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Aerial and aquatic respiration of the Australian desert goby, *Chlamydogobius eremius*

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Abstract

Physiological, anatomical and behavioural adaptations enable the Australian desert goby, *Chlamydogobius eremius*, to live in mound springs and temporary aquatic habitats surrounding the south-eastern rim of the Lake Eyre drainage basin in the harsh inland of Australia. This study describes the desert goby's respiratory and metabolic responses to hypoxic conditions and its use of buccal air bubbles for gas exchange at the water surface. Oxygen consumption for *C. eremius* is significantly higher in water than in air under normoxic and hypoxic conditions. In water, total oxygen consumption ($\dot{V}_{O_2}$) increases from normoxic conditions (253 μl g^{-1} h^{-1}) to 8% ambient O_2 concentration (377 μl g^{-1} h^{-1}), then decreases with increasing hypoxia of 4% O_2 (226 μl g^{-1} h^{-1}) and at 2% O_2 (123 μl g^{-1} h^{-1}). In air (fish were moist but out of water), $\dot{V}_{O_2}$ progressively decreases from normoxic conditions to hypoxic conditions (21% O_2, $\dot{V}_{O_2}$ is 169 μl g^{-1} h^{-1} to 39 μl g^{-1} h^{-1} at 2% O_2). These data indicate oxygen-conforming patterns with increasing hypoxia both in air and in water below 8% O_2. In water, opercular movement rates remain unchanged with increasing hypoxia (139 min^{-1} at 21% O_2, 154 min^{-1} at 8%, 156 min^{-1} at 4% and 167 min^{-1} at 2%) but in air, opercular movement rates are significantly lower than in water, corresponding with the lower metabolic rate (71 min^{-1} at 21% O_2, 53 min^{-1} at 8%, 96 min^{-1} at 4% and 64 min^{-1} at 2%). *Chlamydogobius eremius* can use a buccal air bubble for aerial O_2 uptake, most probably in response to increased aquatic hypoxia. In air, *C. eremius* relies more on the buccal bubble as an oxygen source with increasing hypoxia up to an ambient O_2 of 4% (7.1% of $\dot{V}_{O_2}$ at 21% O_2; 14.5% at 8% O_2; and 27.1% at 4% O_2), then when the available supply of O_2 is further reduced, it decreases (15% of $\dot{V}_{O_2}$ at 2% O_2) and respiration across the skin again makes a higher relative contribution. The Australian desert goby has a higher metabolic rate in higher salinities (336 μl g^{-1} h^{-1} in 35 ppt, 426 μl g^{-1} h^{-1} in 70 ppt) than in freshwater (235 μl O_2 g^{-1} h^{-1}), presumably because of the increased metabolic cost of osmoregulation. There was no significant difference in $\dot{V}_{O_2}$ for fish in air that had come from varying salinities.

Keywords: Desert Goby; *Chlamydogobius eremius*; Metabolism; Operculum movement; Hypoxia; Buccal air bubble

1. Introduction

Air-breathing fishes occur in a variety of habitats. Graham (1997) speculates that aquatic hypoxia and emergence from water are two primary factors that have influenced the evolution of air-breathing fishes. Aquatic hypoxia may be the primary driving force for air-breathing by freshwater fishes, whereas tidal stranding and active exploitation of the littoral-terrestrial boundary may have been the causal factors for marine-brackish littoral fishes. Air-breathing organs that facilitate

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the survival of fishes out of their aquatic environment include lungs or modified swim-bladders, anatomical modifications that increase the buccal, pharyngeal or opercular surface area, gas exchange surfaces along the digestive tract, and modifications to the skin for cutaneous gas exchange (Graham, 1997).

Glover (1971, 1979), Glover and Sim (1978) provided the first description of the basic biology and physiology of the Australian desert goby, *C. eremius*, which is endemic to the Lake Eyre drainage basin of Australia. This taxon has recently been divided into five species (*C. eremius*, *C. micropterus*, *C. squamigenus*, *C. gloveri* and *C. japalpa*) from the Lake Eyre drainage basin and an estuarine species, *C. ranunculus*, from northern Australia (Larson, 1995). *Chlamydogobius eremius* is reported to inhabit waters with a total salinity from 1.0 to 8.0 ppt, but will tolerate a wider range in salinity (1–37.5 ppt), a temperature range from 5 to 41 °C, a pH up to 10, and hypoxia down to 0.8 ppm O_2 (Glover, 1971, 1973; Scott et al., 1974; Glover and Sim, 1978; Merrick and Schmida, 1994). Merrick and Schmida (1994) reported that *C. eremius*, in warm conditions, lie in the shallows almost completely out of the water, suggesting survival by aerial respiration. Gee and Gee (1995) suggested that at very low aquatic P_{O_2} (< 2%) *Chlamydogobius* sp. (and *Mugilogobius*, *Arenigobius* and *Cryptocentroides*) could respire aerially using an air bubble held against the capillary lining of the roof of the buccal cavity, and via skin capillaries on the immersed part of the head, but they provided no direct evidence for aerial respiration.

