RESPIRATORY AND HYDROSTATIC FUNCTIONS OF THE INTESTINE OF THE CATFISHES *HOPLOSTERNUM THORACATUM* AND *BROCHIS SPLENDENS* (CALLICHTHYIDAE)

BY JOHN H. GEE

Department of Zoology, University of Manitoba, Winnipeg, Manitoba, Canada

AND JEFFREY B. GRAHAM

Smithsonian Tropical Research Institute, P.O. Box 2072, Balboa, Canal Zone

SUMMARY

1. The air-breathing behaviour of *Hoplosternum thoracatum* and *Brochis splendens* has been studied and their strategy of coordinating the respiratory and hydrostatic functions of the accessory respiratory organ has been examined.

2. *H. thoracatum* and *B. splendens* are continuous but not obligate air-

additional function of the swimbladder, whereas in other species this organ is diminutive and aerial respiration and buoyancy control are functions of another organ.

Regardless of the specific organ involved, the dual function presents potential conflicts. First, the fish needs to hold a volume of O_2 sufficient for respiration in its accessory respiratory organ without becoming too buoyant. Secondly, O_2 is continually absorbed without equivalent replacement of CO_2 (Johansen, 1970), reducing buoyancy between breaths. Mechanisms to minimize changes in buoyancy between air breaths have not been studied.

This paper analyses the air-breathing behaviour of *Hoplosternum thoracatum* (Valenciennes) and *Brochis splendens* (Castelnau), determines the contribution of aerial and aquatic respiration to total O_2 consumption, and describes the coordination of aerial respiration and hydrostatic control. *B. splendens* is benthic and occurs in still water. It has been collected from drainages in Brazil, Peru and Equador (Nijssen & Isbrücker, 1970). *H. thoracatum* occurs from the Pacific slope of Panama to the Atlantic drainages of Columbia and south to Paraguay (Miller, 1966). Its vertical distribution varies from benthic to midwater. We have collected it during the dry season from isolated pools (23–26 °C) in river channels where waters are hypoxic (< 20% saturation) and turbid. Both species use the intestine as an accessory respiratory organ: air is taken in through the mouth, passed through the gut and let out from the anus. *H. littorale* was reported to be an obligate air breather by Carter & Beadle (1931); but it is not known if *H. thoracatum* or *B. splendens* are also obligate air breathers. Kramer & Graham (1976) observed that air breaths by *H. thoracatum* were clumped in time (synchronous) and suggested that this reduces mortality from predation. Gee (1976) found that *H. thoracatum* breathed air in normoxic (near air-saturated) water and hypothesized that this was done in part to keep the accessory respiratory organ at the requisite volume for optimal buoyancy (assuming that O_2 was removed without equivalent replacement of CO_2). In *H. thoracatum* the swimbladder is minute and gas in the accessory respiratory organ provides nearly all of the buoyant lift (Gee, 1976).

MATERIALS AND METHODS

Fishes (46–57 mm fork length) were obtained from a tropical fish dealer in Winnipeg, Manitoba, and held in the laboratory at 24 (± 1) °C with a 12 h photoperiod and were fed Tetramin flakes. Respirometry studies were carried out in Panama on *H. thoracatum* collected at the Finca Tocumen near Panama City in the Republic of Panama. These were maintained in the laboratory as above.

Air-breathing behaviour

Whether *B. splendens* or *H. thoracatum* is an obligate air breather was tested by placing two individuals of each species in a small aquarium (17 × 14 × 20 cm) that was submerged in a larger aquarium (49 × 24 × 26 cm). A screen on top of the smaller tank prevented access to air. A gently bubbling air-stone circulated normoxic water (partial pressure of O_2 [P_{w,O_2}] *ca.* 150 torr) throughout the system.

The frequency and degree of synchronization of air breathing and frequency of branchial breathing were studied for ten *H. thoracatum* in normoxic and hypoxic (P_{w,O_2} < 40 torr) water when fish were undisturbed and disturbed. Fish (in

49 × 24 × 26 cm aquarium) were observed from behind a blind for a 30 min interval once per hour. From 270 to 450 min of observation were made during the day for each of the four treatments. Time to the nearest second of each air breath was recorded. Fish were disturbed by passing a piece of cardboard between the overhead light source (100 W bulb) and the aquarium three times followed by one splash on the water surface. This was done 15 min prior to the start of each 30 min of observation.

The relationship between activity and frequency of air breathing was also determined in each of these treatments. *H. thoracatum* is a relatively inactive fish spending most of its time resting on the bottom. The number of fish actively swimming at 5 min intervals throughout the observation period was recorded as an index of activity. Occasionally, and after a full day of observation, food was presented to the fish and after 15 min, activity and frequency of air breathing was recorded for an additional 30 min.

A control experiment was done to determine if synchronous air breathing might be attributed to some external stimulus rather than interactions between individuals. Six fish were held in separate aquaria (17 × 14 × 20 cm) and visually isolated from each other. They were observed undisturbed in normoxic water for 240 min and the time to the nearest second of each breath was recorded.

Aquatic and aerial $\dot{V}_{O_2}$ of H. thoracatum

Respirometry studies were carried out to determine how hypoxic water affects the rate of aerial respiration of *H. thoracatum*. The effect of decreasing P_{w,O_2} (progressive hypoxia) on total (aerial and aquatic) routine O_2 consumption rate ($\dot{V}_{O_2}$) was also measured and compared in fish with and without access to air.

A closed respirometer system (1·2 l; Graham, 1973) was used for aquatic respiration measurements. Levels of P_{w,O_2} in the respirometer were monitored by an O_2 electrode that was mounted in a separate chamber to facilitate calibration without disturbing the fish. Water flow was directed at the electrode tip to ensure an accurate, stable signal. The respirometer was submerged in a water bath at 25 ($\pm$0·1) °C. Penicillin (25×10^3 units l^{-1}) was added to reduce microbial respiration.

