Burrow air phase maintenance and respiration by the mudskipper *Scartelaos histophorus* (Gobiidae: Oxudercinae)

Heather J. Lee[1,*], Callie A. Martinez[2], Katherine J. Hertzberg[3], Amy L. Hamilton[4] and Jeffrey B. Graham[1]

[1]*Center for Marine Biotechnology and Biomedicine and Marine Biology Research Division, Scripps Institution of Oceanography, University of California, San Diego, La Jolla, CA 92093-0204, USA,* [2]*Department of Integrative Biology, 3060 VLSB, University of California, Berkeley, Berkeley, CA 94720-3140, USA,* [3]*Biological Oceanography Division, Scripps Institution of Oceanography, University of California, San Diego, La Jolla, CA 92093-0236, USA* and [4]*MEC Analytical Systems Inc., 2433 Impala Drive, Carlsbad, CA 92008, USA*

*Author for correspondence (e-mail: hboyle@iacs5.ucsd.edu)

Summary

A laboratory burrow and mudflat system was used to examine aspects of burrow air-phase maintenance and utilization by the amphibious mudskipper *Scartelaos histophorus*. While confined to its burrow during simulated 'high tide', this species respires both aquatically and aerially, in the latter case utilizing an air phase it had established by transporting air into the burrow during simulated 'low tide'. Over the course of 'high-tide' confinement, burrow-water P_{O_2} declines, making the air phase more important for respiration; the burrow-water O_2 tension eliciting air-phase respiration is 4.8±0.2 kPa. At 'low tide', when the fish has access to air, it deposits new air in the air phase by transporting gulps into the burrow and releasing them. Observed air-deposition rates for both males and females were 12.3±4.5 trips h^{-1}. All of the fish tested (*N*=8 individuals + 2 pairs) deposited air and responded to experimental air-phase withdrawal by replacing the air (72 of 74 tests, 97.3%). Also, repeated tests with one fish showed that experimental reduction of the air-phase P_{O_2} by mixing with N_2 elicited a gas-expelling behavior at O_2 levels less than 10.3 kPa. At O_2 levels greater than 10.3 kPa, the fish left the air phase intact and added to it by depositing surface air.

Key words: Gobiidae, Oxudercinae, mudskipper, *Scartelaos histophorus*, burrowing biology, air phase, air-deposition behavior.

Introduction

Mudskippers constitute a group of 25 air-breathing species in four genera (*Periophthalmodon*, *Periophthalmus*, *Boleophthalmus* and *Scartelaos*) that are the most derived and the most amphibious of the ten genera of the teleost subfamily Oxudercinae (Gobiidae; Murdy, 1989; Clayton, 1993; Graham, 1997; Aguilar, 2000). These fishes spend extensive periods of time out of water and have numerous physiological, morphological and behavioral specializations for amphibious life (Gordon et al., 1969; Clayton, 1993; Graham, 1997; Lee and Graham, 2002). Mudskippers, including *Scartelaos histophorus* (Valenciennes), are unusual because they have been shown to store air in their 'J'-shaped, intertidal burrows, resulting in an air phase (Ishimatsu et al., 1998a,b; Lee and Graham, 2002). Here, we report laboratory observations on the burrow air-deposition behavior of *S. histophorus*. This study also demonstrates the importance of the burrow air phase for respiration during high-tide confinement and shows that this species utilizes both gas removal and addition behaviors to regulate the air-phase P_{O_2}.

To establish an air phase in their burrows, mudskippers must perform the air-deposition behavior. This consists of a rapidly repeated series of actions that includes inflating the buccal chamber (part of the air-breathing organ; Schöttle, 1932; Hora, 1935a,b; Graham, 1997) with air on the mudflat surface, transporting it into the burrow, releasing it, and quickly returning to the surface with a deflated buccal chamber. Because the burrows have an upturned portion that is not connected to the surface (Harms, 1929; Asano, 1936; Kobayashi et al., 1971; Brillet, 1976; Matoba and Dotsu, 1977; Clayton and Vaughan, 1986), deposited air stays under ground and forms an air phase rather than floating back to the surface (Ishimatsu et al., 1998a,b). Both the surface portion of the air-deposition behavior and the presence of a burrow air phase have been documented for several oxudercine species, including *Oxuderces dentatus*, *Scartelaos histophorus*, *Boleophthalmus dussumieri*, *Periophthalmus chrysospilos* and *Periophthalmodon schlosseri* (Ishimatsu et al., 1998a,b; Lee and Graham, 2002), however, detailed observations of the belowground part of the behavior have not yet been described.

The mudskipper burrow air phase has been hypothesized to

be either a source of oxygen for respiration, a medium for embryonic development, or both (Ishimatsu et al., 1998a,b). Here, we examine the role of the air phase in intra-burrow respiration. Mudskippers build their burrows in the littoral zone and most are located in anoxic mud (Scholander et al., 1955; MacNae, 1968a; Takita et al., 1999). During high tide, the burrows are covered by water and the fish remains confined there (for up to 10 h) for protection from predators, namely piscivorous fishes (Milward, 1974; Sasekumar et al., 1984; Clayton and Vaughan, 1988; Clayton, 1993). Many burrow-inhabiting fishes must tolerate burrow-water hypoxia due to inadequate circulation with oxygenated water (Atkinson, 1991) and, indeed, mudskipper burrow water has been shown to be severely hypoxic [less than 0.02 kPa (measurement temperatures unavailable)] (Gordon et al., 1978; El-Ziady et al., 1979; Ishimatsu et al., 1998a,b). The oxygen in the mudskipper burrow air phase may thus be important for intra-burrow aerial respiration.

S. histophorus builds burrows characterized by simple, unadorned entrances (1-2/burrow) and shafts that extend 30 cm or more into the mud (H.J.L., N. M. Aguilar-Roca, N. E. Milward and J.B.G., unpublished). The fish takes refuge in its burrow for the duration of high tide and emerges onto the mudflat a few minutes after the tide recedes. Males and females share burrows during the spawning season and both participate in air deposition, which may occur at the rate of 6–15 trips min^{-1} in the field (Lee and Graham, 2002; H.J.L., N. M. Aguilar-Roca, N. E. Milward and J.B.G., unpublished). While details of surface air transfer to the burrow are known (Lee and Graham, 2002; H.J.L., N. M. Aguilar-Roca, N. E. Milward and J.B.G., unpublished), whether the fish removes used air from its burrow is unknown.

