

Air breathing of aquatic burrow-dwelling eel goby, *Odontamblyopus lacepedii* (Gobiidae: Amblyopinae)

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Summary

Odontamblyopus lacepedii is an eel goby that inhabits both coastal waters and intertidal zones in East Asia, including Japan. The fish excavates burrows in mudflats but, unlike the sympatric amphibious mudskippers, it does not emerge but stays in the burrows filled with hypoxic water during low tide. Endoscopic observations of the field burrows demonstrated that the fish breathed air in the burrow opening; air breathing commenced 1.3 h following burrow emersion, when water P_{O_2} was ~ 2.8 kPa, with an air-breathing frequency (f_{AB}) of 7.3 ± 2.9 breaths h^{-1} (mean \pm s.d., $N=5$). Laboratory experiments revealed that the fish is a facultative air breather. It never breathed air in normoxic water ($P_{O_2}=20.7$ kPa) but started bimodal respiration when water P_{O_2} was reduced to 1.0–3.1 kPa. The fish held air inside the mouth and probably used the gills as gas-exchange surfaces since no rich vascularization occurred in the mouth linings. As is known for other air-breathing fishes, f_{AB} increased with decreasing water P_{O_2} . Both buccal gas volume (V_B) and inspired volume (V_I)

were significantly correlated with body mass (M_b). At a given M_b , V_I was nearly always equal to V_B , implying almost complete buccal gas renewal in every breathing cycle. A temporal reduction in expired volume (V_E) was probably due to a low aerial gas exchange ratio (CO_2 elimination/ O_2 uptake). Air breathing appears to have evolved in *O. lacepedii* as an adaptation to aquatic hypoxia in the burrows. The acquisition of the novel respiratory capacity enables this species to stay in the burrows during low tide and extends the resident time in the mudflat, thereby increasing its chances of tapping the rich resources of the area.

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Key words: Gobiidae, intertidal mudflat, aquatic hypoxia, air-breathing fish.

Introduction

Air-breathing fishes continue to attract the attention of biologists because of their illustrative value as models for understanding the evolution of vertebrate air breathing and the transition from water to land (Graham and Lee, 2004). Many air-breathing fishes occur in freshwater, estuarine and marine habitats (Randall et al., 1981; Graham, 1997; Martin and Bridges, 1999). Estuarine and marine air-breathing fishes, in general, are dominated by highly derived groups of amphibious species (e.g. mudskippers and rockskippers), which routinely emerge during low tide (Graham, 1976). Such a conspicuous lifestyle arguably makes them the most extensively studied group among marine air-breathing fishes (Graham, 1976; Sayer and Davenport, 1991; Bridges, 1993; Clayton, 1993; Martin, 1995). Nevertheless, there appears to be a gap in knowledge concerning the evolutionary transition from aquatic (i.e. does

not emerge from water) to amphibious air breathers in marine fishes. Furthermore, there are no well-established reports on the occurrence of aquatic air breathers in the intertidal mudflat partly because of their fossorial character and the inability to emerge, which make them hardly noticeable in their natural habitat.

The eel goby, *Odontamblyopus lacepedii* (Temminck and Schlegel) (Gobiidae: subfamily Amblyopinae), does not emerge from water but stays inside the burrows during low tide. This fish is widely distributed in East Asia, including southern China, Japan, Korea and Taiwan (Murphy and Shibukawa, 2001). In Japan, the distribution of *O. lacepedii* is limited to Ariake Bay (Dotsu, 1957). Although the physical characteristics of *O. lacepedii* burrows are known – having 4–9 openings and extending to a depth of up to 90 cm (Dotsu, 1957) – the burrow microenvironment has not been previously

described. Since fish burrows, in general, contain hypoxic water (Gordon et al., 1978; Atkinson and Taylor, 1991; Ishimatsu et al., 1998a), burrow-dwelling fishes, including *O. lacepedii*, therefore, have to develop a suite of behavioral, morphological and physiological adaptation mechanisms to maintain their metabolic requirements (Johansen, 1970; Graham, 1997).

Anecdotal evidence suggests the possibility of air breathing in the eel goby. In 1997, Graham classified a species of eel goby from India, *Taenioides rubicundus*, as an air breather (Graham, 1997) based on Hora's description (Hora, 1935) of a group of fish floating with inflated gill chambers at the surface of an aquarium containing foul water. Native fishermen of *O. lacepedii* in the Saga Prefecture, Japan have also constantly observed a similar behavior when they transport live individuals to markets, suggesting the presence of air in the buccal cavity.

The main purpose of the present study was to determine if the eel goby, *O. lacepedii*, breathes air under natural conditions in the mudflat burrows and to examine the effects of aquatic hypoxia as a trigger to switch from aquatic to bimodal gas exchange by laboratory experiments. We also investigated the air-breathing behavior and selected air-breathing variables, such as buccal gas (V_B), inspired (V_I) and expired (V_E) volumes, in order to obtain insights into the aerial respiratory performance of the fish.