Starved (12 h) fish were placed in the respirometer and given 12–24 h to adjust to the chamber and recover from handling stress. In this period the respirometer operated in an open position and aerated water was continually pumped through the chamber. When the respirometer was closed, by connecting its in- and out-flow tubes, water was then recirculated through the system and O_2 decreased as the fish respired. The O_2 electrode was connected to a recorder which provided a continuous trace of O_2 depletion by the fish. Analysis of the slope of this record permitted calculation of $\dot{V}_{O_2}$, (based on respirometer volume, O_2 solubility in water at 25 °C, and fish weight).

Before each determination, the O_2 electrode was calibrated in air and N_2 and standardized for linearity against a Radiometer O_2 electrode. The respirometer chamber was darkened to reduce visual stimulation. An Erlenmeyer flask (1 l) was used as the respirometer chamber and 50 ml of air added (by syringe) to the flask created an air phase at its top and made possible the measurement of aerial $\dot{V}_{O_2}$ respiration in normoxic and hypoxic water. This was done by sampling the air phase at regular intervals and measuring P_{w,O_2} (with the Radiometer). After correcting for diffusion from air to water (by using a non-air breathing fish as a control), aerial $\dot{V}_{O_2}$
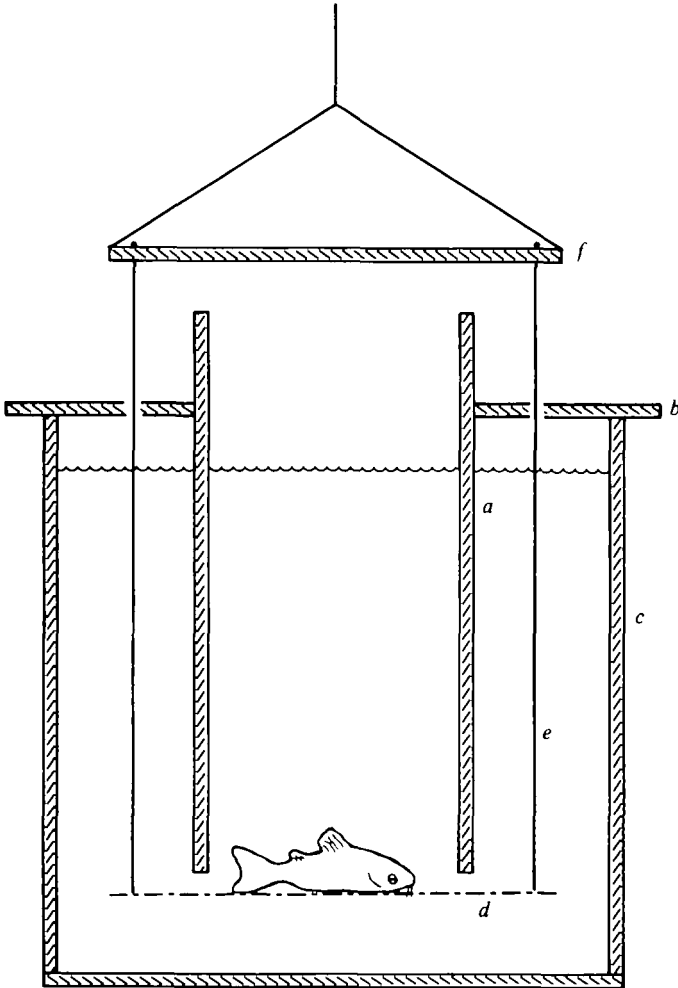
Fig. 1. Aquarium balance system to measure weight of fish in water (buoyancy). The plexiglass cylinder (a) was supported by four horizontal bars (b), on the top of the aquarium (c). The screen (d) was suspended independently of the cylinder by four nylon threads (e), from a plexiglass plate (f) which was suspended from a beneath-the-balance hook. The screen could thus respond to changes in the weight (buoyancy), of the fish in water.

was calculated. A small needle hole in the submerged respirometer tubing was used to equilibrate pressures when the gas was extracted. When the respirometer was closed, P_{w, O_2} gradually decreased and the fish became progressively more dependent on aerial respiration.

Buoyancy

To understand the coordination of aerial respiration and hydrostatic control it is necessary to know the relative contribution of the swimbladder and the accessory respiratory organ to buoyancy and the changes in the volume of the latter between air breaths. Such changes in buoyancy could be measured by recording the weight of the fish in water at regular intervals. These changes in weight can be equated to volume because 1 ml of gas at a given depth will support 1 g of fish tissue in fresh water (Gee

1970). *H. thoracatum* and *B. splendens* were negatively buoyant and relatively inactive. This made it possible to design an aquarium balance system that permitted measurement of fish weight in water at any time (Fig. 1). Fish were contained in a plexiglass cylinder (9 cm ID) that was supported by the walls of the aquarium (22 × 22 × 22 cm). Just below this cylinder a stainless-steel screen was suspended from a below-the-balance hook of a Sartorius balance (Model 2250). Fish rested on the screen and their weight in water (± 0·001 g) could be measured. Individual fish were placed into the cylinder at 17.00 h and observations (from behind a blind) were begun at 09.00 h on the following day. Fish weights in water were recorded at 5 min intervals for about 7 h. After the last recording fish were anaesthetized (MS222) and the volumes (± 0·001 ml) of the accessory respiratory organ and the swimbladder were measured using the procedure of Gee (1976). The weight of the gas-free fish in water was measured and buoyancy was determined by dividing the volume of gas (ml) in both organs intact by the weight (g) of the gas-free fish in water. Measures of buoyancy of individual fish at the end of each day of observation could be used to (*a*) convert the weight-in-water figures obtained at 5 min intervals into buoyancy values, and (*b*) determine the relative contribution of the swimbladder and accessory respiratory organs to buoyancy. Internal pressure of gas in the latter was calculated for each fish at the end of each day of observation by dividing the volume of gas released from it at atmospheric pressure by its intact volume (Gee, 1976).

Effects of breathing N_2 and O_2

An hydrostatic function of the accessory respiratory organ could be demonstrated if fish breathed air in response to a decreased volume of the accessory respiratory organ and not to a change in the composition of gases within it. This information was obtained by observing fish breathing either N_2, O_2 or air.