This study confirms the use of a buccal bubble for gas exchange by *C. eremius* in hypoxic conditions by flow-through respirometry measurement. We follow the suggestion by Graham (1997) to use respirometry to directly demonstrate aerial gas exchange and present data for aerobic metabolism, rate of operculum movements and O_2 consumption by buccal air bubble ejection. Video recordings of respiratory movements and timing of the release and subsequent bursting of the buccal bubble in conjunction with instantaneous graphing of O_2 consumption recordings provided direct evidence for buccal bubble gas exchange.

Glover and Sim (1978) reported *C. eremius* in the Lake Eyre drainage basin. Many of the ephemeral waterways in this system can evaporate, leaving progressively increasing saline conditions until the water disappears. Given the varying salinities and hypoxic conditions that *C. eremius* are likely to encounter, we also examined $\dot{V}_{O_2}$ for this species in air and water at various O_2 concentrations and salinities.

2. Materials and methods

Chlamydogobius eremius (mean body mass for each experiment varied from 0.51 to 0.59 g) were collected from the 'The Bubbler' artesian spring (29°23′23″ S, 136°51′00″ E) on the southern edge of Lake Eyre, then maintained in indoor aquaria, in freshwater, for a period more than 6 months. They were subject to the normal Perth photoperiod at a constant temperature of 23–24 °C. Fish were fed small crustaceans and commercial fish food. All food was withheld for at least 60 h prior to the measurement of aerobic metabolic rate.

Fish were acclimated to distilled water (0 ppt NaCl) and at salinities of 35 and 70 ppt NaCl for at least 7 days prior to experiments. We experimented and found that *C. eremius* were able to survive in 70 ppt NaCl for several weeks in the laboratory and this value was selected to test, as the upper experimental limit, the effects on metabolism and opercular movement.

Unlike most natural conditions where the air above hypoxic water remains normoxic (i.e. 20.94% O_2), we necessarily studied *C. eremius* in conditions where the O_2 concentration of air was the same as in the water (as determined by the gas mixture from compressed air cylinders). Our choice of an experimental protocol is partly a consequence of the difficulty in partitioning gaseous exchange in air and water for such a small fish. However, even if it were technically feasible, measuring the metabolism of gobies in hypoxic water when they were able to aerially respire from normoxic surface air would not permit determination of their metabolic response to hypoxia, other than the extent to which they might increase the use of buccal bubbles and respire across exposed portions of skin. Under these conditions, gobies could simply move to a position that enabled them to place their heads out of the hypoxic water environment and presumably respire normally from the air. Using a flow-through respirometry system enabled us to directly monitor O_2 consumption, while video-recording the release and bursting of the buccal bubbles. *Chlamydogobius eremius* were

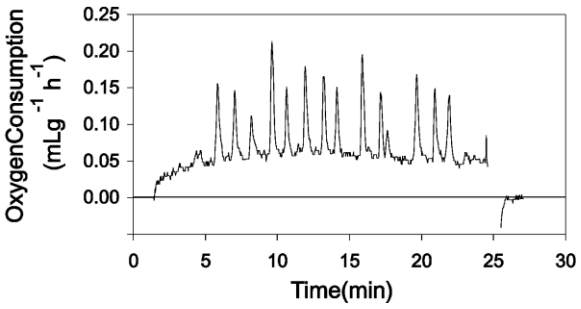

Fig. 1. Total body oxygen consumption for *C. eremius* in air, showing the peaks in O_2 consumption that correspond with the release and bursting of the buccal bubble. The line graph was prepared by joining measured individual total oxygen exchange values (ml h^{-1}) recorded every 2 s and converted to ml g^{-1} h^{-1}.

placed in a 25-ml clear glass flask held at an angle of approximately 45°, containing either 15 ml of freshwater or only air. The sloping flask facilitated the rapid bubbling of air through the water from the bottom of the flask, enabling us to readily monitor changes in O_2 consumption. To measure aerobic metabolism in air, fish were sealed in the sloping glass flask that had wet sides, but a minimal amount of free water. *Chlamydogobius eremius* were noted to use the sides of an aquarium or the rising slope of the bank to raise their head out of the water, so their position in a sloping flask was not unnatural.