Materials and methods

Field-burrow observations and measurement of burrow-water P_{O_2}

In situ video observations of *O. lacepedii* burrows ($N=15$) were conducted from May to June 2005 on an intertidal mudflat at Ashikari, Saga Prefecture, Japan ($33^{\circ}12' N$; $130^{\circ}13' E$). *Odontamblyopus lacepedii* burrows can be readily identified by the presence of a mound in the opening. During the observation period, the burrows were exposed to air for 8–11 h depending on tidal fluctuation, and the air temperature ranged from 20.5 to 30.6°C. Daytime observations (2–8 h) were made during low tide using an endoscope camera (10 m long; model HSCI-S10M; HOGA, Kyoto, Japan) placed directly over a burrow opening. The tip of the endoscope was mounted ~10 cm above the water surface of the burrow, and recording instruments were situated in a vehicle on a nearby platform 3 m above the mudflat surface to avoid interference with the fish. Video images were saved in a 250-GB HDD DVD video recorder (model DR-MX5; Victor Co., Kanagawa, Japan) and analyzed for air-breathing behavior and frequency (f_{AB}).

Measurements of burrow-water P_{O_2} were made during low tide concurrent with the months of field-burrow observations. Water samples were slowly withdrawn at depths of 10–15 cm below the surface into a 5 ml glass syringe tipped with plastic tubing. The syringe was previously cleared of its dead space volume by flushing with burrow water. Samples were placed in an icebox and quickly transported to the Saga Prefectural

Ariake Fisheries Research and Development Center for P_{O_2} analysis (5–10 min after sampling). Burrow-water P_{O_2} was measured with a Blood Gas Meter (Cameron Instruments Co., Port Aransas, TX, USA) thermostatically regulated to the temperature of the burrow water, which ranged from 20 to 24°C.

Laboratory investigations of air breathing in *O. lacepedii* Collection and maintenance of fish

Specimens of *Odontamblyopus lacepedii* were captured by set nets at 3–5 m depths in an estuary at Ariake Bay, Japan ($33^{\circ}10' N$; $130^{\circ}15' E$) between August and October 2003. The area can be characterized as having high-turbidity water with salinity varying from 0.1 to 31.6‰ and temperature from 10.3 to 33.0°C during the collection period. Fish were transported to the Institute for East China Sea Research, Nagasaki University and kept individually in a 60-l glass aquarium, half-filled with 50% seawater (17‰). Each aquarium was provided with an artificial shelter of either clay or polyvinyl chloride (PVC) pipes and was attached to a recirculating system equipped with a thermostat ($25 \pm 1^{\circ}C$) and aeration supply. Fish were fed chopped fish or squid to satiation 4–5 times per week. They were kept in these conditions for at least a month before being subjected to experimentation. All experiments were conducted in 50% seawater (17‰) at a temperature of $25 \pm 0.5^{\circ}C$. Fish were starved for 24 h prior to use. We did not differentiate between sexes.

Observation of air-breathing behavior

Air-breathing behavior was observed in a perforated L-shaped fish chamber submerged in a temperature-controlled water bath. The length (45 cm) and diameter (5.2 cm) of the horizontal bottom section provided enough space for the fish to move freely inside the chamber. The vertical section (length, 25 cm) had a tapered opening (diameter, 3.6 cm) at the top where fish could access air. Two digital video cameras (model DCR-TRV20; Sony Corp., Tokyo, Japan) were mounted on the top and side of the chamber for video observation and recording. The water bath ($65 \times 16 \times 30$ cm depth) was screened off with a black plastic sheet to shield the fish from laboratory activities.

Fish (23.1–128.3 g body mass; $N=14$) were acclimated overnight in the chamber before experiment. A steady flow (5 l min^{-1}) of well-aerated water was gently circulated during acclimation. The same flow rate was used during stepwise exposure to aquatic hypoxia ($P_{O_2}=20.7, 10.4, 6.2, 3.1$ and 1.0 kPa) by bubbling N_2 into the equilibration column (see Fig. 1). Water P_{O_2} was regulated and maintained with a DO controller (model MC-7W; Iijima Electronics Corp., Aichi, Japan) and continuously monitored with an O_2 meter (model DO-14P; TOA Electronics Ltd, Tokyo, Japan). Observation of air-breathing behavior was made for 2 h at each P_{O_2} level. Transition time between P_{O_2} levels was 3–8 min. The air-breathing behavior of individual fish was analyzed with a video cassette recorder (model WV-D9000; Sony Corp., Tokyo, Japan).