Experiment 1. Activity ,air breathing and frequency of branchial breathing with gas phases of either air, N_2 or O_2 were compared for ten *B. splendens*. Fish, held in a 49 × 24 × 26 cm aquarium, were observed from behind a blind for 30 min at hourly intervals for at least 600 min for each treatment. Number of air breaths, index of activity (as above), and number of branchial breaths in 15 sec were recorded for 5 fish during the 30 min period. A continuous supply of either O_2 or N_2 was maintained in the 5 cm space between the water surface and a sealed glass lid (with an exhaust vent) on the top of the aquarium. Water was kept normoxic by constant addition of aerated water.

Experiment 2. Individual *B. splendens* were observed in the aquarium balance system over 5 days to compare the effect of breathing either N_2, O_2 or air on buoyancy change between breaths and the buoyancy at which an air breath was initiated. On the first 2 days fish were provided with an air phase above the water but on the last 3 days air was replaced by either N_2 or O_2. These gases were continuously supplied to the top of the cylinder and a plastic cap with a small exhaust vent was used to seal the top of the cylinder when these gases were used. Two fish were used in each treatment and were observed for about 8 h each day with their weight in water recorded every 5 min. At the end of the 5-day test each fish was anaesthetized and the above buoyancy-related measurements were made. Fish were fed at the end of each day's test and it is assumed that gain/loss of fish tissue was negligible. Water in the system was kept normoxic by a continuous inflow of aerated water.
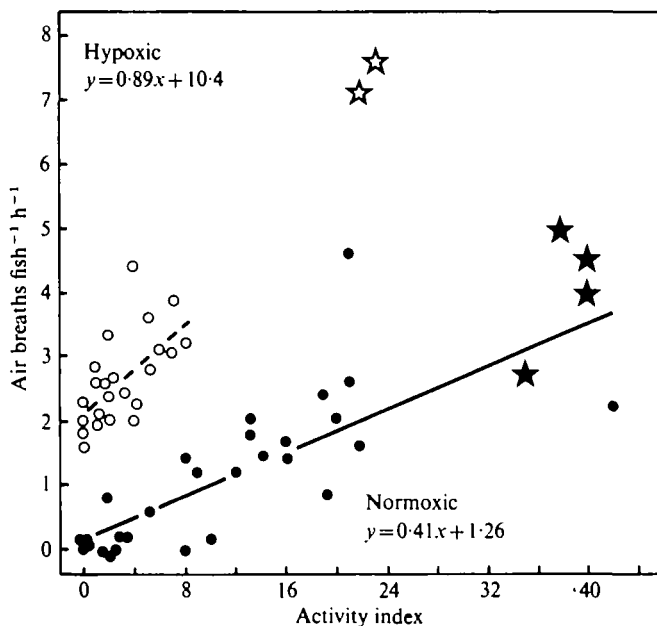
Figure. 2. Relationship between activity and frequency of air breathing in normoxic (closed symbols, $n = 31$) and hypoxic (open symbols, $n = 24$) water. Each point shows the data obtained for ten fish over a 30 min period. Maximum index of activity = 70. Stars indicate a 30 min observation following feeding and these were ommitted from regression analyses.
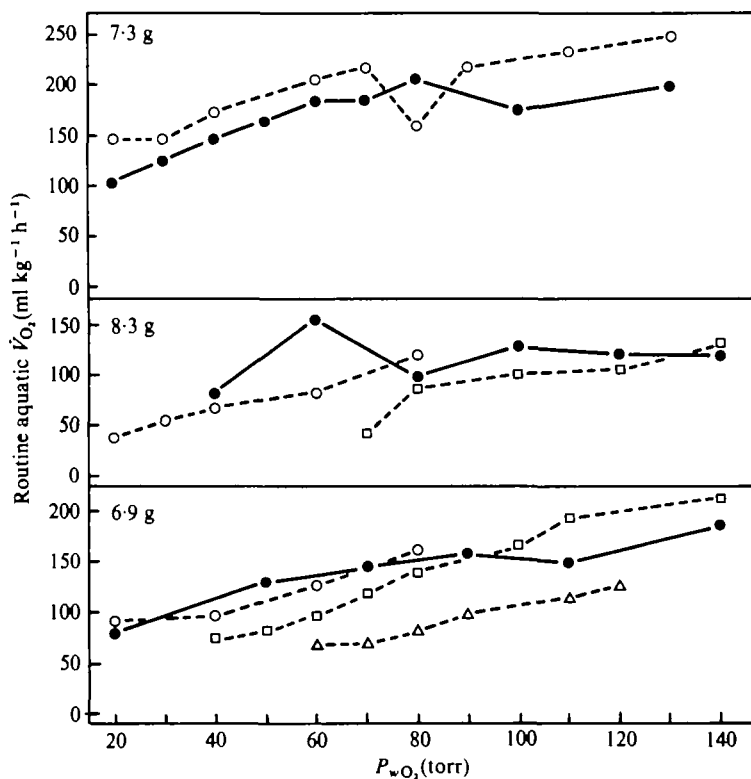
RESULTS

Air-breathing behaviour

All individuals of both species tested survived 48 h without access to air and *H. thoracatum* survived 7 days in the respirometer in near normoxic water (100–150 torr).

H. thoracatum breathed air in normoxic and hypoxic water and when frightened or undisturbed. Air breathing was most frequent when activity was high (Fig. 2). Four regression equations were calculated for unfrightened and frightened fish in normoxic and in hypoxic water. Slopes and intercepts of lines for unfrightened and frightened fish were similar ($P > 0.05$) in both situations and were combined yielding the two equations shown in Fig. 2. Slopes of these lines did not differ significantly ($P > 0.10$) but intercepts did ($P < 0.005$). Disturbance had no effect on the frequency of branchial breathing or the relationship between activity and frequency of air breathing in either normoxic or hypoxic water. In hypoxic water activity was generally lower and frequency of air breathing was higher than in normoxic water (Fig. 2). Branchial breathing in hypoxic water (96 min^{-1}) was greater than in normoxic water (72 min^{-1}). There was little air breathing in normoxic water when activity was low. Fish were very active during and after feeding and the frequency of air breathing was close to that extrapolated from regression equations.