Oxygen consumption was measured for fish in water at 0, 35 and 70 ppt NaCl and in air having been removed from water with these salinities. The aerobic metabolism of these fish was also measured in water and in air with the percentage O_2 in ambient air/water stabilised at 1.92%, 4.0%, 8.12% or 20.94% by bubbling an appropriate O_2/N_2 mixture of compressed air (BOC Gases Australia) from the bottom of the flask through the chamber at the rate of 50 ml min^{-1}. $\dot{V}_{O_2}$ was measured continuously by flow-through respirometry for each goby until a steady state was established; this could take 10–60 min. Stable O_2 recordings before and after each goby was introduced into the flask convinced us that we had achieved equilibration between P_{O_2} in the metabolic chamber before the goby was introduced and after the goby had been removed, enabling us to measure O_2 consumption levels when each fish was in the flask (Fig. 1). Fish remained in the chamber long enough to establish an O_2 consumption recording that was stable for a minimum of 10 min. Occasional brief periods of activity were obvious in the O_2-consumption recordings and these were excluded from the measurement of $\dot{V}_{O_2}$. The percentage O_2 in expired air was measured every 2 or 3 s. *Chlamydogobius eremius* generally remained motionless, resting on the sloping side of the flask once they had settled.

During each experiment, 50 operculum movements were counted visually and timed with a stopwatch for each fish while it was still. Each experiment was recorded by video camera (Canon TR 303E). Graphic pictures of consecutive video frames showing buccal air bubble intake and release were obtained on a PC with a Genius HiVideo Pro capture card. For fish in air, pulses of increased O_2 consumption coincided with the release of buccal air bubbles. The release and bursting of a buccal air-bubble (see Figs. 2 and 3) corresponded with a measured increased in consumption of O_2 in the flask (see Fig. 1). This indicated *C. eremius* had extracted O_2 from the bubble while it was in the buccal cavity.

The excurrent air from the glass flask was passed through a Drierite column to remove water vapour and an Ascarite column to remove CO_2. The O_2 content of the excurrent airstream was then measured with a Servomex 571 paramagnetic O_2 analyser and its voltage output was monitored by a Thurlby 1905A digital voltmeter and a PC GWBASIC program via an RS232 connection. The air-flow rate was controlled by a Sierra Instruments Model 90IC-PE control box and model 840-L-I-VI-S1 controller. Inflow air and water temperature were held at a constant 23.5 °C ($\pm$0.5). $\dot{V}_{O_2}$ was calculated at standard temperature and pressure in dry air after Withers (1977). A base-line record of O_2 consumption was obtained before and after the experiment with ambient air passing through the chamber to allow correction of barometric pressure changes and minor shifts in analyser calibration during the experiment. For recordings with no obvious pulses in O_2 consumption reflecting buccal air bubble release (see Section 3), an average aerobic metabolic rate was calculated, typically over 5–10 min of recording, excluding any periods that obviously corresponded with activity.

The rate of buccal air bubble release was calculated from the number of $\dot{V}_{O_2}$ pulses (see Fig. 1) in the continuous recording of aerobic metabolism over a measured period. These metabolic

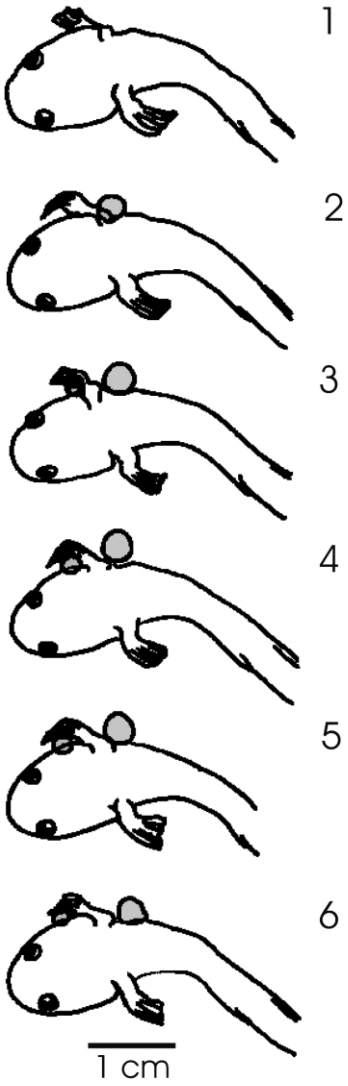

Fig. 2. Buccal bubble being released from the operculum by *C. eremius*. Drawn from a video recording; images are consecutive frames separated by 1/25 of a second (50 frames s^{-1} interlaced).

recordings were time-averaged to determine the overall aerobic metabolic rate, and then the average aerobic metabolic rate ascribed to the release of buccal air bubbles was determined from the area of the air bubble trace with a Numonics graphics digitizer tablet (model 2210). Subtraction of the buccal air bubble metabolic rate from the total aerobic metabolic rate yielded the average non-buccal bubble metabolic rate, which presumably reflected mainly cutaneous exchange for fish in air.