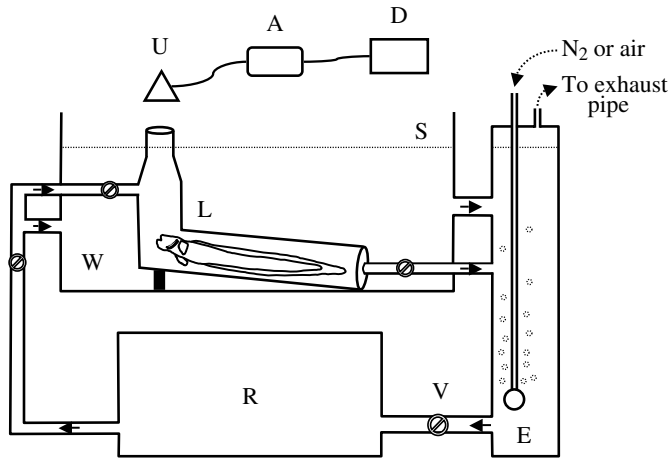


Fig. 1. Schematic diagram of the experimental setup used for the determination of inspired (V_I) and expired (V_E) volumes in *Odontobutypus lacepedii*. A, amplifier; D, data-acquisition system; E, equilibration column; L, L-shaped fish chamber; R, water reservoir; S, water surface; U, ultrasonic sensor; V, ball valve; W, water bath. Sensors for a DO controller and a DO meter, thermoregulator and pump are located inside the water reservoir. Arrows indicate the direction of water flow.

Measurement of V_B

Fourteen fish weighing 20.8–113.5 g were placed individually in a PVC pipe (length \times diameter; 34 \times 3.8 cm or 44 \times 4.4 cm, depending on fish size). The pipe was diagonally immersed in a water tank (80 \times 60 \times 50 cm depth), and the lower opening was covered with a plastic screen to hold the fish inside while allowing water to flow in and out of the pipe. A portion of the upper opening was positioned above the water level so that fish had access to air and at the same time preventing the fish from escaping. After 3–4 h acclimation, water P_{O_2} was rapidly lowered and maintained at 1 kPa. When fish gulped a mouthful of air, the whole PVC pipe was slowly immersed in a vertical position, and an inverted glass funnel filled with water was immediately positioned above the upper opening. The funnel was fitted with a 20-gauge syringe needle attached to PE-90 tubing. Because the fish has a tendency to hold its breath for a long period of time and the volume of gas reduces with time (see Results), the mouth was poked with a blunt rod immediately after inspiration to forcibly expel the newly inhaled air. The gas trapped in the funnel was withdrawn and its volume measured in a 5 or 10 ml plastic syringe. Suctioning of gas from the funnel was done rapidly after expiration to prevent the fish from re-gulping the air. Air in the buccal cavity was considered to be completely expelled when fish started gill ventilation. Buccal gas volume was measured 5–7 times in each fish.

Measurement of V_I and V_E

V_I and V_E were measured in an L-shaped fish chamber (Fig. 1). After overnight acclimation in recirculating normoxic water, the fish (21.2–113.9 g body mass; $N=14$) was exposed to aquatic hypoxia ($P_{O_2}=1.0$ kPa) by bubbling N_2 into the

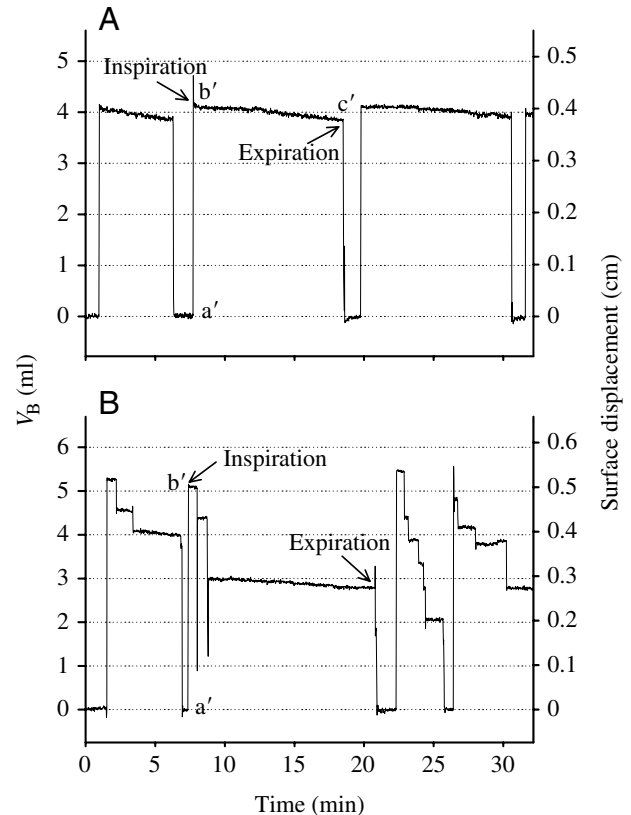


Fig. 2. Representative traces of air-breathing cycles in *O. lacepedii*. Inspired (V_I) and expired (V_E) volumes were obtained by subtracting a' from b' and a' from c' , respectively. Note that V_E can be obtained only in (A), when the inspired air was kept intact in the buccal cavity until expiration. In (B), the fish released several bubbles prior to expiration. Body mass of fish used for A and B were 60.6 g and 81.1 g, respectively.

equilibration column. When fish commenced air breathing, both inlet and outlet valves of the L-shaped chamber were closed.