Synchronous air-breathing was observed in all four treatments. Table 1 shows that observed air-breathing frequencies differed from the expected frequencies of a Poisson distribution at all time intervals. Variance/mean ratios were significantly greater than 1·0 ($P < 0.001$; t test), indicating a contagious distribution of air breaths over time

Table 1. *Synchronization of air breaths of a group of ten* H. thoracatum *in normoxic and hypoxic water when disturbed and undisturbed*

	Frequency distribution of breaths min⁻¹ of observation:				Time (min)	Breath min⁻¹	No. of breaths	$S^2/\bar{x}$
	0	1	2	3–6				
Normoxic								
Undisturbed					420	0·093	39	1·32
Observed	388	26	5	1				
Expected	383	35	2	0				
Disturbed					360	0·217	78	1·27
Observed	298	49	10	3				
Expected	290	63	7	0				
Hypoxic								
Undisturbed					450	0·427	192	1·60
Observed	328	74	32	16				
Expected	294	125	27	4				
Disturbed					270	0·459	124	1·94
Observed	193	54	9	14				
Expected	171	78	18	3				

Fig. 3. The effect of P_{w,O_2} on aquatic $\dot{V}_{O_2}$ of three *H. thoracatum* with access to surface (open symbols) and without access to the surface (closed symbols). Open symbols of different shapes represent different trials on the same fish.

(Table 1). The frequency distribution of air breaths by isolated fish was not different from a Poisson (variance/mean = 1·097) and thus were random. Disturbance had no effect on the degree of synchrony of air breathing by fish in either normoxic or hypoxic water. There was, however, a tendency for a higher degree of synchrony in hypoxic water as evidenced by the larger variance/mean ratios (Table 1).

Aquatic and aerial $\dot{V}_{O_2}$ of H. thoracatum

H. thoracatum has a mean aquatic $\dot{V}_{O_2}$ of 155 ml kg^{-1} h^{-1} at $P_{w,O_2} \leqslant$ 100 torr. With progressive hypoxia aquatic $\dot{V}_{O_2}$ is reduced and below 40 torr it averaged 95 ml kg^{-1} h^{-1} (Fig. 3). With reduced P_{w,O_2} the $\dot{V}_{O_2}$ dropped steadily, indicating that *H. thoracatum* is an O_2 conformer, but the absolute reduction of aquatic $\dot{V}_{O_2}$ at 40 torr was only 40 %. In some cases access to air lowered aquatic $\dot{V}_{O_2}$ but the rate of decrease in $\dot{V}_{O_2}$ with P_{w,O_2} was not affected by whether or not the fish could breathe air.

With access to air *H. thoracatum* increased reliance upon aerial respiration and maintained a more or less constant $\dot{V}_{O_2}$ as P_{w,O_2} decreased (Fig. 4). There was a slight reduction of total $\dot{V}_{O_2}$ at lower P_{w,O_2} levels but the total bimodal (air and water) $\dot{V}_{O_2}$ was
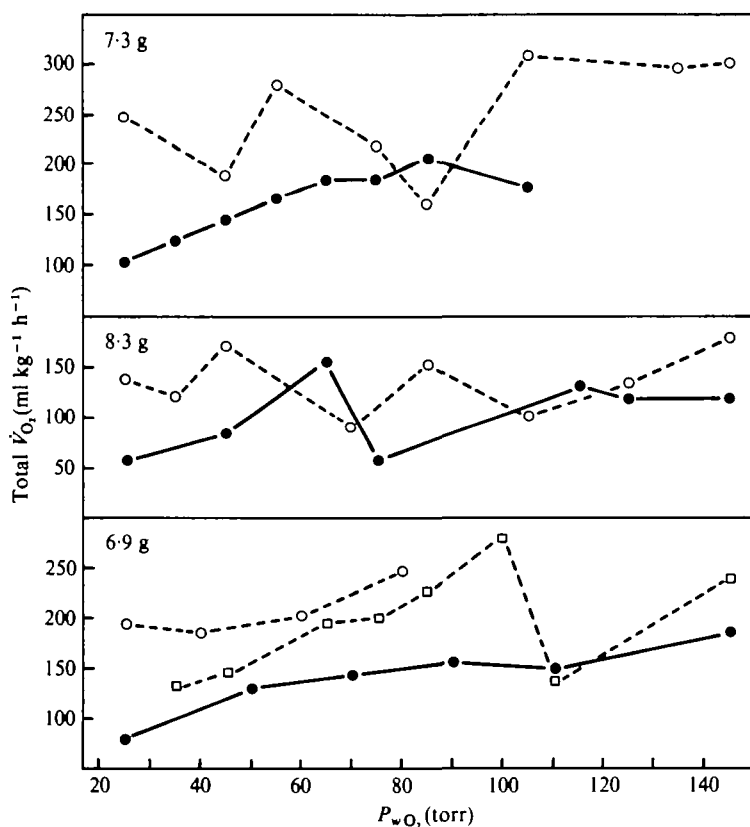