One objective of this study was to determine if *S. histophorus* would deposit air in a laboratory burrow system, and if this behavior occurs, to quantify the rate of air-deposition. Another objective was to measure the threshold partial pressure of oxygen (P_{O_2}) at which *S. histophorus* switches from water to air breathing and determine if the fish uses the air phase for respiration during burrow confinement. The last objective was to describe how the burrow gas is refreshed after its oxygen is depleted by respiration and diffusion into the water. The results will provide insight into three important questions about mudskipper burrowing biology. (1) What is the function of the mudskipper burrow air phase? (2) How is burrow gas refreshed? (3) What volume of burrow air is needed to meet the O$_2$ requirements of *S. histophorus* during high tide?

Materials and methods

Collections

Scartelaos histophorus Valenciennes were collected by hand net from mudflats in Cardwell (18°16′S; 146°01′E) and Townsville (19°15′S;

146°50′E), Australia, and transported to the Australian Institute of Marine Science, Cape Ferguson, where they were housed in flow-through seawater aquaria for 1–3 weeks. Fish were then transported to Scripps Institution of Oceanography, La Jolla, California, and maintained in aquaria (25‰ at 25°C). Captive fish were fed *Spirulina* algal flakes (Ocean Star International, Inc., Snowville, UT, USA) until satiation three times per week.

Field observations

Field studies of *S. histophorus* were conducted during austral spring, 1999, on the mudflats of Cardwell. Binoculars were used to observe low-tide behaviors, some of which were also recorded with a digital video camera at normal shutter speed. Observation periods extended from before low tide to the time when water began to cover the habitat. Tidal data were obtained from the National Tidal Facility, Flinders University of South Australia, and the Queensland Department of Transport.

Burrow system

Above- and belowground aspects of the behavior of *S. histophorus* related to the burrow air phase were studied in the laboratory using a specially constructed burrow system (Fig. 1). The system consisted of a 625 ml plastic bottle (the burrow chamber) connected to a rectangular plastic container (the mudflat) (30 cm×15.5 cm×11 cm, l × w × d) by a 27 cm plastic tube (2 cm diameter) (Fig. 1). Silicone adhesive was used to join the tubing and container. Bottle neck and tubing diameters were slightly larger than the body diameter of the largest specimens studied. The angle of the tube was adjusted so that the fish had to exit the burrow at least partway in order to release air to the surface.

Continuous water-phase P_{O_2} sampling was achieved with a burrow-system modification in which a 1 l plastic bottle with
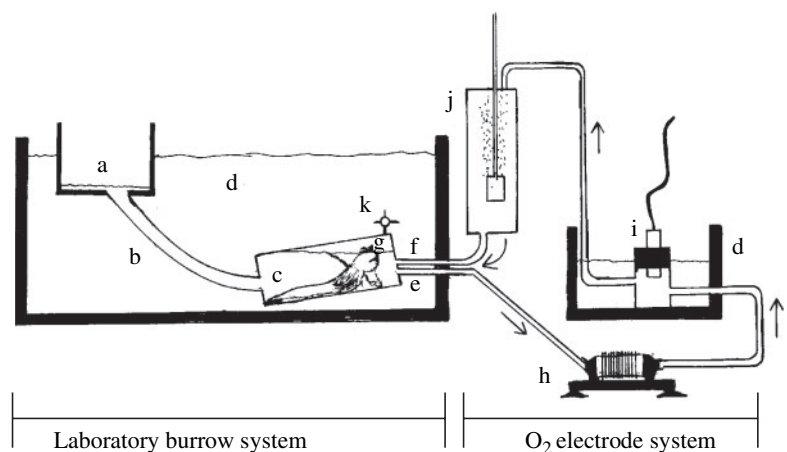

Fig. 1. Laboratory burrow system with the O$_2$ electrode circulation loop for continuous measurement of water-phase P_{O_2}. a, mudflat with shallow water; b, connecting tube; c, burrow chamber filled with water; d, temperature-controlled water bath; e, water-phase outlet; f, and inlet; g, air phase; h, oscillating pump; i, O$_2$ electrode in housing; j, N$_2$ gas-equilibration chamber; k, stopcock for air-phase sampling. Arrows indicate direction of water flow.

sampling ports and a stopcock in the ceiling replaced the chamber. Two ports, an inlet and outlet, were used for water-phase sampling and when the chamber was elevated, gas was trapped beneath the stopcock for sampling. In another system modification, a narrower 200 ml burrow chamber allowed fish to access the air phase more easily.

The system was assembled by filling the burrow chamber with mud 3 cm deep, connecting it to the tubing and adding seawater (25‰ at 25°C) to a depth of 2 cm in the plastic container (the 'mudflat'; Fig. 1) to simulate the low-tide conditions during which air would normally be deposited in the burrow by fish on mudflats. The system was then immersed in a temperature-controlled (25°C) water bath with an observation window and inlet/outlet ports in one side wall to permit tube connections to the burrow chamber for water-phase P_{O_2} measurements (Fig. 1). Observations of air-deposition behavior and rate were made in a dark room by sitting quietly between a black curtain and the burrow chamber, which was illuminated from behind by a low intensity light source.

Most behavioral observations were made in conjunction with continuous measurements of burrow-water P_{O_2} (Fig. 1) made with an O_2 meter (model OM 200, Cameron Instrument Company, Port Aransas, TX, USA). The O_2 electrode (model DO-051) was fitted into a plastic housing and connected to the in- and outlet burrow-chamber ports through the wall of the water bath. An oscillating pump (model 14925-005, Gorman-Rupp Industries, Bellville, OH, USA) with variable autotransformer (Staco Energy Products Corporation, Dayton, OH, USA) was used to circulate water through the system at a rate sufficient to maintain a stable P_{O_2} reading (Fig. 1).