Fish inspired air from the top opening (diameter, 3.6 cm) of the chamber and, upon descent, displaced the water level upwards (Fig. 2). Conversely, downward displacement occurred during expiration. An ultrasonic sensor (model E4DA-LS7; Omron Corp., Tokyo, Japan), securely positioned directly on top of the opening (about 4 cm above the water surface), continuously detected and output the changes in the water level. The output signals were amplified (model E4DA-WL1C; Omron Corp.) before recording on a data-acquisition system (model NR-1000; Keyence Corp., Osaka, Japan) at 1-s intervals. V_I and V_E were obtained by taking the difference of the deflection caused by inspiration and expiration, respectively, from the baseline value (Fig. 2A). *In situ* calibration of the system was done after each experiment by stepwise addition of 1 ml water from a pipette into the chamber while the fish was gill-ventilating at the bottom. The slope of the line derived from the linear regression of the calibration curve was used to calculate V_I and V_E . Some of the fish subjected to V_B measurement were used in this experiment

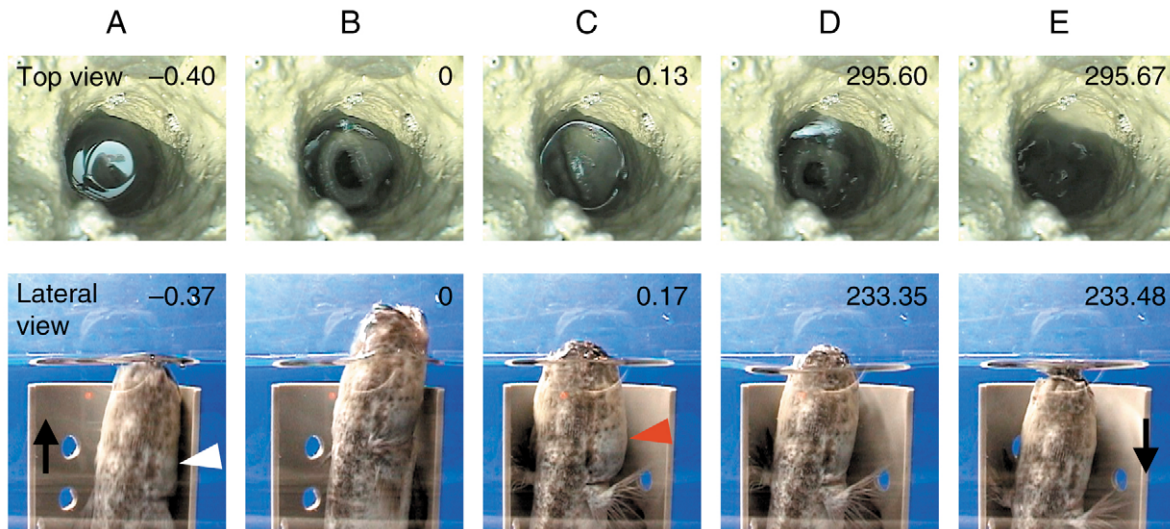


Fig. 3. Representative video sequences of an air-breathing cycle in *O. lacepedii* in the field burrow (top view), with corresponding footage taken from the laboratory chamber (lateral view). To breathe air, the fish approaches the surface with the mouth closed and the buccal floor and opercula adducted (white arrowhead, A). Upon breaking the surface, the mouth opens to gulp air (B) and closes immediately, with the buccal floor and opercula fully abducted (red arrowhead, C). After several minutes of breath holding, the mouth reopens to release the air (D) and the fish submerges (E). Values in the top right corner of each frame indicate the time interval (s) from the onset of inspiration. Black arrows indicate the movement of the fish. See supplementary material for further details.

more than a month after the last time they had been used. Measurement lasted for 4 h, which was sufficient to obtain at least seven ventilatory replicates per fish.

Statistical analyses

Values are reported as means \pm standard deviation (s.d.), wherever appropriate. Changes in the burrow-water P_{O_2} over time were fitted to the equation for exponential decrease: $y = y_0 + ae^{-bx}$, where y = burrow-water P_{O_2} , x = time after burrow emersion, y_0 = asymptote of a curve (Riggs, 1963). Statistical differences of air-breathing frequency (f_{AB}) and breath-holding duration at different levels of hypoxia were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's test to identify the data that significantly differed from control values. Significant correlation was determined between body mass (M_b) and V_B and between M_b and V_I by linear regression analysis. The slopes and y-intercepts of V_B and V_I were compared using analysis of covariance (ANCOVA) and t -test for comparing two elevations (Zar, 1984), respectively. Results were considered statistically significant at $P < 0.05$.