Fish acclimated in freshwater for 7 days were transferred to a salinity of 35 ppt NaCl, and fish acclimated at 70 ppt NaCl for a period of 7 days were transferred to a salinity of 35 ppt NaCl, and their $\dot{V}_{O_2}$ was immediately measured. The concentration of NaCl in water was measured by refractometer (Scientific Instruments, 10419). No fish were lost during the experiments. Probability for significance in all cases was $P < 0.05$.

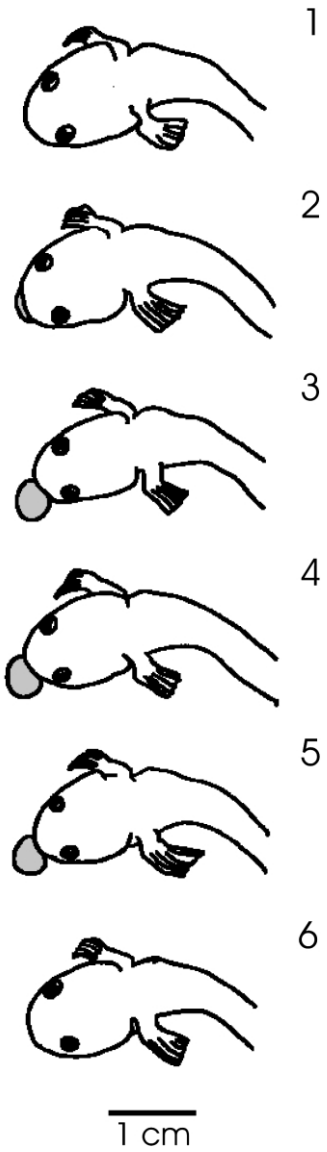

Fig. 3. Buccal bubble being released and recaptured from the mouth by *C. eremius*. Drawn from a video recording; images are consecutive frames separated by 1/25 of a second (50 frames s^{-1} interlaced).

Table 1
Oxygen consumption rate and the rate of operculum movements of *C. eremius* in freshwater and in air

Medium	Ambient concentration of O_2 (%)				$F_{d.f.}$, P value
	1.92	4.00	8.12	20.94	
Total O_2 consumption ($\mu l\ g^{-1}\ h^{-1}$)					
Water	123±8.0 (8)	226±16.1 (16)	377±34.2 (8)	253±24.3 (14)	$15.45_{3,42}$, <0.001
Air	39±4.0 (16)	91±17.5 (16)	136±18.5 (8)	169±15.8 (14)	$16.13_{3,50}$, <0.001
$t_{d.f.}$, P value	9.53_{11}, <0.001	5.68_{29}, <0.001	6.18_{10}, <0.001	2.91_{22}, <0.01	
Opercular movements (min^{-1})					
Water	167±8.23 (8)	156±3.7 (8)	154±5.6 (7)	139±9.2 (8)	$2.74_{3,27}$, 0.063
Air	64±7.35 (8)	96±11.0 (7)	53±9.1 (7)	71±5.6 (8)	$5.41_{3,27}$, <0.01
$t_{d.f.}$, P value	9.31_{13}, <0.001	5.18_{7}, <0.005	9.85_{8}, <0.001	6.31_{11}, <0.001	

Values are mean±1 S.E., with the sample size (n); mean body mass 0.51–0.59 g. P values are for comparisons among different O_2 concentrations (ANOVA) and t-test values are for comparisons between water and air for each ambient O_2 concentration.

3. Results

3.1. Respiration in freshwater

There was a significant difference among the $\dot{V}_{O_2}$ values for *C. eremius* at the four O_2 concentrations in freshwater (Table 1). $\dot{V}_{O_2}$ was higher at 8% than at 21% O_2 ($t_{13}=2.93$), indicating an initial lack of oxygen-conformity, then declined in an essentially linear fashion below 8% O_2 indicating oxygen-conformity; oxygen conformers passively allow their $\dot{V}_{O_2}$ to drop with a decrease in P_{O_2}, whereas O_2 regulators adjust gill ventilation and O_2 extraction and regulate $\dot{V}_{O_2}$ at near or above normal levels down to a critical $\dot{V}_{O_2}$, below which $\dot{V}_{O_2}$ is reduced (Graham, 1997). Similarly, there was a significant difference among the $\dot{V}_{O_2}$ values at the four different O_2 concentrations in air, but with only a slightly lower $\dot{V}_{O_2}$ in 8% than 21% O_2, then a fairly linear oxygen-conforming decline in $\dot{V}_{O_2}$ below 8% O_2. The $\dot{V}_{O_2}$ was always significantly higher in water than in air at the same O_2 concentration.