Air-phase volume and P_{O_2} measurements were made by withdrawing air through the stopcock on the elevated end of the burrow chamber into a syringe from which the dead space volume had been cleared. Volume was measured using the scale on the syringe barrel. Sample P_{O_2} was measured by injecting the air into an O_2 electrode (model 1302, Strathkelvin Instruments, Bearsden, Glasgow, UK) connected to an O_2 meter (model 781) and sample injection was verified by the displacement of excess air at the electrode overflow port. Readings were taken when stable (approximately 1 min). The temperature-controlled (25°C) O_2 electrodes were calibrated daily in water-vapor saturated air and N_2 at atmospheric pressure (measured with a mercury barometer) with appropriate correction for water-vapor pressure.

'Low-tide' air-deposition studies

To determine if *S. histophorus* would deposit air in the laboratory burrow, fish were housed in the burrow for 1–11 days under the simulated low-tide conditions. The air-deposition behavior and the rate of air accumulation in the burrow were determined by continuous direct observations and by removing the air phase and then measuring the volume of air accumulated over periods ranging from 0.05 to 54 h (successful air-phase restorations were defined as occurring in 24 h or less). Selected air-deposition behavior was recorded with a digital video camera. Air-deposition studies were

conducted on females, males and male/female pairs. After a period over which air-deposition occurred, the entire air-phase volume was withdrawn and measurements of its volume and P_{O_2}, and water-phase P_{O_2} were made. The hourly air-deposition rate was estimated by dividing the end air volume by the number of hours over which it was deposited and the buccal chamber volume of the experimental fish. *Scartelaos* uses its buccal chamber to transport air and the relationship between buccal volume and body size is described by $y=0.088x+0.33$ ($r^2=0.48$), where y=buccal chamber volume and x=mass (H.J.L. and J.B.G., unpublished). Air-deposition rate data were grouped into categories (0.05–1.5 h, 1.5–8 h and >8 h) based on the time intervals over which fish were left undisturbed. These relate to the amount of time the fish and its burrow entrance are exposed on the mudflat surface during low tide, which varies with tidal amplitude and burrow position in the intertidal zone. Tests were done on individual fish and male/female pairs; sex was determined by examination of the urogenital-papilla shape under a dissecting microscope. Fish mass was measured at the conclusion of each study.

'Low-tide' response to gas-phase P_{O_2}

The behavioral response of *S. histophorus* to burrow air-phase P_{O_2} was also observed in one fish that had been carrying out routine air-deposition behavior under simulated low-tide conditions in the small burrow chamber (200 ml). After removing an air phase, a series of mixes of N_2 gas and air (20 ml) ranging in P_{O_2} from 0–20.7 kPa were introduced in place of the original air phase. Such gas mix tests were conducted over the course of five different experimental periods in the burrow system, each lasting from 4–10 days. On each day of an experimental period, one to eight gas phases were presented to the fish (depending on how rapidly the fish removed each gas phase). Observation began at the time of gas phase introduction and the response of the fish was assessed. All tests lasted <1 h, and the exact duration varied depending on fish behavior. At the conclusion of each test, the experimenter removed any small volumes (<2 ml) of remaining gas and a new gas phase, either the same or a different gas mix, was introduced. In most cases, the new tests were done with a gas phase that differed by approximately 1 kPa from the previous gas phase. The first gas phase presented each day was chosen randomly. Fish were left overnight either with no gas phase or with the last gas phase presented that day. Fish mass was measured after removal from the burrow.

'High-tide' response to burrow-water P_{O_2}

Threshold air-breathing studies were conducted on *S. histophorus* to determine how it might respond to declining water P_{O_2} during high-tide burrow confinement. Under normal conditions, the fish would be able to swim through the water column to reach air during high tide. However, the threat of predation confines mudskippers to their burrows at this time and so high-tide conditions were simulated by blocking the tube connecting the mudflat and burrow chambers, thereby

trapping the fish in the burrow with a 120 ml air phase. The air-breathing threshold, the P_{O_2} at which the fish ceased aquatic ventilation and began air breathing, was measured by direct observation, and fish were housed in the system for the duration of each study (approximately 5 days).

At the start of each threshold measurement, burrow water ($P_{O_2} \geqslant 14.7$ kPa) was pumped through the O_2 electrode system and P_{O_2} was gradually reduced by adding N_2 gas in the equilibration chamber (Fig. 1). Rate of P_{O_2} decline (determined over approximately 30 min from start to threshold) was recorded for each measurement. The reduction of burrow-water P_{O_2} continued until the fish took its first air breath, which was readily observed as the fish extended its head into the air phase, inflated its buccal chamber, and subsequently had a buoyant head. The number and duration of air breaths and ventilation periods during the first hour post-threshold were recorded. After 1 h, the burrow water was bubbled with air until P_{O_2} was greater than 13.3 kPa. Up to four replicate measurements of threshold (1/day) were made for each fish and mass was measured at the conclusion of each study.

To account for other factors contributing to burrow-water P_{O_2} decline, bacterial respiration control experiments were run in the burrow system with no fish and an air phase (180 ml). Air-phase diffusion control experiments lasting 24 h were also run under the same conditions.

Data analysis and statistics

Values are reported as means ± standard error (S.E.M.). Mean air-deposition rates over different time intervals were compared by using a randomized-block analysis of variance (ANOVA), in which the time intervals were the treatments and the individuals were the blocking factor. Individuals for which data were missing in one of the treatments were omitted from the ANOVA. A two-way ANOVA was used to determine if significant differences in air-deposition rates over different time intervals existed among males, females, and pairs. The two factors in this analysis were the different time intervals and the sex/paired status of the fish. An r^2 value was calculated from a linear regression to determine if threshold P_{O_2} was affected by the rate of P_{O_2} decline in the burrow system. A 5% level of significance was used in all cases.

