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THE TRANSITION TO AIR BREATHING IN FISHES

III. EFFECTS OF BODY SIZE AND AQUATIC HYPOXIA ON THE AERIAL GAS EXCHANGE OF THE SWAMP EEL *SYNBRANCHUS MARMORATUS*

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SUMMARY

Synbranchus marmoratus (Bloch) breathes air during terrestrial excursions and while dwelling in hypoxic water and utilizes its gills and adjacent buccopharyngeal epithelium as an air-breathing organ (ABO). This fish uses gills and skin for aquatic respiration in normoxic (air-saturated) water but when exposed to progressive aquatic hypoxia it becomes a metabolic O_2 conformer until facultative air breathing is initiated. The threshold Pw_{O_2} (aquatic O_2 tension or partial pressure in mmHg) that elicits air breathing in *S. marmoratus* is higher in larger fish. However, neither air-breathing threshold nor the blood haemoglobin (Hb) concentration of this species were changed following hypoxia ($Pw_{O_2} < 20$ mmHg) acclimation. In hypoxic water *S. marmoratus* supplies all of its metabolic O_2 requirement through air breathing. ABO volume scales with body weight raised to the power of 0·737 and the amount of O_2 that is removed from each air breath depends upon the length of time it is held in the ABO. Ambient Pw_{O_2} directly affects the air-breath duration of this fish, but the effect is smaller than in other species. Also, average air-breath duration (15·7 min at Pw_{O_2} 0–20 mmHg) and the average inter-air-breath interval (15·1 min) of *S. marmoratus* are both longer than those of other air-breathing fishes. Although the gills of *S. marmoratus* are involved in aerial O_2 uptake, expelled air-breath CO_2 levels are not high and always closely correspond to ambient Pw_{CO_2}, indicating that virtually no respiratory CO_2 is released to air by this fish. CO_2 extrusion therefore must occur aquatically either continuously across another exchange surface or intermittently across the gills during intervals between air breaths. This study with *S. marmoratus* from Panama reveals physiological differences between this population and populations in South America. The greater Hb content of South American *S. marmoratus* may be the result of different environmental selection pressures.

INTRODUCTION

Aerial respiration has evolved independently among many species of fishes which, as a result, possess a variety of anatomical, physiological, biochemical and behavioural

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specializations for aerial gas exchange (Carter & Beadle, 1931; Johansen, 1970; Graham, 1976; Kramer, Lindsey, Moodie & Stevens, 1978; Randall, Burggren, Farrell & Haswell, 1981). Despite a considerable diversity of air-breathing adaptations, fishes that breathe air can be separated into two groups, amphibious and aquatic air breathers (Graham, 1976). Amphibious air breathers rely on this respiratory mode during their frequent and routine terrestrial excursions, whereas aquatic air breathers, which for the most part remain confined to water, periodically surface and gulp air to obtain supplemental O_2 (Johansen, 1970; Graham, 1976).

Because they seldom leave water, aquatic air-breathing fishes lack specializations such as for aerial vision, terrestrial locomotion, desiccation resistance and nitrogen excretion usually present in amphibious fishes (Graham, 1976; Gordon, Ng & Yip, 1978; Iwata *et al.* 1981). Also, aquatic air breathers, unlike most amphibious forms, typically have separate anatomical structures and gas exchange surfaces for aerial and aquatic respiration (Johansen, 1970). The air-breathing organ (ABO) of these fishes is usually highly specialized and sequestered in the body away from direct contact with ambient water (Graham, 1976). This allows a fish to consume O_2 from the ABO while simultaneously ventilating its gills in water for CO_2 and N_2 release, electrolyte and volume regulation and, depending upon aquatic conditions, some O_2 uptake (Johansen, 1970; Graham, 1983).