There was no significant difference in the mean rate of operculum movement over the four O_2 concentrations in water (Table 1). However, operculum movement rates differed significantly among the four O_2 concentrations in air. They remained about the same (Tukey test, $P>0.05$) for ambient O_2 concentrations of 21 and 8%, then increased significantly (Tukey test, $P<0.05$) from 8 to 4%, but did not significantly change further at 2% O_2 (Tukey test, $P>0.05$). The rates were always significantly lower in air than in water at the same O_2 concentration.

The quantity of oxygen extracted per opercular beat increased initially with aquatic hypoxia from 21 to 8% O_2 from 0.030 to 0.036 $\mu l\ O_2\ g^{-1}\ beat^{-1}$ ($253\ \mu l\ g^{-1}\ h^{-1}/139$ beats $min^{-1}\times 60 = 0.030\ \mu l\ O_2\ g^{-1}\ beat^{-1}$), then declined at 4% O_2 ($0.024\ \mu l\ O_2\ g^{-1}\ beat^{-1}$) and further at 2% O_2 ($0.012\ \mu l\ O_2\ g^{-1}\ beat^{-1}$). However, when expressed relative to the ambient fractional O_2 concentration (F_{O_2}; which reflects the O_2 content of inspired water) the relative amount of O_2 extracted per opercular beat increased from approximately $0.144\ \mu l\ O_2\ g^{-1}\ beat^{-1}\ F_{O_2}^{-1}$ at 21% O_2 ($0.03\ \mu l\ O_2\ g^{-1}\ beat^{-1}/0.2094\ F_{O_2} = 0.144\ \mu l\ O_2\ g^{-1}\ beat^{-1}\ F_{O_2}^{-1}$) to 0.449 at 8% then to $0.604\ \mu l\ O_2\ g^{-1}\ beat^{-1}\ F_{O_2}^{-1}$ at 4%, indicating a substantial improvement in relative O_2 extraction per opercular movement with hypoxia down to 4% O_2. However, at 2%, the relative opercular extraction remained at $0.639\ \mu l\ O_2\ g^{-1}\ beat^{-1}\ F_{O_2}^{-1}$ at 2% O_2. In air, *C. eremius* would normally remain motionless, only leaving their heads to intake or release a buccal air bubble.

3.2. Buccal air bubbles

In water, *C. eremius* has never been observed to gulp or release air bubbles. In air, 36 of 46 fish released an obvious bubble (see Figs. 2 and 3) that could be seen (and video-recorded) for a fraction of a second before bursting. In air, *C. eremius* would release a bubble from either the mouth or the opercular chamber (Fig. 2); and those from the mouth were occasionally rapidly

Table 2
Oxygen consumption via buccal air bubble release and across the skin

	Ambient concentration of O_2 (%)				$F_{d.f.}$, P value
	1.92	4.00	8.12	20.94	
Rate of air bubble release (h^{-1})					
Air	52 (±12.1) [7/8]	56 (±4.4) [16/16]	43 (±13.6) [4/8]	38 (±14.5) [9/14]	$0.81_{3,32}$, 0.50
Air bubble O_2 consumption ($\mu l\ g^{-1}$ bubble^{-1})					
Air	0.15 (±0.075) [6/8]	0.36 (±0.05) [13/16]	0.62 (±0.16) [3/8]	0.38 (±0.23) [4/8]	$2.67_{3,22}$, 0.072
Air bubble O_2 extraction ($\mu l\ g^{-1}$ bubble$^{-1}\ F_{O_2}^{-1}$)					
Air	7.9 (±3.91) [6/8]	9.1 (±1.16) [13/16]	7.6 (±1.94) [3/8]	1.8 (±1.09) [4/8]	$1.71_{3,22}$, 0.194
O_2 consumption rate via bubbles ($\mu l\ g^{-1}\ h^{-1}$)					
Air	5.8 (±1.34) [6/8]	19.7 (±2.68) [13/16]	16.6 (±1.83) [3/8]	10.3 (±2.16) [4/8]	$5.21_{3,22}$, <0.01
Percentage of total O_2 consumption via bubbles					
Air	15.0 (±2.45)	27.1 (±2.72)	14.5 (±2.52)	7.1 (±1.72)	$8.11_{3,22}$, <0.01

Values are mean±1 S.E., n is the number of specimens that released a bubble; N is the total number of specimens examined. P values are for comparisons among different O_2 concentrations (ANOVA) and t-test values are for comparisons between water and air for each ambient O_2 concentration.

recaptured by the mouth (Fig. 3). When the bubble burst there was a corresponding increase in the measured O_2 consumption (Fig. 1) that represents the lower concentration of O_2 in the bubble compared to that in the metabolic chamber. Although the measured consumption indicated 'pulses' in consumption, the $\dot{V}_{O_2}$ at the level of the tissues is presumably a continuous process operating at a fairly constant rate.