Fig. 4. Comparison of total $\dot{V}_{O_2}$ of three *H. thoracatum* respiring bimodally (air and water; open circles) with those respiring without access to air (solid circles). Comparisons made at different levels of P_{w,O_2}. Open symbols of different shapes represent different trials on the same fish.
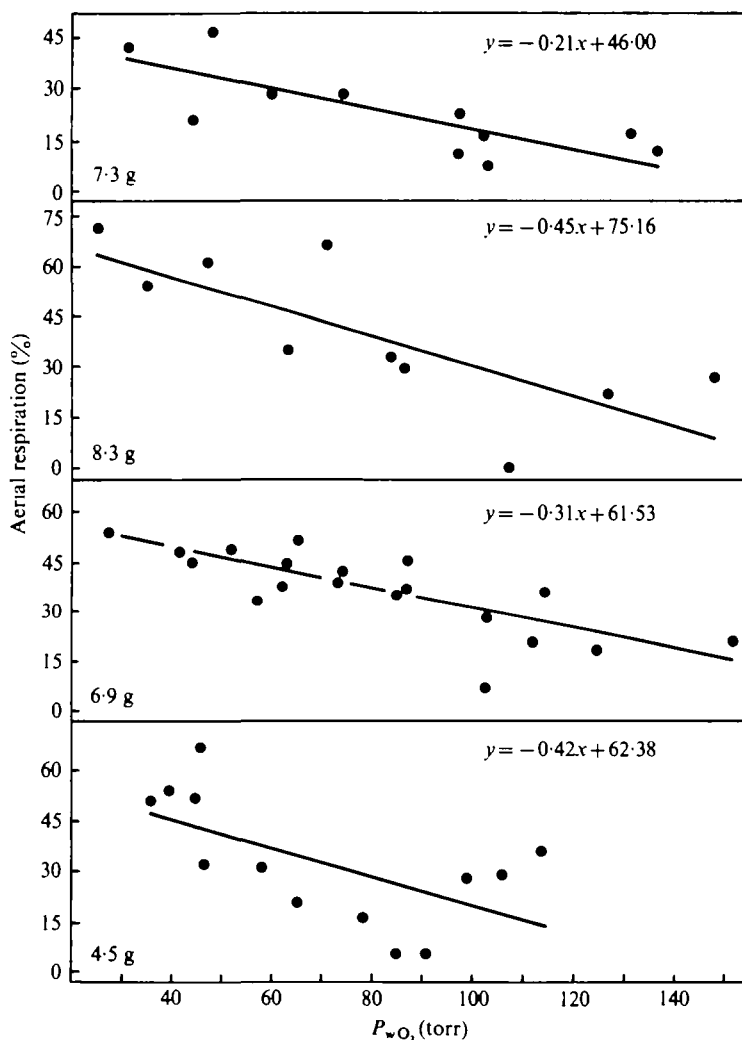
Fig. 5. Effect of P_{w,O_2} on the percentage contribution of air breathing to total O_2 consumption of four *H. thoracatum*.

much higher than the $\dot{V}_{O_2}$ measured for fish respiring in water only. In normoxic water aerial respiration was irregular. In some cases it contributed to over 35 % of total $\dot{V}_{O_2}$ but there were periods when no aerial respiration occurred. In progressively hypoxic water aerial $\dot{V}_{O_2}$ became less variable and increased its percentage contribution total $\dot{V}_{O_2}$ (Fig. 5) although its absolute rate did not markedly increase as aquatic respiration decreased. At $P_{w,O_2} < 40$ torr aerial $\dot{V}_{O_2}$ ranged from 42–72 % of total $\dot{V}_{O_2}$ (Fig. 5).

Buoyancy

B. splendens and *H. thoracatum* are negatively buoyant. The accessory respiratory organ provides about 75 % of the lift required to attain neutral buoyancy whereas the swimbladder provides less than 5 %. Internal pressures of gas in the accessory respira-

Table 2. *Mean buoyancy derived from the accessory respiratory organ and the swimbladder of* H. thoracatum *and* B. splendens

(Data on buoyancy show the contribution of each organ to lift. Neutral buoyancy is attained when total lift is 1·0 ml g^{-1}. Figures in parentheses are 95 % confidence intervals on means.)

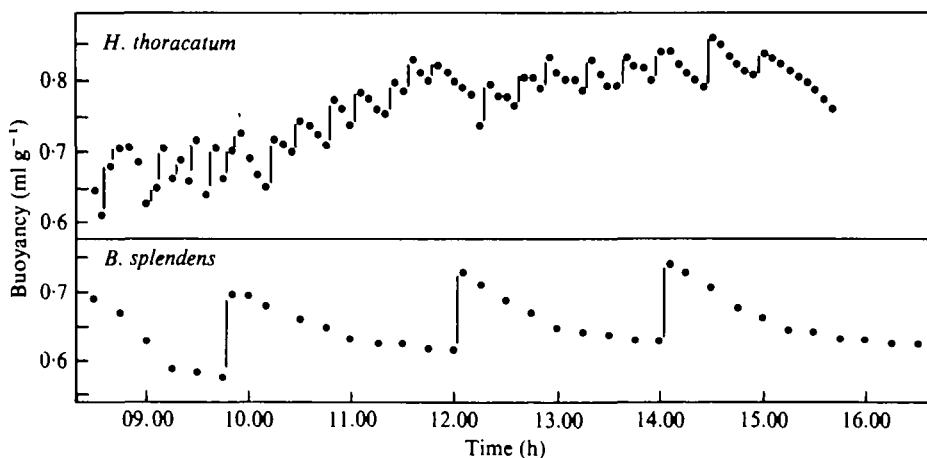|	*H. thoracatum*	*B. splendens*
Number examined	6	12
Mean buoyancy (ml g^{-1})		
Accessory respiratory organ	0·76 (±0·01)	0·73 (±0·05)
Swimbladder	0·02 (±0·007)	0·03 (±0·003)
Mean internal pressure (atm)		
in accessory respiratory organ	1·036 (±0·025)	1·051 (±1·018)

Fig. 6. Representative changes in buoyancy between air breaths for an individual *H. thoracatum* and an individual *B. splendens*. Buoyancy values for the latter are given only at 15 min intervals. Vertical lines indicate a breath of air.

tory organ were just above atmospheric (Table 2). Although the mean buoyancy of both species was similar, the mean decline in volume of the accessory respiratory organ between breaths was greater in *B. splendens* (13·2 %) than in *H. thoracatum* (7·8 %). On average, *H. thoracatum* breathed air more frequently (every 22·5 min) than *B. splendens* (every 84·4 min, Table 3). *H. thoracatum* maintained more precise control over its buoyancy while *B. splendens* often remained at low buoyancy for long periods before taking a breath of air (Fig. 6). Both species showed a gradual increase in buoyancy throughout the day.

Effects of O_2 and N_2 on air breathing and buoyancy in B. splendens

Activity and frequency of branchial breathing were lowest when *B. splendens* was breathing O_2, highest when breathing N_2 and intermediate when breathing air (Table 4a). The frequency of air breathing was variable in all treatments and differences were not evident between air, O_2 or N_2 (Table 4a, b). The decline in buoyancy between breaths was variable when breathing O_2 (Fig. 7) but on average exceeded that when breathing air (Table 4b). When breathing N_2, the decline in

Table 3. *Changes in buoyancy of individual* H. thoracatum *and*
B. splendens *during daily observations*

	H. thoracatum				
Fish	1	2	3	4	5
Time observed (min)	425	420	385	430	430
No of air breaths	22	15	22	14	20
Mean decrease in buoyancy between breaths (%)	7·2	10·5	9·1	7·1	5·1
Mean time between breaths (min)	19·3	34·7	17·5	30·7	21·5

	B. splendens					
Fish	1	2	3	4	5	6
Time observed (min)	480	445	525	435	420	900*
No of air breaths	4	11	3	8	5	7
Mean decrease in buoyancy between breaths (%)	14·3	7·3	18·3	14·9	9·2	15·4
Mean time between breaths (min)	120·0	40·5	175·0	54·4	84·0	128·6

* Observed over two days.