Results

Air breathing in the burrow

Seven out of the 15 burrows contained *O. lacepedii*, but in only five burrows did fish perform air breathing. In the other two burrows with fish, water level intermittently moved up and down, suggesting activity inside. It is possible that the fish were breathing in other openings of the burrow. Air breathing was characterized by rapid inspiration followed by variable

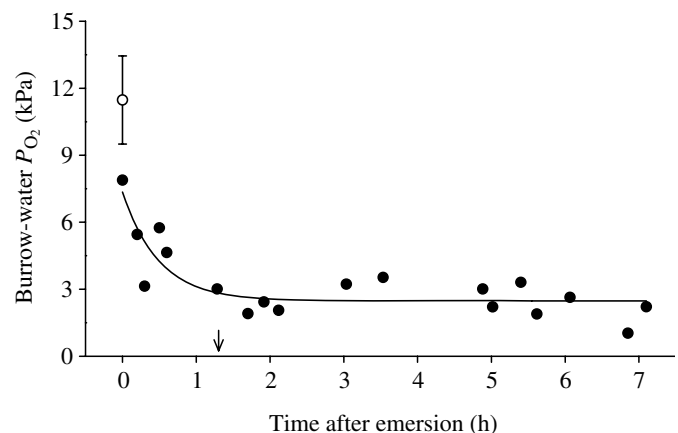


Fig. 4. Temporal profile of water P_{O_2} of *O. lacepedii* burrows (filled circles). Each filled circle corresponds to an individual burrow. Data for burrow-water P_{O_2} were fitted to the exponential decrease equation ($y = 2.48 + 4.87e^{-2.02x}$; $r^2 = 0.73$, $P < 0.0001$). The P_{O_2} of free water over the mudflat during high tide is represented by the open circle with vertical line (mean \pm s.d.; $N = 6$). The arrow indicates the onset (1.3 h) of air breathing.

periods of breath holding either in the water or at the surface before expiration (Fig. 3, top row).

There was a significant correlation between duration of burrow emersion and water P_{O_2} (Fig. 4). Burrow-water P_{O_2} was approximately 3 kPa lower than free-water P_{O_2} at the time of emersion and subsequently stabilized at a lower P_{O_2} of 2.5 kPa. Fish commenced air breathing after 1.3 h of burrow emersion, and f_{AB} was 7.3 ± 2.9 breaths h^{-1} (Table 1). Breath holding constituted $53.7 \pm 28.7\%$ of the total time.

Table 1. Effects of water P_{O_2} on aerial respiratory parameters of *Odontamblyopus lacepedii*

Water P_{O_2} (kPa)	N	% Air-breathing fish	f_{AB} (breaths h^{-1}) ^a	Breath-holding duration (% of total time of observation) ^a
Field burrow 2.5 ^b	7	71.4	7.3±2.9	53.7±28.7
Laboratory chamber				
1.0	14	100	10.8±6.1*	88.4±6.3*
3.1	14	35.7	7.9±5.5*	58.4±38.0*
6.2	14	35.7	1.5±0.9	0.1±0.1
10.4	14	21.4	2.0±2.6	0.1±0.2
20.7	14	0	0	0

^aValues presented are means ± s.d. of the air-breathing individuals.

^bBased from the asymptote of a curve of burrow-water P_{O_2} (see legend for Fig. 4).

*Significant difference from normoxic water (P_{O_2} =20.7 kPa) (Dunnett's test, $P<0.05$).

Effects of aquatic hypoxia on air breathing

Aquatic hypoxia triggered air breathing in *O. lacepedii*. The air breathing observed at a water P_{O_2} of 6.2 (five fish) and 10.4 (three fish) kPa probably has little respiratory significance considering the short breath-holding duration (Table 1). At a water P_{O_2} of 3.1 kPa, five out of 14 fish breathed air with highly variable breath-holding duration ranging from 1 to 88% of the total time. At a water P_{O_2} of 1.0 kPa, all fish breathed air, with much smaller variability in breath-holding duration ranging from 76 to 97%. Although only at a water P_{O_2} of 1.0 kPa did all fish breathe air, a significantly higher f_{AB} and breath-holding duration were found at 1.0 and 3.1 kPa than in normoxia (Table 1). There was no significant difference in f_{AB} (t -test, $t=-0.22$, $P>0.05$) or breath-holding duration (t -test, $t=-0.22$, $P>0.05$) between the burrow water (P_{O_2} =2.5 kPa) and the laboratory condition of 3.1 kPa water P_{O_2} , which implies that our laboratory protocol did not impose noticeable stress on the fish. Furthermore, the air-breathing behavior observed in the laboratory was comparable with the endoscopic data (Fig. 3). There was no significant correlation between f_{AB} and M_b ($P>0.05$). On return to normoxia, fish immediately stopped air breathing and resumed gill ventilation.

Laboratory air-breathing behavior

When preparing to inhale air, the fish approached the water surface with the mouth closed and the buccal floor and opercula adducted (Fig. 3A). After breaking the surface, the mouth opened and air was taken in quickly by the sudden distension of the buccal floor and expansion of the opercular cavity (Fig. 3B). The mouth closed, with the buccal floor and opercula fully adducted, while still above the water surface (Fig. 3C).