Results

'Low-tide' air-deposition behavior

Fig. 2A shows an 8 h period during which a single *S. histophorus* (5.1 g male) was observed to repeatedly deposit air in the burrow chamber. Each time the experimenter removed an air phase (air phase volume in Fig. 2A is 0 after each removal), the fish created another one. Between 0900 and 1530 h, the fish deposited 4.8 ml of air in the chamber. Following experimental removal of this air phase, the fish added the same volume of air in 1.2 h. Experimental removal of this air phase was again followed by air addition, of 0.3 ml in 0.7 h. These three observations of burrow air deposition took
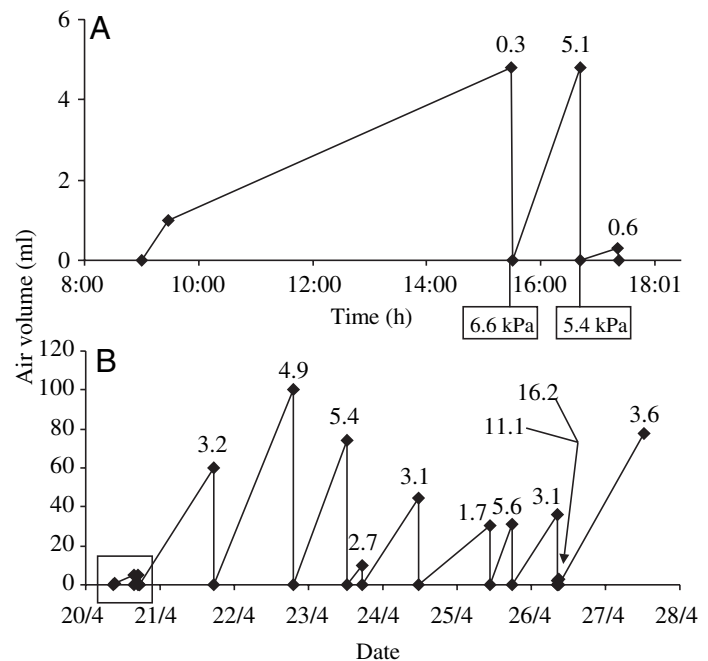

Fig. 2. Record of burrow air-deposition behavior by one *S. histophorus* (5.1 g male) over an 8 day period. Diagonal lines show increased air-phase volume deposited by the fish over time. Vertical lines indicate when all deposited air was removed by the experimenter. Zero air-phase volume occurred when the fish was first placed in the system and immediately after each air phase was withdrawn. Numbers above air-deposition events indicate air-deposition rate (trips h^{-1}). (A) The three air phases added during the first 9 h period (burrow-water P_{O_2} indicated in boxes). (B) Entire observation record for the fish showing 14 separate air-phase depositions. (Boxed section is the compressed record shown in A.)

place at the beginning of an 8 d period documenting this behavior (Fig. 2B). The record for this period shows that, during the first night in the burrow system, 60 ml of air was deposited and, over the entire experimental period, the fish re-established an air phase 13 more times after each was withdrawn by the experimenter.

Observations of air deposition and air-phase restoration after withdrawal by the experimenter were documented for 12 *S. histophorus* (*N*=8 individuals + 2 pairs; Table 1) in burrow water ranging in P_{O_2} from 0.4 kPa to 6.6 kPa. In a total of 74 air-phase withdrawals, air-phase restoration occurred 72 times (97.3%), and the volume of each restored phase ranged from 2-100 ml. In most cases air-phase replacement began within a few minutes of removal and males, females and paired fish did this. Continuous observations on three *S. histophorus* showed that one made five air-deposition trips in 2 min and another made seven trips in 57 min. The third fish made 14 trips in 49 min and, following another withdrawal, made five trips in 42 min. The mean number of hourly air-deposition trips for these three fish was 12.3±4.5 trips h^{-1} (*N*=31 trips). While many trips could be made in a short interval, there were also periods as long as 14 min when no trips occurred.

Also shown in Fig. 2 are the estimated hourly air-deposition

Table 1. *The total number of air-phase depositions by laboratory burrow-dwelling* S. histophorus *for periods ranging from 1–11 days*

Fish	Mass (g)	Sex	No. days observed	Water P_{O_2} range (kPa)	No. air-phase depositions*
1	3.7	M	2	0.4–1.8	4
	6.2	F			
2	5.8	M	1	2.9	1
	3.1	F			
3	2.8	F	4	0.5–0.7	7
4	5.9	F	5	0.5–0.9	9
5	4.6	F	11	0.5–4.4	12
6	5.3	F	5	3.1–3.4	6
7	5.9	F	5	1.2–3.9	8
8	5.1	M	8	1.1–6.6	14
9	3.7	M	6	0.7–2.7	5
10	3.5	M	3	1.5–3.4	6

Fish were initially placed in the burrow system with no air phase. When observations at a later time indicated that air had accumulated in the chamber, the entire volume was removed and measured. Each subsequent air deposition was recorded and its volume measured.

All fish and pairs of fish tested deposited an air phase and most did this repeatedly.

*Each air-phase deposition occurred within 24 h of withdrawal of the previous air phase by the experimenter.

rates for each air phase formed by a 5.1 g male. The highest air-deposition rates were 11.1 and 16.2 trips h^{-11} (Fig. 2B). Calculated air-deposition rates were also obtained for two other males, five females and two male/female pairs, and means are shown in Fig. 3. For the 0.05–1.5 h deposition periods, the air-deposition rate for 15 air phases deposited by seven fish was 6.7±1.8 trips h^{-1}. This rate was significantly greater than that observed for 1.5–8 h periods (3.0±0.8 trips h^{-1}, 17 air phases; eight fish) and for periods longer than 8 h (1.9±0.3 trips h^{-1}, 31; 10) (Fig. 3). No significant rate differences were found among males, females, or pairs. [Note: real-time images of the air-deposition behavior can be found at: http://mbrd.ucsd.edu/labpages/graham2.cfm. Also contained in these images are subsurface, 'in-the-burrow' views of *S. histophorus* with an inflated buccal chamber and positive buoyancy, which then releases air into the burrow chamber (H.J.L., N. M. Aguilar-Roca, N. E. Milward and J.B.G., unpublished).]