There was considerable variation in the rate of buccal bubble expulsion from mouth or operculum at any ambient O_2 concentration (see SE in Table 2), and there was no significant difference in the rate of buccal bubble release among the four concentrations of O_2 in air (Table 2). The mean O_2 consumption from each buccal air bubble (μl O_2 g^{-1} bubble^{-1}; i.e. area under each pulse in Fig. 1) was not significantly different for the four different O_2 concentrations in air. However, the O_2 consumed per buccal air bubble relative to the ambient O_2 content (μl O_2 g^{-1} bubble$^{-1}/F_{O_2}^{-1}$) was higher at 4% than 8%, and higher at 8% than at 21% O_2 (Table 2). This suggests that O_2 extraction from the air bubble can be increased with hypoxia, presumably reflecting physiological mechanisms (e.g. increased vasodilatation and blood flow, reduced blood P_{O_2}, etc). There was also a significant inverse correlation ($R=-0.60$, $P<0.05$) between the $\dot{V}_{O_2}$ g^{-1} bubble^{-1} value and the rate of bubble release, which suggests that there is greater O_2 extraction from an air bubble if it is retained longer in the buccal cavity. Corresponding to these changes, the percentage of total $\dot{V}_{O_2}$ accounted for by buccal air bubble O_2 consumption increased from approximately 7% at normal ambient O_2 to 27% at 4% O_2, then declined to 15% at 2% O_2. At 2% O_2 concentration, the metabolic rate of *C. eremius* decreased significantly ($t_{16}=2.9$) from that at 4% O_2, along with the rate of opercular movements ($t_{10}=2.41$), the percentage of O_2 consumption extracted from buccal bubble ($t_{15}=3.31$) and the rate of O_2 consumption via buccal bubbles ($t_{16}=4.64$) but not the rate of buccal bubble release ($t_7=0.33$).

In air, if the O_2 consumption via buccal bubbles is subtracted from total O_2 consumption then the remainder is presumably O_2 consumption across the skin, assuming there is no transfer of O_2 via the gills. Therefore, in normoxic air the goby has a cutaneous $\dot{V}_{O_2}$ of approximately 140 μl g^{-1} h^{-1}; it was lower at 8% (105 μl g^{-1} h^{-1}), and lower again at 4% (51 μl g^{-1} h^{-1}) and at 2% (31 μl g^{-1} h^{-1}) (Table 2). The amount of O_2 extracted cutaneously relative to the fractional ambient O_2 concentration (F_{O_2}; i.e. at 2% O_2, $F_{O_2}=0.031\div0.0194=1.6$) increases with hypoxia; it was lowest at 670 μl $F_{O_2}^{-1}$ g^{-1} h^{-1} at 21%, higher (1300) at 8% and 4%, and highest (1600) at 2% O_2.

Table 3
Oxygen consumption rate and the rate of operculum movements for *C. eremius* at varying salinities in water and air

Salinity (ppt NaCl)	Medium	O_2 consumption rate ($\mu l\ O_2\ g^{-1}\ h^{-1}$)	Operculum movements (min^{-1})
0	Water	253±24.3 (14)	139±9.2 (8)
	Air	169±15.8 (14)	71±5.6 (8)
0→35	Water	388±46.2 (7)	144±7.5 (8)
35	Water	336±39.4 (7)	145±3.7 (8)
70→35	Water	326±66.2 (4)	128±14.4 (4)
	Air	136±26.8 (5)	105±8.0 (8)
70	Water	426±34.0 (6)	162±10.2 (6)
	Air	188±30.5 (6)	198±29.9 (6)

Values are mean±1 S.E., with the sample size (n); the mean body mass varied between experiments from 0.41 to 0.94 g.

3.3. Effect of salinity

There was a significant difference ($F_{2,23}=7.63$) among $\dot{V}_{O_2}$ values for *C. eremius* in water at 0, 35 and 70 ppt NaCl, with fish at the higher salinities having a higher metabolic rate. There was no significant difference among $\dot{V}_{O_2}$ values ($F_{2,22}=0.96$) for fish in air having come from water containing 0, 35 or 70 ppt NaCl. $\dot{V}_{O_2}$ was significantly higher in water than in air (Table 3) for salinities of 35 ppt NaCl ($t_{10}=3.60$) and 70 ppt NaCl ($t_9=5.4$, Table 3) as also reported above for fish in freshwater.