This paper investigates aspects of the aquatic aerial respiration of the swamp eel *Synbranchus marmoratus* one of only a few fish species that, owing to conditions in its natural habitat, has evolved the capability for both amphibious and aquatic aerial respiration (Liem, 1980; Heisler, 1982). The swamp eel ranges from southern Mexico to southern Brazil and is found in streams, rivers, lakes, ponds and swamps (Breder, 1927; Carter & Beadle, 1931; Luling, 1958; Rosen & Greenwood, 1976; Kramer *et al.* 1978). This species breathes air while inhabiting hypoxic water and during terrestrial excursions in search of prey and new habitats as well as when it is confined to its relatively dry mud burrow during the tropical dry season (Carter & Beadle, 1931; Bicudo & Johansen, 1979; Heisler, 1982). *Synbranchus marmoratus* is also one of the few species that utilize gills and adjacent buccopharyngeal epithelium for air breathing (Liem, 1980). This means that unlike other aquatic air-breathers this fish cannot ventilate its gills while it holds air in its ABO (Heisler, 1982).

The major objective of this research was to compare the swamp eel's ability for aquatic aerial respiration with that of other species. Previous papers in this series (Graham & Baird, 1982; Graham, 1983) demonstrated that a suite of biochemical and physiological compensations for hypoxia are initiated by the onset of facultative air-breathing in the armoured catfishes *Ancistrus chagresi* and *Hypostomus plecostomus*. As a result of hypoxia acclimation, both these species were able to reduce their air-breathing frequency in hypoxic water (Graham & Baird, 1982). It was further shown that hypoxia-acclimated *Ancistrus* has an increased air-breathing efficiency and a heightened capability for aquatic respiration in hypoxia (Graham, 1983). Acclimation to hypoxia, however, did not change the threshold aquatic O_2 partial pressure (Pw_{O_2}, mmHg) that elicited air breathing in these species (Graham & Baird, 1982). Gee (1980) also reported no effect of hypoxia acclimation on the Pw_{O_2} air-breathing threshold of the mud minnow, *Umbra limi*.

In contrast to these findings Bicudo & Johansen (1979) reported that, following

6 weeks of air breathing and acclimation to hypoxia, *S. marmoratus* from Brazil initiated air breathing at a significantly higher Pw_{O_2} (54·0 mmHg) than did normoxia-acclimated control fish (30·1 mmHg). Aquatic hypoxia also appears to have less of an effect on the gas exchange capacity of *S. marmoratus*. Both the total blood haemoglobin (Hb) concentration and the Hb-O_2 affinity of air-breathing *Ancistrus* and *Hypostomus* are elevated in hypoxic water (Graham, 1983 and unpublished data). Weber, Wood & Davis (1979) also reported that 4–7 days of air breathing and hypoxia increased the Hb-O_2 affinity of two Brazilian armoured catfish species. However, this treatment did not affect either the total Hb or the Hb-O_2 affinity of *S. marmoratus*.

These observations suggest that aspects of respiratory control and the response mechanisms to aquatic hypoxia, including air breathing, in *S. marmoratus* are not the same as in other air-breathing fishes. To test this, we studied this species using specimens captured in Panama. Our first objectives were to confirm both the absence of a Hb concentration change and the presence of air-breathing threshold shifts, both of which have been previously reported for hypoxia-acclimated Brazilian *S. marmoratus*. We also determined the effect of body size on the air-breathing threshold and ABO volume and examined how Pw_{O_2} and Pw_{CO_2} affect aerial and aquatic gas exchange. Our preliminary studies revealed differences between the air-breathing physiology of *S. marmoratus* from Panama and what has been reported for fish from Brazil. Thus an additional objective of this investigation was to determine if differences between South American and Panamanian populations could be attributable to geographic isolation and possibly different environmental selection pressures or to the experimental protocols of different investigators.

MATERIALS AND METHODS

Swamp eels weighing between 0·5 and 900 g were collected by hand net and with live-baited minnow traps in the Burunga and Mandinga Rivers near Arraijan, Republic of Panama and transported by air to the Physiological Research Laboratory, Scripps Institution of Oceanography, La Jolla, California. Fish were maintained in dimly lighted aquaria (25–27 °C) on a natural photoperiod and fed beef liver or live goldfish at least once each week.