'Low-tide' response to gas-phase P_{O_2}

A series of observations totaling over 177 h and spanning 33 d showed the sensitivity to burrow gas P_{O_2} of one female *S. histophorus* (7.9 g) (Table 2). When 20 ml gas phases of various P_{O_2} values less than 9.3 kPa were added to the chamber, the fish responded by first taking a breath, holding it for a few seconds and then releasing it back into the gas phase. It then removed most or all of the gas phase (18–20 ml) within 12–235 min of introduction (*N*=32 replicates). In contrast, each time a 20 ml gas phase of P_{O_2} >9.3 kPa was introduced (*N*=6
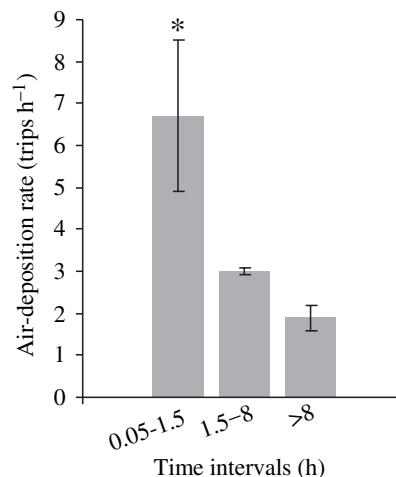

Fig. 3. Mean air-deposition rates of *S. histophorus* over different treatments (the time intervals over which the fish was left undisturbed) (*N*=8 individuals + 2 pairs) based on the volume of air accumulated, fish buccal chamber volume and the time interval between withdrawals. Values are means ± S.E.M. *Significant difference from the 1.5-8 h and >8 h intervals.

Table 2. *Results of repeated testing of the behavioral response of one* S. histophorus *to the experimental introduction of gas phases (20 ml) of different* P_{O_2} *into the laboratory burrow system*

Gas-phase P_{O_2} (kPa)	Minimum response time (min)	No. tests	Fish behavior	
			Gas-phase removal	Gas addition
0	12	11	11	0
2.1[2]	23	6	6	0
2.6[3]	34	1	1	0
3.1[1,4]	58	2	2	0
4.1	31	1	1	0
5.2[5]	37	3	3	0
6.2[6]	42	1	1	0
7.3[7]	27	3	3	0
8.3	133	2	2	0
9.3	36	2	2	0
10.4	13	4	0	4
20.7	248	2	0	2

On each day of an experimental period, 1–8 gas phases were presented to the fish (depending on how rapidly the fish removed each phase). The sequence on day 1 of gas-phase testing is indicated by the superscript numbers next to the gas-phase P_{O_2} values. For example, tests on that day began with a gas phase of 3.1 kPa, followed by a 2.1 kPa gas phase, etc. Two gas phases of 3.1 kPa (the first phase and the fourth) were introduced on day 1.

replicates), the fish did not remove the gas phase, but rather added surface air to it (Table 2). Burrow-water P_{O_2} for these studies was 1.6-8.9 kPa.

The fish removed gas by taking a mouthful and moving a sufficient distance into the burrow-access tubing (Fig. 1) for

the bubble to ascend to the surface upon release. The mean number of successful gas-release trips needed to clear a 20 ml gas phase was 18.3±2.2, as would be expected based on a buccal chamber volume of 1.02 ml for an *S. histophorus* of 7.9 g mass (H.J.L. and J.B.G., unpublished). However, the fish did not always swim a sufficient distance into the tube for gas release, so that when released, the gas returned to the chamber.

'High-tide' response to burrow-water P_{O_2}

Studies of the effects of declining burrow-water P_{O_2} on the respiration of *S. histophorus* defined a threshold P_{O_2} at which the fish switched from aquatic to aerial respiration. The mean air-breathing threshold measured for eight fish was 4.8±0.2 kPa. This value was not significantly affected by experimental rate of P_{O_2} decline (0.4 kPa min^{-1}, N=29 replicates, r^2=0.007).

During the first hour after the air-breathing threshold was reached, burrow-confined *S. histophorus* took 32.0±3.9 air breaths (*N*=7). Individual fish varied in the number of breaths taken from 11–49. The average time breaths were held was 1.4±0.07 min (*N*=10; range=0.8–4.4 min) and the inter-breath interval (i.e., the period when air was not in the buccal chamber and gill ventilation was occurring) was 0.39±0.02 min (*N*=7). Only two fish took air breaths after burrow-water P_{O_2} was restored to pre-threshold levels; these breaths occurred at 6.5 and 8.1 kPa.

Above and below ground aspects of burrow life

Experimental controls showed that 1.1 h after the burrow system was sealed with water, the background bacterial respiration of the mud in the chamber (0.0035 ml^{-1} O$_2$ ml^{-1} H$_2$O h^{-1}) was sufficient to reduce burrow-water (1 l air-saturated water with a 180 ml air phase) P_{O_2} to the air-breathing threshold level (4.8 kPa) of *S. histophorus*. Controls also showed that threshold P_{O_2} water levels caused negligible (1.4×10^{-4} ml^{-1} O$_2$ ml^{-1} air h^{-1}) O$_2$ diffusion from the air phase to the water.

Fig. 4 combines these control observations with the field observations and respiratory parameters described for *S. histophorus* in this study to depict the course of events likely to take place in the natural environment of the fish over a 24 h period. Field observations showed that *S. histophorus* was active on the mudflat surface when its burrow entrance was uncovered, which was 23% (5.5 h) of the 24 h shown in Fig. 4 (this time budget depends on tidal periodicity and burrow height in the intertidal). During the time it was active on the mud surface, *S. histophorus* breathed and deposited air in its burrow. It took refuge in the burrow as the tide covered the entrance and remained confined there until the tide receded.