Table 4. *Effects of breathing air,* O_2 *or* N_2 *on air breathing behaviour of*
B. splendens

(Figures in parentheses represent 95 % confidence limits on the mean. In Exp 1 fish were observed for at least 10 h for each treatment. In Exp 2 fish were observed for at least 14 h when breathing air and at least 21 h when breathing either N_2 or O_2.)

(a) On a group of 10 fish (Exp 1)

Gas inspired ...	Air	O_2	N_2
Mean air breaths h^{-1}	0·3	1·1	0·2
Mean branchial breaths min^{-1}	105·2	91·2	131·2
Mean index of activity	4·5	2·2	9·2

(b) On individual fish (Exp 2)

	Fish no. 1		Fish no. 2	
Gas inspired	Air	O_2	Air	O_2
No. of breaths	35	36	11	17
Mean air breaths h^{-1}	2·5	1·8	0·8	1·0
Mean decrease in buoyancy (ml g^{-1})	6·7 (±1·7)	9·1 (±1·9)	9·5 (±1·5)	17·0 (±4·1)
Rate of decrease (ml g^{-1} min^{-1})	0·28	0·27	0·12	0·28

	Fish no. 3		Fish no. 4	
Gas inspired	Air	N_2	Air	Air
No. of breaths	26	10	4	13
Mean air breaths h^{-1}	2·1	0·7	0·4	0·7
Mean decrease in buoyancy (ml g^{-1})	4·6 (±1·1)	2·1 (±1·2)	14·0 (±2·3)	2·7 (±1·2)
Rate of decrease (ml g^{-1} h^{-1})	0·16	0·02	0·10	0·03

buoyancy was consistently very slight (Fig. 7). The rate of decrease in buoyancy between breaths was variable when breathing air or O_2 but was very low when breathing N_2 (Table 4b). The mean buoyancy at which individual *B. splendens* took an air breath varied within and between individuals and no consistent patterns were seen for fish breathing air, O_2 or N_2 (Fig. 7).
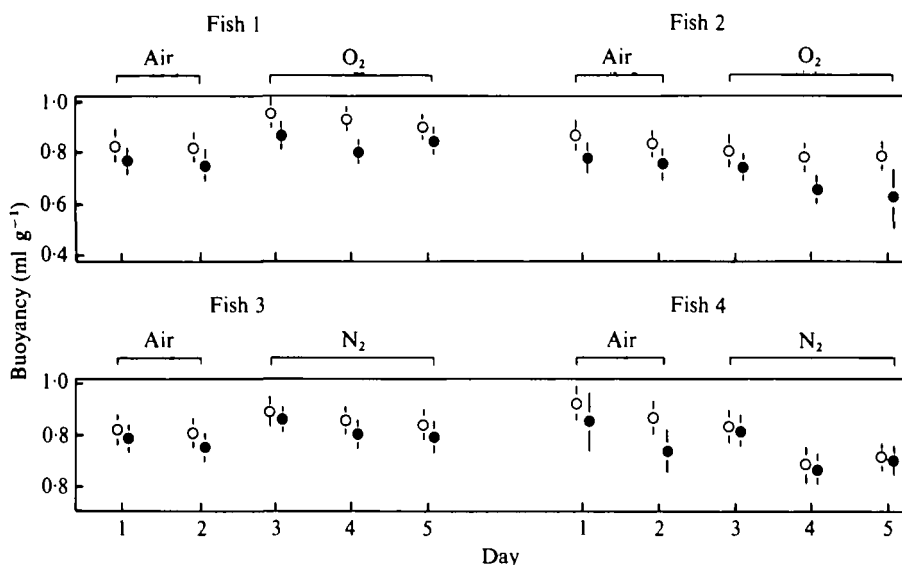
Fig. 7. Mean change in buoyancy between inspiration (open circles) and expiration (closed circles) of individual *B. splendens*. Each fish was observed for 2 days with access to air, followed by 3 days with access to O₂ or N₂. Identification numbers for each fish correspond to those in Table 3 (*b*). Vertical lines represent 95 % confidence limits on the mean.

DISCUSSION

Factors affecting air breathing

B. splendens and *H. thoracatum* are continuous but not obligatory air-breathers. Individuals of *H. thoracatum* breath air in synchrony with each other, both in normoxic and hypoxic water, and when disturbed and undisturbed. The level of synchrony increased slightly when fish in hypoxic water were disturbed. Kramer & Graham (1976) suggested synchronous air-breathing may reduce susceptibility to attack from aquatic and terrestrial predators and likened its effect to schooling which reduces the probability of contact between predator and prey. Field tests of this hypothesis by Kramer & Graham (1976) showed that disturbance temporarily halted air breathing by *H. thoracatum* but it was not determined if the level of synchrony changed.

The air-breathing frequency of *H. thoracatum* increased with activity and when fish were in hypoxic water. Also, total $\dot{V}_{O_2}$ increased when fish were able to breathe bimodally in air and water. From this it could be expected that the percentage contribution of air breathing to total $\dot{V}_{O_2}$ would increase with activity and that typical levels of activity would remain within the scope of aerobic metabolism of the fish regardless of P_{w,O_2}. In this manner, air breathing would have the important function of increasing the metabolic scope of *H. thoracatum* for activity beyond the limits imposed by aquatic respiration. Grigg (1965) suggested this as one function of air breathing in the Australian lungfish (*Neoceratodus*) which also increased $\dot{V}_{O_2}$ and breathed more frequently when active. Similar correlations between activity and air-breathing frequency have been found for the bowfin, *Amia* (Johansen, Hanson, & Lenfant, 1970; Horn & Riggs, 1973).