No significant difference ($F_{2,19}=1.93$, $P=0.17$) was noted among the gill ventilation rates in water at the three salinities (0, 35 and 70 ppt NaCl) despite the difference in $\dot{V}_{O_2}$, but there was a significant difference ($F_{2,18}=19.73$, $P<0.05$) among opercular movement rates in air. The rate of operculum movement was significantly different in air and in water with a salinity of 0 ppt NaCl ($t_{11}=6.90$, $P<0.05$) and 35 ppt NaCl ($t_9=4.54$, $P<0.05$) but not at 70 ppt NaCl ($t_4=1.17$, $P=0.3$).

There was no difference between the $\dot{V}_{O_2}$ ($F_{2,16}=0.76$, $P=0\ 48$) and the rate of operculum movement ($F_{2,17}=2.06$, $P=0.16$) of *C. eremius* when moved from 0 to 35 ppt NaCl, or when moved from 70 to 35 ppt NaCl, compared with those held at 35 ppt NaCl (Table 3).

4. Discussion

4.1. Aerobic metabolism and respiration in water and air

In water, $\dot{V}_{O_2}$ for *C. eremius* is higher in 8% O_2 than normoxia, but was lower at 4% and 2% O_2 than at 8% O_2, indicating O_2-conformation below 8% O_2. The opercular pumping rate was not significantly altered with increased hypoxia (21–2% O_2). Oxygen extracted per opercular beat matched the $\dot{V}_{O_2}$ pattern, being higher at 8% than 21% but lower at 4% and 2% O_2. However, when expressed relative to ambient fractional O_2 concentration (which determines the O_2 content of inspired water), the relative amount of O_2 extracted per opercular beat increased from 21% to 8% O_2, then again to 4%, but remained unchanged between 4% and 2% O_2. These data suggest that at 4% ambient O_2, *C. eremius* has maximised its relative O_2 extraction and there was no further possible increase in relative O_2 extraction at 2% O_2 or there was a change in ventilation amplitude which was not measured. This is consistent with the observation of Gee and Gee (1991) that *Chlamydogobius* sp. (probably *C. gloveri*; Gee and Gee 1995) begins aerial respiration at approximately 0.7 ppm O_2 (approx. 1.8% O_2).

In air, $\dot{V}_{O_2}$ was slightly lower at 8% than 21% O_2, but it decreased below 8% O_2 indicating an O_2-conforming pattern. *Chlamydogobius eremius* in 8% O_2 was able to extract sufficient O_2 to maintain an aerial metabolic rate slightly lower than at 21% O_2. At 4%, $\dot{V}_{O_2}$ decreased and opercular rate increased, absolute O_2 extraction decreased and the use of buccal air bubbles increased, indicating respiratory stress and compensatory responses. At an O_2 concentration of 2%, the $\dot{V}_{O_2}$ of *C. eremius* was significantly lower than at 4%. The opercular pumping rate had also declined, the rate of release of buccal air bubbles had declined although not significantly (but it did not increase further), and the percentage O_2

extracted by buccal air bubbles declined (despite the overall decrease in metabolic rate). These results suggest that *C. eremius* is in severe and insufficiently compensated respiratory distress at 2% O_2.

Although there is no obvious general pattern in the O_2 consumption rate of fish when respiring in water or aerially at the surface, Graham (1997) in his comprehensive review of the metabolism of air-breathing fish, suggested that for active amphibious fishes the $\dot{V}_{O_2}$ is generally higher in air than in water, but for aquatically-active species that generally remain quiescent during their exposure to air (such as *C. eremius*) the metabolism in air would be lower than in water. The metabolic rate of *C. eremius* in normoxic air is significantly less than that in water, even with hypoxia down to 4% O_2 and over a wide range of ambient salinity and O_2 levels. There are a number of possible explanations for this. There may be a substantial metabolic cost in water for the high opercular pumping rate of aquatic ventilation, for ionoregulation or for activity. In air, *C. eremius* remained relatively motionless, only lifting their head to intake and release a buccal air bubble. In water, *C. eremius* mainly rested on the bottom but they did seem to move more than when in air, and their operculum movement rate was higher. The goby might become partially anaerobic when in air, but we did not examine this possibility.