The air-breathing threshold and control data for rate of decline in burrow-water P_{O_2} indicate that a burrow-confined *S. histophorus* would be able to respire aquatically for the first 65.2 min of burrow confinement (Fig. 4). Once burrow-water P_{O_2} declined from low-tide levels of 14.9 kPa (H.J.L., N. M. Aguilar-Roca, N. E. Milward, and J.B.G., unpublished) to the air-breathing threshold, the fish would use the burrow air phase for aerial respiration. Fig. 4 shows that, depending on tidal periodicity, the fish would spend from 8–10 h in the burrow, and, for about 90% of this time, burrow conditions would require air breathing. Based on an estimated routine aerial O$_2$
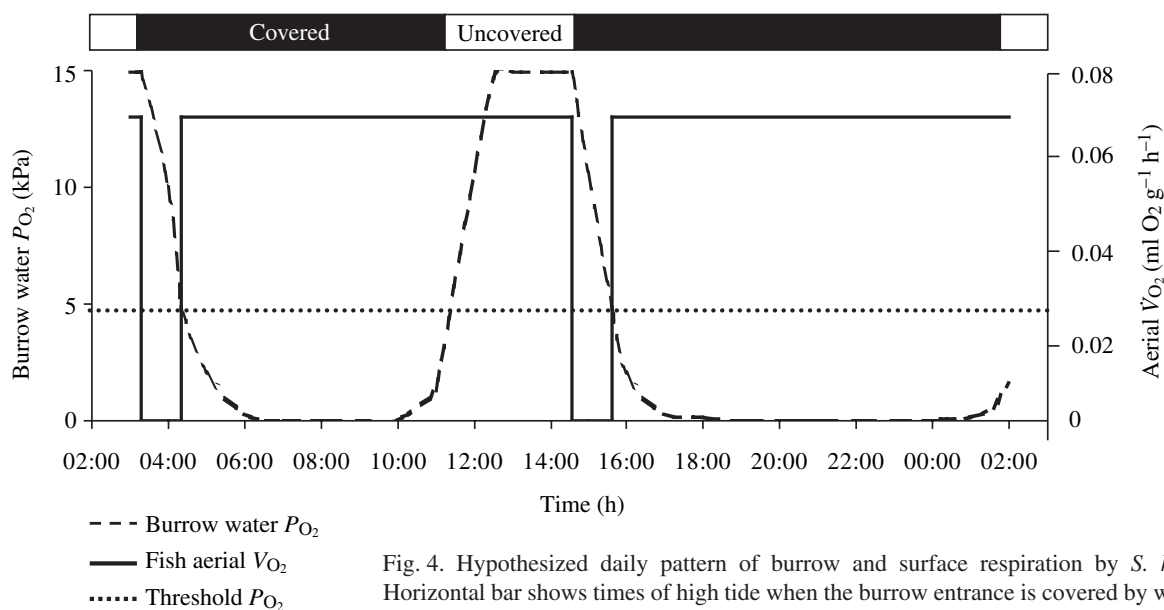

Fig. 4. Hypothesized daily pattern of burrow and surface respiration by *S. histophorus*. Horizontal bar shows times of high tide when the burrow entrance is covered by water (black) and when the burrow is uncovered at low tide (white). Dashed line shows tidal variation in burrow-water P_{O_2}, which is highest at the time the tide rises to cover the burrow and lowest when the tide recedes. The fish remains in the burrow during high tide. Solid black line shows fish aerial V_{O_2} (Milward, 1974); when burrow-water P_{O_2} is at or below air-breathing threshold P_{O_2} (horizontal dotted line), the fish requires air-phase breathing. When burrow-water P_{O_2} is above threshold, aquatic respiration would occur. During low tide, the fish is up on the mudflat where it breathes air. Figure compiled with laboratory data on threshold P_{O_2} and burrow-water P_{O_2} decline, and field data for burrow position in the intertidal zone, low-tide burrow P_{O_2}, and tide tables for the area of study.

consumption rate of 0.072 ml O_2 g^{-1} h^{-1} (Milward, 1974), an 8 g fish would require a minimum air-phase volume of 20–25 ml.

Discussion

This study provides the first record of burrow air-deposition behavior by *S. histophorus* in the laboratory and demonstrates its use of the air phase for intra-burrow respiration. We found that, if an air phase was experimentally removed, fish in the burrow consistently re-established the air phase and that one *S. histophorus* would remove a burrow gas phase containing little or no oxygen. This study also determined the threshold burrow-water P_{O_2} at which the fish switches from water to air breathing, and these results, when combined with control experiment and field data, allowed us to posit a 24 h scenario of activity and respiration related to tidal cycle for this fish.

Air-deposition behavior function and rate studies

Repeated (up to 13 in 8 days) air phase restorations by *S. histophorus* show that this fish deliberately creates air phases that are not the result of accidental air breath release. Because the burrow air-deposition behavior of *S. histophorus* occurred in the laboratory, even when fertilized eggs were not present, our findings suggest that the air phase is important for adult respiration, and this was confirmed by our observations that the fish made use of the air phase for respiration during simulated high tide when the laboratory burrow-water P_{O_2} was low. If the burrow air phase was used primarily for oxygenation of developing eggs, one might expect that males would be the primary depositor of air because, as with the majority of intertidal fishes, it is the male of most mudskipper species that provides parental care for the eggs (Gibson, 1982). However, our results show that male and female air-deposition rates do not differ, giving further support to the idea that the air phase is also important for adult intra-burrow respiration. In addition, field observations show that gravid females will deposit air in burrows they share with a male, and, unless males spawn with more than one female, this would clearly indicate that air-deposition precedes egg-deposition (H.J.L., N. M. Aguilar-Roca, N. E. Milward, and J.B.G., unpublished).

The air phase most probably also serves its second proposed function, that of a steady oxygen supply for developing eggs (Ishimatsu et al., 1998a,b). Many gobies, including mudskippers, use burrows for egg incubation (MacNae, 1968a,b; Brillet, 1969; Hudson, 1977; Gibson and Ezzi, 1978; Atkinson, 1991) and all gobies whose reproductive behaviors have been studied guard their fertilized eggs (Reese, 1964; Gibson, 1969, 1982; Kobayashi et al., 1971; Brillet, 1976; Gibson and Ezzi, 1978; Clayton, 1993). The eggs of some mudskipper species have been shown to require periods out of water for proper development (Brillet, 1976) and, if bathed in burrow water throughout the developmental period, would be exposed to severely hypoxic conditions.

Our laboratory results confirmed the field observations of air-deposition behavior by *S. histophorus*. Directly observed

laboratory rates (0.2 ± 0.08 trips min^{-1}) were an order of magnitude less than those observed in the field (6–15 trips min^{-1}). The difference in rates may arise because field air-deposition activity is concentrated into periods of a few minutes and interspersed with prolonged bouts of feeding, courtship and territorial defense over the course of an hour. Laboratory fish were not involved in any other activities while under observation, and thus may have deposited air at a slower rate than fish in the field. Our results also show that calculated air-deposition rates decreased over longer time intervals (Fig. 3), suggesting that air-deposition most probably occurs in the first few hours after the previous air phase is withdrawn and then slows down.

'Low-tide' response to gas-phase P_{O_2}

Our observations of the behavioral response to low-P_{O_2} gas phases ($\leqslant10.4$ kPa) suggest that *S. histophorus* may remove low-P_{O_2} gas from its burrow. Gas-phase (20 ml) removal time (12 min in some cases) shows that, in the limited time available during low tide, when the entrance is exposed to air, a fish could easily remove and restock a burrow air phase of the minimum required volume for high-tide respiration (approximately 20–25 ml).