Four distinct types of behavior were observed following inspiration. First, the fish immediately descended while releasing bubbles through the mouth and instantaneously started gill ventilation (type 1). This was the exclusive air-breathing behavior at a water P_{O_2} of 6.2 and 10.4 kPa, where only a small percentage of fish breathed air (Table 1). Second, the fish descended and rested all the way to the horizontal

section of the chamber while holding its breath (type 2). When maneuvering to position itself underwater, several bubbles could be released through the gill slits (Fig. 2B), while some fish retained the full volume of air in the buccal cavity until expiration in the water (Fig. 2A). Third, the fish descended while holding its breath, then slowly returned to the surface and stayed motionless with part of the snout protruding out of the water until expiration in air (type 3). Fourth, the fish remained breath holding at the surface, protruding its snout out of the water until expiration in air (type 4; Fig. 3). In all cases, expiration was through the mouth and opercular pumping was often observed prior to the next inspiration. Some individuals (eight of 14) exhibited a combination of the latter three types of post-inspiratory behavior under severe aquatic hypoxia (P_{O_2} =1.0 kPa).

Fig. 5 shows the relationships of M_b and V_B and V_I . A significant correlation was found between V_B and M_b

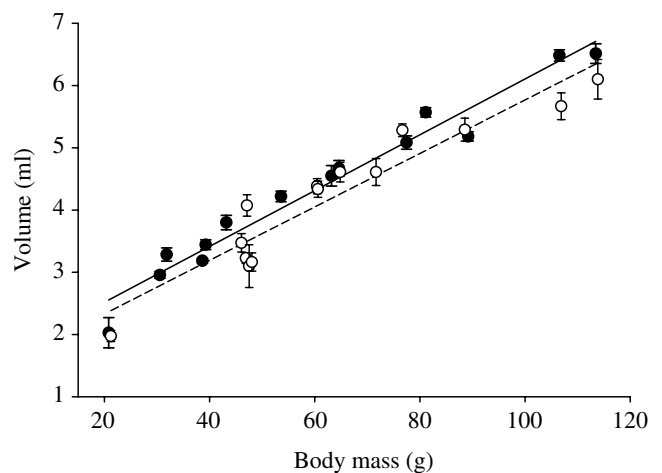


Fig. 5. Buccal gas volume (V_B) and inspired volume (V_I) in relation to body mass (M_b) for *O. lacepedii*. Filled circles and solid regression line represent V_B ($V_B=0.05M_b+1.62$; $r^2=0.96$, $N=14$). Open circles and broken regression line represent V_I ($V_I=0.04M_b+1.47$; $r^2=0.90$, $N=14$). Each symbol corresponds to mean ± s.d. of 5–7 (V_B) and at least 7 (V_I) replicates per fish.

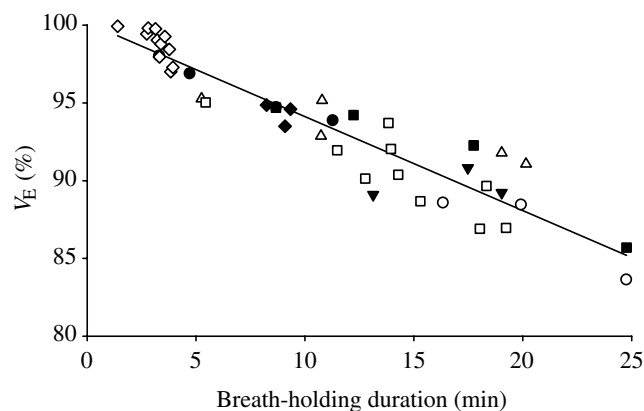


Fig. 6. Decline in V_E (expressed as % of inspired volume) as a function of breath-holding duration (BHD) in *O. lacepedii* during air breathing. Regression equation: $V_E = -0.60\text{BHD} + 100.17$; $r^2 = 0.88$, $P < 0.001$. V_E was calculated only from fish that retained the full amount of air in the buccal cavity until expiration (see Fig. 2A). Different symbols represent individual fish ($N=8$).

($V_B = 0.045M_b + 1.621$; $r^2 = 0.96$, $P < 0.001$, $N=14$) and between V_I and M_b ($V_I = 0.043M_b + 1.472$; $r^2 = 0.90$, $P < 0.001$, $N=14$). There was no significant difference between the slopes (ANCOVA, $F=0.14$, $P>0.05$) or the y-intercepts (t -test, $t=1.92$, $P>0.05$) of the regression lines of V_B and V_I .

During breath holding, the volume of gas kept in the buccal cavity diminished gradually with time (0.02 ml min^{-1}), as depicted by the composite plot of the percentage of V_E from eight individuals (Fig. 6). Breath-holding duration ranged from a few seconds to ~30 min.

