. L\(^{-1}\) (mean D.O. ± SD = 3.22 ± 0.74 ml L\(^{-1}\)). This variation in D.O. concentration did not result primarily from differences in water temperature (24.9–26.6°C), as the percent saturation also varied (43.8 to 91.9%). Two out of 12 D.O. values were below 50% oxygen saturation.

RESPONSES OF MALE FANNING ACTIVITY TO D.O. FLUCTUATION.—In the aquarium, both the frequency of fanning and the time spent fanning increased with decreasing D.O. concentration in the burrow (Fig.2). Below about 1.2 ml D.O. L\(^{-1}\) (about 25% saturation), fanning was performed without stopping, but its frequency gradually decreased (Fig.2). Under these conditions, the male sometimes displayed fanning near the burrow entrance away from the egg mass. The frequency of fanning differed significantly among egg ages in

![Figure 2](image-url)  

Figure 2. Relationship between D.O. concentration in the burrow and (a) the frequency of fanning, and (b) time spent fanning by one male held in an aquarium.
Table 1. (A) Stepwise multiple regression analysis to estimate contributions of the stage of egg development, the number of survived and dead eggs in each bottle, DO at the start of incubation and the end of incubation to the oxygen consumption of an egg. NS indicates no statistically significant association between variables. (B) ANOVA regression summary.

(A) Stepwise multiple regression (adjusted $r^2 = 0.90$)

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(B) ANOVA summary

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due to three DO ranges (below 1.2 ml D.O. L$^{-1}$: one-way repeated measures ANOVA, $F = 6.31$, P < 0.05; 1.2–2.8 ml D.O. L$^{-1}$: $F = 5.83$, P < 0.05; over 2.8 ml D.O. L$^{-1}$: $F = 16.78$, P < 0.01). Although the duration of fanning also differed significantly among egg ages in higher two D.O. ranges (1.2–2.8 ml D.O. L$^{-1}$: $F = 8.81$, P < 0.05; over 2.8 ml D.O. L$^{-1}$: $F = 16.59$, P < 0.01), it had no variance below 1.2 ml D.O. L$^{-1}$: male fanned without resting. Both behavioral responses gradually increased with egg age (Fig.2).

Oxygen Consumption of an Egg.—Oxygen consumption of an egg was influenced by both the stage of egg development and the number of live eggs in each bottle (Table 1). Both factors could account for 90% of the variance in oxygen consumption of an egg. The sign of the regression coefficient indicated that oxygen consumption of an egg increased with the development of the eggs (mean consumption ± SD = 0.008 ± 0.002 µml h$^{-1}$ on spawning day, 0.011 ± 0.003 µml h$^{-1}$ and 0.019 ± 0.005 µml h$^{-1}$ on 2nd and 3rd day, respectively) and decreased with the number of live eggs in each sample bottle. The number of dead eggs and the D.O. concentration at the start and end of incubation did not contribute significantly to the variance in the oxygen consumption of an egg.

Discussion

D.O. concentration in the egg-tending burrow of V. longipinnis gradually decreased from high tide to low tide during the daytime, while D.O. concentration outside of the burrow increased. At low tide, the exposed reef-edge divided the moat from the outer-reef, and the water in the moat stagnated as in a tidepool. The decrease of D.O. concentration in the burrow appeared to be caused by the reduction in exchange of water between the inside and outside of the burrow during this stagnant period. D.O. concentrations in two out of 12 egg-tending burrows at low tide were below 50% oxygen saturation.

The egg-tending male rapidly increased the frequency of fanning and time spent fanning with decrease of D.O. concentration in the burrow. One of the roles of fanning is to supply oxygenated water to eggs for their survival and normal development (Keenleyside,
1978, 1979, 1991; Potts, 1984). Therefore, a male should increase its fanning frequency and time in response to low oxygen concentration in the water, as reported in some sticklebacks (van Iersel, 1953; Potts, 1983; Reebs et al., 1984) and a freshwater goby (Torricelli et al., 1985). The results of D.O. fluctuation in the egg-tending burrows suggest that the male’s burden of egg care at low tide is much larger than that at high tide. Below 1.2 ml D.O. L\(^{-1}\) (about 25% D.O. saturation), the frequency of fanning decreased irrespective of the developmental stage of the eggs. This seemed to be caused by the physiological constraint to the male because of the limited oxygen.

Moreover, the frequency and duration of fanning increased with the development of eggs. This may be a response to increase of oxygen requirement of the eggs with their stage of development. Increased fanning with the development of eggs has also been reported for some stickleback fishes (van Iersel, 1953; Sevenster, 1961; Reebs et al., 1984) and gobiid fishes (Torricelli et al., 1985; Lindström and Wennström, 1994). Lindström and Wennström (1994) also suggested that the egg-tending male Pomatoschistus minutus should be more willing to invest in older eggs with higher fitness value to the male because of their higher probability of hatching successfully. The rapid adjustments of fanning behavior by male V. longipinnis to D.O. concentration in the burrow and developmental stage of the eggs showed indirectly that the primary role of fanning is a supply of oxygenated water to eggs.

The oxygen consumption of an egg decreased with the number of live eggs sealed in the incubation bottles. Under hypoxic conditions, not only adult and larval fish but also eggs consume less oxygen (respiratory dependence; Fry, 1957). The oxygen consumption of an egg in this study did not correlate with D.O. concentration in the bottles at the start or the end of the experiments. Thus, the decreased oxygen consumption of an egg with the number of live eggs in each bottle should be caused not by the low oxygen in the bottles but by the insufficient supply of oxygen to the inside of the egg mass in the bottles, despite of use of a shaker in this experiment. Similar results were reported in Atlantic herring Clupea harengus harengus (McQuinn et al., 1983) and stickleback Gasterosteus aculeatus (Reebs et al., 1984) as a density or crowding effect. Many amphibians also lay their eggs in gelatinous masses and the eggs deep inside the mass are exposed to lower D.O. concentration than those closest to the surface of the mass (e.g., Burggren, 1985; Seymour and Bradford, 1995). Oxygen deprivation may lead to development retardation or death of eggs. Some dead or undeveloped V. longipinnis eggs were actually observed near the substrate in an egg mass (Takegaki unpubl. data). Therefore, male V. longipinnis may have to supply oxygenated sea water not only to the surface of the egg mass but also to the inside of the egg mass. This was also suggested by the fanning behavior under almost oxygen saturated conditions.

Male V. longipinnis fanned near the burrow entrance away from an egg mass under hypoxic conditions. Although the burrow entrance was covered with dead coral-fragments, rubble, shells, sand and algae (Takegaki and Nakazono, 1999a), it may have had enough openings to exchange the water. Similar parental behavior was reported in ice goby Leucopsarion petersi, fanning eggs beneath the eggs or near the burrow entrance alternately in the closed burrow (Akiyama and Ogasawara, 1994). For these substrate brooders, the promotion of water exchange in the burrow may be one of the important roles of fanning.
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LITERATURE CITED


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