Aerial and aquatic respiration

The pattern of aquatic respiration seen in *H. thoracatum* in response to progressive hypoxia is different from most non-air-breathing fishes which initially increase their $\dot{V}_{O_2}$ until P_{w,O_2} reaches a critical level and they become O_2 conformers (Hughes, 1973). *H. thoracatum* has specializations that favour aquatic respiration such as developed gills and moderately high blood haemoglobin concentration (9 g%, Graham, in prep.). In hypoxic water it increases frequency of aquatic breathing by 25% and although an O_2 conformer, it can still respire aquatically (in hypoxia) at a rate over 60% of its routine $\dot{V}_{O_2}$ in normoxic water. This pattern is similar to that found in other air-breathing fishes (*Anabas* and *Electrophorus*, Hughes & Singh, 1970; Farber & Rahn, 1970). *Saccobranchus* and *Clarias* are also O_2 conformers under certain conditions (Hughes & Singh, 1971, Jordan, 1976; Singh, 1976).

It is important to consider how air breathing in *H. thoracatum* relates to its aquatic respiration and other factors such as activity and how this relationship compares with other air-breathing fishes. There is a tendency in the literature to consider air-breathing fishes purely on the basis of their degree of dependence upon aerial O_2 (Carter, 1957; Johansen, 1970; Singh, 1976). Facultative air breathers, for example, normally respire aquatically and use air breathing only when poor conditions for aquatic respiration prevail. Obligate air breathers must continually respire bimodally, with some species showing much more dependence on aerial O_2. Our study demonstrates that *H. thoracatum* is typical of a group of fishes in which air breathing, in addition to supplying O_2, seems to have assumed an important role in other functions. This species continually respires aerially and aquatically but never exclusively in either medium although the relative contribution of the two modes varies with P_{w,O_2}. When breathing bimodally its total $\dot{V}_{O_2}$ is higher. *H. thoracatum*, however, is not an obligate air breather and its reliance upon aerial respiration in hypoxic water is not particularly heavy (not exceeding 65% of total $\dot{V}_{O_2}$ at $P_{w,O_2} = 20$ torr), thus air breathing in this fish is also not purely facultative. Our study further demonstrates that air breathing correlates with both activity (and presumably metabolic demand) and buoyancy control (discussed elsewhere) in *H. thoracatum*. A factor that seems to be very important in allowing the diversification of the role of air breathing in *H. thoracatum* is its rather efficient aquatic respiratory system which allows the same level of aquatic $\dot{V}_{O_2}$ whether or not air breathing is occurring, and remains moderately independent of hypoxia (i.e. aquatic $\dot{V}_{O_2}$ was only reduced 40% from 150 to 40 torr), even though the fish is an O_2 conformer.

Buoyancy control

B. splendens averaged a 13·2% decrease in buoyancy between air breaths as opposed to 7·8% in *H. thoracatum*. Variations in buoyancy of this magnitude exceed differences between individual non-air-breathing fish in still water (Gee, 1977). The difference between *B. splendens* and *H. thoracatum* is related to their behaviour patterns. The latter is more active and often swims in mid-water, which is reflected in its more precise control of buoyancy (i.e. a minimum change between breaths) to permit more efficient locomotion. *B. splendens* remains on the bottom much of the time. Even when its lowest buoyancy was reached this fish usually did not surface immediately. It

presumably would be less sensitive to buoyancy changes and precise buoyancy control would not be required.

A reduction in excess internal pressure of gas in the accessory respiratory organ between breaths could compensate for only part of the decline in buoyancy between breaths because the capacity to increase the volume of the accessory respiratory organ by pressure reduction is exceeded by the volume of O_2 absorbed from it. Internal pressures of gas in *H. thoracatum* and *B. splendens* (1·036 and 1·051 atm respectively; just after an air breath) are low when compared to those found in the swimbladder of temperate and tropical fishes (1·12 atm and higher; Gee, Machniak & Chalanchuk, 1974; Gee & Gee, 1976). If this excess pressure was lowered as O_2 was absorbed from the accessory respiratory organ then this would minimize its overall reduction by up to 3·5% (*H. thoracatum*) and 5% (*B. splendens*) of its volume. Reductions of 7·8% and 13·2% respectively were observed. It is not known if changes in internal pressure did occur between breaths and if so how much effect they had on the observed decrease in volume of the accessory respiratory organ between breaths. An individual electric eel (*Electrophorus*) was observed to reduce the volume of its accessory respiratory organ by 10·9% between breaths (Farber & Rahn, 1970) but the reduction of buoyancy would be much less as the buccal chamber is used in aerial respiration and is separated from the hydrostatic organ (swimbladder) whose volume would not be directly affected by respiration.

Is the air-breathing response evoked by a change in gas composition within the accessory respiratory organ or by a change in its volume (buoyancy)? Our data for *B. splendens* indicate the latter. When it was breathing only O_2, the decrease in buoyancy was more than when breathing air but the difference was not great. Thus even with an unusually high P_{O_2} in the accessory respiratory organ, *B. splendens* continued to inspire gas. The rate of gulping gas was very infrequent when breathing N_2. Although some CO_2 could accumulate in the accessory respiratory organ between air breaths it is not likely that this could stimulate inspiration because the period between breaths was prolonged when breathing only N_2, but *B. splendens* continued to inspire gas under this condition even though optimal buoyancy had not been completely lost. It is possible that abnormally high levels of CO_2 were reached in the accessory respiratory organ due to the prolonged period between breaths and this may have stimulated inspiration. These data indicate that *B. splendens* does not breathe air primarily in response to normal changes in gas composition in the accessory respiratory organ but that a change in volume probably initiates a breath. This response has been found in *Amia* where a change in volume of the swimbladder, here functioning as an accessory respiratory organ, alters the pattern of its breathing (Johansen, 1970).

Obligate and facultative air breathers can be differentiated on the basis of whether or not P_{w,O_2} controls air breathing (Johansen, Hanson & Lenfant, 1970). Obligate air breathers respond to hypoxia in the accessory respiratory organ by increasing the frequency of air breathing without altering branchial breathing. They do not appear to be affected by a low P_{w,O_2}. Facultative air breathers, however, increase air-breathing frequency and branchial breathing in response to low P_{w,O_2}.