Discussion

Air-breathing capability of *O. lacepedii*

The present study demonstrated for the first time the capability of *O. lacepedii* to directly utilize atmospheric air for respiration. In the field, the fish did not commence air breathing until after 1.3 h of burrow emersion when burrow-water P_{O_2} was ~2.8 kPa (Fig. 4). Similar to some freshwater aquatic mouth breathers [e.g. *Synbranchus marmoratus* (Johansen, 1966), *Amphipnous cuchia* (Lomholt and Johansen, 1974)], *O. lacepedii* inspires first and, following variable periods of breath holding, expires through the mouth leaving no residual gas.

After inspiration, some individuals immediately retreat into the water (type 2 post-inspiratory behavior) whereas others retreat into the water and return (type 3) or remain (type 4) at the surface until expiration. During breath holding, the fish stays motionless with its head floating at the surface. *Odontamblyopus lacepedii* has a V_B of 6.1% of body volume (assuming body density=1), which is larger than the air volume needed to make the whole fish body positively buoyant (Gee and Gee, 1991).

During the early phase of air-breathing episodes, the type 2 post-inspiratory behavior was usually observed, but there was an apparent tendency to shift to types 3 and/or 4 when the air-breathing cycle continued. These three types of air-breathing behavior were similarly observed in the field burrows. The shift in respiratory behavior is probably employed to minimize energy expenditure associated with movement in the water column and maintenance of buoyant head under water. Aerial exposure, however, could be highly dangerous in the field unless the fish can hide itself in some shelter (e.g. burrows). The presence of several openings in one burrow system of the eel goby (Dotsu, 1957) may also provide alternative outlets for air breathing when situation in the other openings becomes precarious for predators such as wading birds.

Like most brackish water and marine air-breathing fishes (Graham, 1997), *O. lacepedii* does not possess any diverticulum specialized for aerial gas exchange (Fig. 7). Furthermore, visual examination of the buccal cavity of freshly sacrificed *O. lacepedii* revealed the apparent lack of vascularization on the palatine and tongue epithelia (Fig. 7). The gills therefore can be inferred as the potential sites of O_2 absorption, as similarly suggested in more than 30 species of marine air-breathing fishes (Graham, 1976). However, the gills are generally considered not to be suitable for aerial gas exchange due to a reduction of functional surface area caused by gravitational collapse of the filaments. When hypoxic stress is not severe enough to necessitate bimodal respiration, such as during migration (see the section below on burrowing behavior and air breathing in *O. lacepedii*), the gills of *O. lacepedii* must meet most, if not all, respiratory and other functional requirements (e.g. ammonia release, acid-base regulation and ionic transfer). However, once *O. lacepedii* is subjected to severe hypoxia, such as during burrow confinement, the gills probably engage in aerial gas exchange, while other functions of the gills might be translocated to other sites or suspended momentarily.

The reduction in V_E further supports the capacity of *O.*

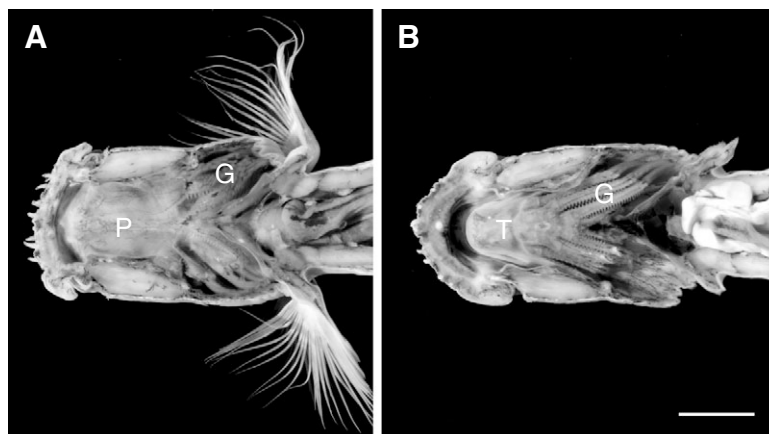


Fig. 7. Horizontal section of the head of *O. lacepedii* showing the absence of diverticulum and the apparent lack of respiratory vascularization in the upper (A) and lower (B) jaws. G, gill arches; P, palatine region; T, tongue. Fish body mass, 51 g. Scale bar, 1 cm.

lacepedii to extract O_2 from air. The temporal decline in V_E during aquatic air breathing is generally attributed to the low gas respiratory exchange ratio ($RER = CO_2$ elimination/ O_2 uptake) through the gas-exchange surfaces in the air-breathing organ (ABO), as shown for many freshwater air-breathing fishes (Abdel Magid et al., 1970; Rahn et al., 1971; Lomholt and Johansen, 1974). On the contrary, some intertidal fishes, especially the amphibious air breathers, have high aerial RER (Bridges, 1993), indicating the efficiency of their ABO not only in O_2 extraction but also in CO_2 excretion. It should however be noted that the aerial RER of these amphibious marine species was determined while the fish were exposed to air. The air breathing of *O. lacepedii* shares the same functional trait as in freshwater species, demonstrating that the early role of air breathing in fishes is oxygen uptake irrespective of the habitat salinity conditions.