4.2. Buccal air bubble respiration

Gee and Gee (1995) report the buccal bubble has two functions for *C. eremius*; maintaining head and body lift at the water surface and gas exchange. Gee and Gee (1995) reported an increased abundance of blood-filled capillaries in the lining of the roof of the buccal cavity for *C. eremius* held at <0.5 ppm O_2 for 5.5 h compared with those held at 8.0 ppm O_2, suggesting aerial respiration across the buccal lining. The release of buccal air bubbles by *C. eremius* when out of water (Figs. 2 and 3) corresponds with a pulsed increase in $\dot{V}_{O_2}$ (Fig. 1) providing direct evidence for the view of Gee and Gee (1991, 1995) that *C. eremius* uses its buccal air bubble for O_2 exchange based on their observations of buccal capillary abundance and engorgement during hypoxia. It is possible that a buccal air bubble is used to promote gas exchange across the gills in water, although we did not observe *C. eremius* gulping air at the water surface. The reason for the occasional oral release and recapture of a bubble is unclear, because a fresh bubble should contain more O_2 than one just expelled and rapidly re-engulfed. Oxygen consumption of gobies in air via the buccal air bubble varied between 27% and 7% of the total metabolic requirement, between 2% and 21% ambient O_2, with the lowest buccal air bubble $\dot{V}_{O_2}$ being in normoxic conditions. Oxygen extraction from the buccal air bubble is clearly insufficient to meet the normal metabolic requirements of *C. eremius* when it is out of water.

The amount of O_2 extracted from each buccal air bubble was lower at 2% O_2 than 4%, which, in turn, was lower than at 8% O_2 (Table 2). A decrease is expected, as the amount of O_2 available for extraction from each bubble would decrease in proportion to the percentage of O_2. However, the amount of O_2 extracted relative to the fractional ambient O_2 concentration was higher with hypoxia. This suggests either an increase in the fractional O_2 extraction at lower percentage O_2 to compensate for the lower amount of O_2 present in the buccal bubble, an increase in the buccal air bubble volume, or both. The highest rate of buccal air bubble release and operculum movements was at 4% O_2, presumably to compensate for the lower O_2 extraction levels. This pattern of increased gill ventilation rate with hypoxia then a decrease with severe hypoxia is consistent with the pattern of gill ventilation reported for *Chlamydogobius* sp. by Gee and Gee (1991). The level of hypoxia (2% O_2) at which we observed the above changes in gas exchange corresponds closely to the O_2 level of 0.7 ppm ($\approx$1.8% O_2) observed by Gee and Gee (1991) for aerial respiration by aquatic gobies (i.e. 10% of gobies using aquatic surface respiration).

4.3. Effects of salinity

Chlamydogobius eremius in the Lake Eyre drainage are subject to increases in salinity up to and over 100 ppt as the waterholes they inhabit evaporate (Glover, 1982), leaving a dry salt-encrusted depression in many areas. Hence, some fish would be subject to total salinities greater than 70 ppt NaCl. Increasing the salinity from 0 to 35 ppt NaCl or higher should increase the metabolic effort for osmoregulation for a teleost fish that maintains its internal ionic balance (e.g. Rao,

1968). For *C. eremius*, the $\dot{V}_{O_2}$ was higher at 35 and 70 ppt NaCl salinities than in freshwater, presumably reflecting in part the cost of osmoregulation (Table 3). Although the rates of operculum movements were slightly higher for gobies at higher salinities, these differences were not significant, indicating that *C. eremius* extracts more O_2 per operculum movement at higher salinity. Operculum movement rates were higher in fish that came from higher salinities than those from lower salinities when measured in air, although $\dot{V}_{O_2}$ did not differ. However, the role of opercular movements for a fish in air is unclear and these results are difficult to interpret. The $\dot{V}_{O_2}$ of *C. eremius* moved quickly from 0 to 35, or 75 to 35 ppt NaCl, and are similar to those of gobies at 35 ppt NaCl, suggesting that these gobies rapidly adapt to their new ionic environment. The absence of a difference among the rates of operculum movements for gobies held at 35 ppt NaCl and those transferred from freshwater to 35 ppt NaCl, and 70–35 ppt NaCl, are, therefore, consistent with a constant $\dot{V}_{O_2}$.

There are differing conclusions regarding the aerobic metabolic response of fishes to changes in salinity (see Nordlie et al., 1991) compared to the predicted effect of increased salinity and higher cost of osmoregulation (Potts, 1954). In general, over a wide range of salinities, the metabolic rate increases with higher salinity, reflecting the increased metabolic cost of iono- and osmo-regulation (as we observed for *C. eremius*). However, metabolic rate has not been observed to increase as expected with hypersalinity (>35 ppt NaCl) in some fishes, suggesting possible changes in ionic/osmotic regulation, changes in osmotic permeability, or changes in activity (see Nordlie et al., 1991). It will, therefore, be of interest to examine in more detail the patterns of iono/osmoregulation and metabolism for *C. eremius* over a wide range of hyper-saline conditions.

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