H. thoracatum and *B. splendens* appear to fit the above pattern for facultative air breathers. *H. thoracatum* responds to aquatic hypoxia by increasing frequency of aerial and branchial breathing. Its response to hypoxia in the air is unknown. *B. splendens*

does not increase air-breathing frequency in response to either hypoxia (N_2) or hyperoxia (O_2) in the air or in its accessory respiratory organ. However, the rate of branchial breathing was increased when breathing N_2 and decreased when breathing O_2. The electric eel (*Electrophorus electricus*), an obligate air breather, responded to breathing O_2 by prolonging the interbreath interval by 2·5 times (Gary & Rahn, 1970).

Carter & Beadle (1931) described *H. littorale* as an obligate air breather but this may not be the case. Examination of their study reveals that only one fish was tested and that P_{w,O_2} was assumed to be near saturation but not actually measured. Their description of the response of *H. littorale* to progressive hypoxia (i.e. increased branchial breathing) is typical of facultative rather than obligatory air breathers.

We conclude that *H. thoracatum* and *B. splendens* are continuous non-obligate air breathers. Aerial respiration normally supplements branchial respiration and during increased activity may enable these fishes to increase their metabolic scope beyond the limits of branchial respiration. The volume of gas in the accessory respiratory organ is regulated within limits and this organ assumes an hydrostatic function. Co-ordination of respiration and hydrostatic control is achieved by frequent air breaths taken at least in part in response to changes in the volume of the accessory respiratory organ (buoyancy). Even so, buoyancy control is not precise as changes in the volume of the accessory respiratory organ between breaths are considerable. Lack of precise buoyancy control may not be that critical for those species that are relatively inactive and remain negatively buoyant on the bottom part of the time.

REFERENCES

CARTER, G. S. (1957). Air breathing. In *Physiology of Fishes*, vol. 2 (ed. M. Brown), pp. 65–97. New York: Academic Press.

CARTER, G. S. & BEADLE, L. C. (1931). The fauna of the swamps of the Paraguayan Chaco in relation to its environment. II. Respiratory adaptations in the fishes. *J. Linn. Soc. (Zool.)* **37**, 327–368.

FARBER, J. & RAHN, H. (1970). Gas exchange between air and water and the ventilation pattern in the electric eel. *Resp. Physiol.* **9**, 151–161.

GAREY, W. F. & RAHN, H. (1970). Normal arterial gas tensions and pH and the breathing frequency of the electric eel. *Resp. Physiol.* **9**, 141–150.

GEE, J. H. (1970). Adjustment of buoyancy in blacknose dace, *Rhinichthys atratulus*. *J. Fish .Res. Bd Can.* **27**, 1855–1859.

GEE, J. H. (1976). Buoyancy and aerial respiration: factors influencing the evolution of reduced swimbladder volume of some Central American catfishes (Trichomycteridae, Callichthyidae, Loricariidae, Astroblepidae). *Can. J. Zool.* **54**, 1030–1037.

GEE, J. H. (1977). Effects of size of fish, water temperature and water velocity on buoyancy alteration by fathead minnows, *Pimephales promelas*. *Comp. Biochem. Physiol.* A **56**, 503–508.

GEE, J. H. & GEE, P. A. (1976). Alteration of buoyancy by some Central American stream fishes, and a comparison with North American species. *Can. J. Zool.* **54**, 386–391.

GEE, J. H., MACHNIAK, K. & CHALANCHUK, S. M. (1974). Adjustment of buoyancy and excess internal pressure of swimbladder gases in some North American freshwater fishes. *J. Fish. Res. Bd Can.* **31**, 1139–1141.

GRAHAM, J. B. (1973). Terrestrial life of the amphibious fish *Mnierpes macrocephalus*. *Mar. Biol.* **23**, 83–91.

GRIGG, G. C. (1965). Studies on the Queensland lungfish, *Neoceratodus forsteri* (Krefft). III. Aerial respiration in relation to habits. *Aust. J. Zool.* **13**, 413–421.

HORN, M. H. & RIGGS, C. D. (1973). Effects of temperature and light on the rate of air breathing of the bowfin, *Amia calva*. *Copeia* 653-657.

HUGHES, G. M. (1973). Respiratory responses to hypoxia in fish. *Am. Zool.* **13**, 475-489.

HUGHES, G. M. & SINGH, B. N. (1970). Respiration in an air-breathing fish, the climbing perch *Anabas testodineus* Bloch. I. Oxygen uptake and carbon dioxide release into air and water. *J. exp. Biol.* **53**. 265-280.

HUGHES, G. M. & SINGH, B. N. (1971). Gas exchange with air and water in an air breathing catfish *Saccobranchus fossilis*. *J. exp. Biol.* **55**, 667-682.

JOHANSEN, K. (1970). Air breathing in fishes. *In Fish Physiology*, vol. 4 (ed. W. S. Hoar and D. J. Randall), pp. 361-411. New York, London: Academic Press.

JOHANSEN, K., HANSON, D. & LENFANT, C. (1970). Respiration in a primitive air breather, *Amia calva*. *Resp. Physiol.* **9**, 162-174.

JORDAN, J. (1976). The influence of body weight on gas exchange in the air breathing fish *Claris batrachus*. *Comp. Biochem. Physiol.* **53**A, 305-310.

KRAMER, D. L. & GRAHAM, J. B. (1976). Synchronous air breathing, a social component of respiration in fishes. *Copeia*, pp. 689-697.

MILLER, R. R. (1966). Geographical distribution of Central American freshwater fishes. *Copeia*, pp. 773-802.

NIJSSEN, H. & ISBRÜCKER, I. J. H. (1970). The South American catfish genus *Brochis* Cope, 1872 (Pisces, Siluriformes, Callichthyidae). *Beaufortia* **18**, 151-168.

SINGH, B. N. (1976). Balance between aquatic and aerial respiration. In *Respiration of Amphibious Vertebrates* (ed. G. M. Hughes), pp. 125-164. London: Academic Press.