S. histophorus took a single, rapid breath (held for 1–2 s) from each gas phase before removing or adding to it. This suggests that it was able to sense the gas-phase P_{O_2} immediately upon inspiration, a reasonable assumption based on the occurrence of O_2 chemoreceptors on the branchial arches and in the buccal cavities of most fishes (Jones and Milsom, 1982; Smatresk, 1988; Graham, 1997; Milsom et al., 2002). The mudskipper aerial respiratory surfaces line the buccal, branchial, pharyngeal and opercular cavities (Schöttle, 1932; Hora, 1935a,b; Stebbins and Kalk, 1961; Gibson, 1969; Graham, 1997; Clayton, 1993), so it is reasonable to expect that air held in the mouth would contact the O_2 sensors.

'High-tide' component of the air-deposition behavior: response to burrow-water P_{O_2}

Our findings show that if the P_{O_2} of the laboratory burrow water was low ($\leqslant4.8\pm0.2$ kPa), *S. histophorus* breathed regularly from the air phase. This result supports the hypothesis that the air phase is used for respiration when, over the course of high-tide burrow confinement, *S. histophorus* exhausts its burrow-water O_2 supply. Our field measurements of burrow-water P_{O_2} made near the end of low tide indicate a P_{O_2} greater than the air-breathing threshold (6.1–14.9 kPa; H.J.L., N. M. Aguilar-Roca, N. E. Milward and J.B.G., unpublished). This probably results from burrow water contact with atmospheric air and fish activity (frequent entrance or exit would cause water mixing). However, other field measurements indicate severely hypoxic burrow water (Gordon et al., 1978; El-Ziady et al., 1979; Ishimatsu et al., 1998a,b), and our control experiments show that, because of fish and infaunal respiration, burrow-water P_{O_2} would decline rapidly after the burrow entrance is covered by high tide, thus making the air phase essential for high-tide respiration.

Once we determined that the air phase was important for the intra-burrow respiration of *S. histophorus*, the question arose of whether or not the burrow air-storage chamber could hold the minimum air volume necessary for high-tide respiration. While burrow structure is not known for *S. histophorus*, air-phase volumes (approximately 274 ml in smaller burrows; Ishimatsu et al., 1998b) found in the burrows of another mudskipper, *Periophthalmodon schlosseri*, are much greater than the minimum required respiratory volume of *S. histophorus*. It therefore seems likely that the burrow of *S. histophorus* could hold the required minimum 20–25 ml, ensuring sufficient O_2 in the air phase for respiration throughout high tide.

Implications for the evolution of air breathing

This study has shown that burrow air deposition by *S. histophorus* is critically important for its respiration during high-tide burrow confinement. This finding is especially important because many species of water-breathing, burrow-dwelling fishes tolerate hypoxia instead of alleviating it by air deposition (Congleton, 1974; Pullin et al., 1980; Atkinson, 1991). The capacity of mudskippers to use air deposition to overcome burrow hypoxia may have resulted from their proximity to the air surface during low tide and the extreme hypoxia that develops within burrows during high tide. This behavior is also widespread among the basal oxudercines, which occur in mud and probably have hypoxic burrows. One species (*Oxuderces*) has a burrow air phase (Ishimatsu, 1998a,b). This implies that air gulping for burrow air deposition may have preceded the terrestrial radiation of mudskippers and their use of amphibious air breathing. Our control studies suggest that air deposition would not necessarily increase the burrow-water P_{O_2}. Thus, if the early oxudercines gulped air, they would have needed to utilize the O_2 in the air either by aquatic surface respiration (ASR; gill ventilation of surface water under hypoxic conditions) or by gulping air. Studies by Gee and Gee (1995) demonstrated that non-oxudercine gobies gulped air to increase their buoyancy during ASR and such a mechanism may have enabled the early oxudercines to acquire an aerial respiratory capacity. This evolutionary scenario is consistent with the morphology of the mudskipper air-breathing organ (Schöttle, 1932; Hora, 1935a,b; Stebbins and Kalk, 1961; Gibson, 1969; Graham, 1976; Clayton, 1993).

References

Aguilar, N. M. (2000). Comparative physiology of air-breathing gobies. PhD dissertation, University of California, San Diego, USA.

Asano, N. (1936). The egg laying habits of the South Sea mudskipper, *Periophthalmus* spp. *Ecol. Rev. (Sendai)* **2**, 137-139.

Atkinson, R. J. A. and Taylor, A. C. (1991). Burrows and burrowing behaviour of fish. In *The Environmental Impact of Burrowing Animals and Animal Burrows* (ed. P. S. Meadows and A. Meadows), pp. 133-155. Oxford: Oxford University Press.

Brillet, C. (1969). Etude du comportement constructeur des poissons amphibies Periopthalmidae. *Terre et Vie* **23**, 496-520.

Brillet, C. (1976). Structure du Terrier, reproduction et comportement des jeunes chez le poisson amphibie *Periophthalmus sobrinus* Eggert. *Terre et Vie* **30**, 465-483.

Clayton, D. A. (1993). Mudskippers. *Oceanogr. Mar. Biol. Annu. Rev.* **31**, 507-577.

Clayton, D. A. and Vaughan, T. C. (1986). Territorial acquisition in the mudskipper *Boleophthalmus boddarti* (Pisces: Gobiidae) on the mudflats of Kuwait. *J. Zool.* **209**, 501-519.

Clayton, D. A. and Vaughan, T. C. (1988). Ethogram of *Boleophthalmus boddarti* (Teleostei: Gobiidae), a mudskipper found on the mudflats of Kuwait. *J. Univ. Kuwait (Sci.).* **15**, 115-138.

Congleton, J. L. (1974). The respiratory response to asphyxia of *Typhlogobius californiensis* (Teleostei: Gobiidae) and some related gobies. *Biol. Bull. Mar. Biol. Lab. Woods Hole* **146**, 186-205.

El-Ziady, S., Eissa, S. M. and Al-Naqi, Z. (1979). Ecological studies on the mud-skipper fish *Periophthalmus chrysospilos* (Bleeker) in Kuwait. *Bull. Fac. Sci., Cairo Univ.* **48**, 113-134.