Air-breath variables

The gradual increase of f_{AB} in *O. lacepedii* with decreasing water P_{O_2} is in agreement with observations for other facultative air-breathing fishes (Graham and Baird, 1982; Mattias et al., 1998; Takasusuki et al., 1998). Fishes have O_2 receptors in the gills (Perry and Gilmour, 2002), which may be particularly important in signaling a shift from gill ventilation to air breathing. When confronted with extreme aquatic hypoxia, a facultative air-breathing fish normally shifts to aerial respiration, and subsequent modulation of its aerial ventilatory responses is employed (e.g. V_B or V_I). Since V_I is equal to V_B in *O. lacepedii*, air ventilation can be augmented exclusively by way of increasing f_{AB} , assuming that the fish fully distends the buccal cavity.

The complete expiration ($V_I = V_B$) found in *O. lacepedii* can be explained by the vertical position assumed by the fish during air breathing and the apparent lack of anatomical dead space of the buccal cavity (Fig. 7). The large renewal of gas may favor its intermittent breathing pattern, which is characterized by extended periods of breath holding following inspiration. Complete expiration of each breath is common in many aquatic air-breathing species with the ABO situated in the mouth or the buccal cavity [e.g. *Synbranchus marmoratus* (Johansen, 1966), *Electrophorus electricus* (Farber and Rahn, 1970), *Amphipnous cuchia* (Lomholt and Johansen, 1974), *Ctenopoma kingsleyae* and *Osphronemus goramy* (Peters, 1978) and four species of *Channa* (Liem, 1984)]. The mudskipper, *Periophthalmodon schlosseri*, has a gas renewal of 54% (Aguilar et al., 2000). Even though it uses the buccopharyngeal cavity as its ABO, this fish normally breathes air in a horizontal position (Aguilar et al., 2000) so that incomplete expiration is highly possible.

Burrowing behavior and air breathing in *O. lacepedii*

Odontamblyopus lacepedii appears both in the coastal water, where exigency for air breathing is unlikely, and in the intertidal mudflat burrows, where strong selection for air breathing exists. Presently, it is unclear whether there are two

distinct populations (one migrating and the other burrowing) or the same individual switches between the two modes of existence depending on some internal and/or external conditions. Our limited observations demonstrated seasonal changes in burrow density on the mudflat, being high in June to August. Since these months correspond to the breeding season of the species (Dotsu, 1957), it is possible that the burrowing behavior of *O. lacepedii* is related to reproduction. Many intertidal burrowing fishes have been reported to spawn in the burrows (Clayton, 1993; Ishimatsu et al., 1998a; Ishimatsu et al., 1998b). However, as shown in this study, the water in *O. lacepedii* burrows is severely hypoxic so that eggs may not be able to develop normally without some mechanisms to ensure O_2 supply. If *O. lacepedii* does indeed spawn in the burrows, then air breathing offers a potential advantage not only for sustaining respiratory requirements of the adult during burrow confinement but also for maintaining adequate supply of O_2 to the developing embryos, as has been suggested for the mudskippers (Ishimatsu et al., 1998a; Ishimatsu et al., 1998b).

Another potential benefit of burrowing is to extend the resident time in the intertidal mudflat in order to increase the chance of tapping the rich resources of the area. Intertidal zones, including estuaries, are among the most biologically productive ecosystems in the world but, at the same time, are characterized by strong physico-chemical gradients (Levinton, 2001). By staying in the burrows during low tide, *O. lacepedii* could avoid both the fluctuations in environmental conditions (such as salinity and temperature) and the threat of aquatic predations in the estuary. Thus, air breathing is a necessary trade-off to ensure survival in the hypoxic burrow environment. The elongated body shape and highly degenerated eye structure (Murdy and Shibukawa, 2001) both suggest that selective pressure on this species has been towards a fossorial mode of life and not towards amphibious existence. The fact that *O. lacepedii* does not emerge from its burrow during low tide indicates that the fish feeds on either entrapped organisms in the burrow, as suggested for a similar eel goby, *Taenioides rubicundus* (Hora, 1936), or on some infauna of the mudflat. Alternatively, *O. lacepedii* may come out from the burrows and feed during high tide. In either case, the fish exploits the rich biological resources of the mudflat in such a way that competition with sympatric mudskippers (*Boleophthalmus pectinirostris* and *Periophthalmus modestus*) is minimized. The limited knowledge on the ecophysiology of this fish necessitates more field studies.

List of abbreviations

ABO	air-breathing organ
f_{AB}	air-breathing frequency
M_b	body mass
P_{O_2}	partial pressure of oxygen
V_B	buccal gas volume
V_E	expired volume
V_I	inspired volume

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