Gee, J. H. and Gee, P. A. (1995). Aquatic surface respiration, buoyancy control and the evolution of air breathing in gobies (Gobiidae: Pisces). *J. Exp. Biol.* **198**, 79-89.

Gibson, R. N. (1969). The biology and behaviour of littoral fish. *Oceanogr. Mar. Biol. Ann. Rev.* **7**, 367-410.

Gibson, R. N. (1982). Recent studies on the biology of intertidal fishes. *Oceanogr. Mar. Biol. Ann. Rev.* **20**, 363-414.

Gibson, R. N. and Ezzi, I. A. (1978). The biology of a Scottish population of Fries' goby, *Lesueurigobius friesii. J. Fish Biol.* **12**, 371-389.

Gordon, M. S., Boetius, I., Evans, D. H., McCarthy, R. and Oglesby, L. C. (1969). Aspects of the physiology of terrestrial life in amphibious fishes I: the mudskipper, *Periophthalmus sobrinus. J. Exp. Biol.* **50**, 141-149.

Gordon, M. S., Ng, W. W.-S. and Yip, A. Y.-W. (1978). Aspects of the physiology of terrestrial life in amphibious fishes. III. The Chinese mudskipper *Periophthalmus cantonensis. J. Exp. Biol.* **72**, 57-75.

Graham, J. B. (1976). Respiratory adaptations of marine air-breathing fishes. In *Respiration of amphibious vertebrates* (ed. G. M. Hughes), pp. 165-187. London: Academic Press.

Graham, J. B. (1997). *Air-Breathing Fishes*. San Diego: Academic Press.

Harms, J. W. (1929). Die realization von genen und die consecutive adaptation. I: Phasen in der differenzierung der anlagen komplexe und die frage der landtierwerdung. *Z. Wiss. Zool.* **133**, 211-397.

Hora, S. L. (1935a). Ecology and bionomics of the Gobioid fishes of the Gangetic Delta. *Int. Cong. Zool.* **12**, 841-865.

Hora, S. L. (1935b). Physiology, bionomics, and evolution of the air-breathing fishes of India. *Trans. Natn. Inst. Sci. India* **1**, 1-16.

Hudson, R. C. L. (1977). Preliminary observations on the behaviour of the gobiid fish *Signigobius biocellatus* Hoese and Allen, with particular reference to its burrowing behaviour. *Z. Tierpsychol.* **43**, 214-220.

Ishimatsu, A., Khoo, K. H. and Takita, T. (1998a). Deposition of air in burrows of tropical mudskippers as an adaptation to the hypoxic mudflat environment. *Sci. Prog.* **81**, 289-297.

Ishimatsu, A., Hishida, Y., Takita, T., Kanda, T., Oikawa, S., Takeda, T. and Khoo, K. H. (1998b). Mudskippers store air in their burrows. *Nature* **391**, 237-238.

Jones, D. R. and Milsom, W. K. (1982). Peripheral receptors affecting

breathing and cardiovascular function in non-mammalian vertebrates. *J. Exp. Biol.* **100**, 59-91.

Kobayashi, T., Dotsu, Y. and Takita, T. (1971). Nest and nesting behavior of the mudskipper *Periophthalmus cantonensis*, in Ariake Sound. *Bull. Fac. Fish., Nagasaki Univ.* **32**, 27-39. (In Japanese with English abstract.)

Lee, H. J. and Graham, J. B. (2002). Their game is mud. *Nat. Hist.* **9/02**, 42-47.

MacNae, W. (1968a). A general account of the fauna and flora of mangrove swamps and forests in the Indo-west Pacific region. *Adv. Mar. Biol.* **6**, 73-270.

MacNae, W. (1968b). Mudskippers. *African Wildlife* **22**, 240-248.

Matoba, M. and Dotsu, Y. (1977). Prespawning behavior of the mud skipper *Periophthalmus cantonensis* in Ariake Sound. *Bull. Fac. Fish., Nagasaki Univ.* **43**, 23-33.

Milsom, W. K., Reid, S. G., Rantin, F. T. and Sundin, L. (2002). Extrabranchial chemoreceptors involved in respiratory reflexes in the neotropical fish *Colossoma macropomum* (the tambaqui). *J. Exp. Biol.* **205**, 1765-1774.

Milward, N. E. (1974). studies on the taxonomy, ecology, and physiology of Queensland mudskippers. PhD dissertation, University of Queensland, Australia.

Murdy, E. O. (1989). A taxonomic revision and cladistic analysis of the oxudercine gobies (Gobiidae: Oxudercinae). *Rec. Austr. Mus.*, **Supp. 11**, 1-93.

Pullin, R. S. P., Morris, D. J., Bridges, C. R. and Atkinson, R. J. A. (1980). Aspects of the respiratory physiology of the burrowing fish *Cepola rubescens* L. *Comp. Biochem. Physiol.* **66A**, 35-42.

Reese, E. S. (1964). Ethology and marine zoology. *Oceanogr. Mar. Biol. Ann. Rev.* **1964**, 455-488.

Sasekumar, A., Ong, T. L. and Thong, K. L. (1984). Predation of mangrove fauna by marine fishes. In *Proceedings of the Asian Symposium on the Mangrove Environment – Research and Management* (ed. E. Soepadmo et al.), pp. 378-384. Kuala Lumpur: University of Malaya and UNESCO.

Scholander, P. F., van Dam, L. and Scholander, S. I. (1955). Gas exchange in the roots of mangroves. *Amer. J. Bot.* **42**, 92-98.

Schöttle, E. (1932). Morphologie und physiologie der atmung bei wasser-, schlamm- und landlebenen Gobiiformes. *Z. Wiss. Zool.* **140**, 1-114.

Smatresk, N. J. (1988). Control of the respiratory mode in air-breathing fishes. *Can. J. Zool.* **66**, 144-151.

Stebbins, R. C. and Kalk, M. (1961). Observations on the natural history of the mudskipper *Periophthalmus sobrinus*. *Copeia* **1961**, 18-27.

Takita, T., Agusnimar and Ali, A. B. (1999). Distribution and habitat requirements of oxudercine gobies (Gobiidae: Oxudercinae) along the Straits of Malacca. *Ichthyol. Res.* **46**, 131-138.