Hypoxia acclimation

Groups of *S. marmoratus* were kept in hypoxic ($Pw_{O_2} \leqslant 20$ mmHg) water and thus forced regularly to breathe air for up to 10 weeks. Light and temperature conditions and feeding regimen were the same as for control fish (above). The absence of aeration resulted in hypoxic conditions. Electric filters fitted with extensions on the in- and out-flow tubes were used to clean and mix water without aeration. Plastic sheeting draped snugly over the water surface of the aquarium and filter reservoir minimized the surface area for O_2 diffusion from air but provided space, along the edges of the tank, for fish to surface and gulp air. Water temperature and Pw_{O_2} in the hypoxic tanks were monitored daily with a Yellow Springs Instrument (Model 54) O_2 meter and probe.

Contrasts of hypoxia-acclimated and control fish

Control and hypoxia-acclimated *S. marmoratus* were compared for blood Hb content and for their Pw_{O_2} threshold. Contrasts were made between fish of similar body size. Blood comparisons were made using fish (125–450 g) that had been in hypoxia for 14–28 days, a time determined to be sufficient for an erythropoietic response to occur in other air-breathing fishes (Graham, 1983). Blood samples (0·2–1·0 ml), taken by cardiac puncture, were withdrawn into tuberculin syringes that were flushed with heparin and dried prior to use. To facilitate handling, fish were cooled in water at 10–12 °C for 5–10 min before blood sampling. Total Hb was estimated using the cyanmethaemoglobin procedure (Graham, 1983).

Air-breathing thresholds in progressive hypoxia (i.e. the Pw_{O_2} at which fish initiate air breathing) were compared in control and hypoxia-acclimated groups of *S. marmoratus* using procedures described by Graham & Baird (1982). Fish acclimated to hypoxia for 6–10 weeks were used in these tests in order to ensure comparability with Bicudo & Johansen (1979). Groups ($N = 5$–6) of test fish (40–152 g) were transferred to a 30 l experimental aquarium (26 ± 1 °C) containing aerated ($Pw_{O_2} >$ 130 mmHg) water. The aquarium was back lighted and positioned behind a visual blind. Following a 12–24 h adjustment period, aeration was stopped and aquarium Pw_{O_2} was slowly (about 0·7 mmHg min^{-1}) reduced by bubbling N_2 gas into the tank through an air stone. Pw_{O_2} at the time of the first air breath by each test fish was recorded and at least three replicate threshold tests were made on consecutive days for each group. In the 24–30 h interval between replicate testing, control fish were maintained in aerated water. Hypoxia-acclimated fish were re-exposed to hypoxic water soon after transfer to the experimental tank and, in successive threshold tests, 6–10 h prior to testing. Neither previous threshold tests nor the treatment between tests affected subsequent threshold determinations.

Body size and air-breathing threshold

Air-breathing threshold determinations were also carried out on control fish weighing between 6 and 850 g in order to learn the effects of body size.

Respiration

Aerial gas exchange

Gas exchange measurements were made using an L-shaped Lucite respirometer submerged (except for the upper end of the vertical section) in a constant temperature (25·0 ± 0·1 °C) water bath (Fig. 1). The chamber was darkened with black plastic and its uppermost section was covered by a dark cloth, dimly back lighted, and observed through a blind. Depending on fish body size, either 1·8 or 4·0 l respirometers were used. Fish that had been starved 24 h were placed in the chamber and then allowed a 24 h adjustment period before experiments were begun. During this time filtered, aerated water was continually pumped through the respirometer from a reservoir also located in the water bath. When the respirometer was closed, water was recirculated and, because of fish respiration, Pw_{O_2} decreased. In air-breathing experiments a fish was allowed access to air at the top of the vertical section (Fig. 1) and, once Pw_{O_2} had

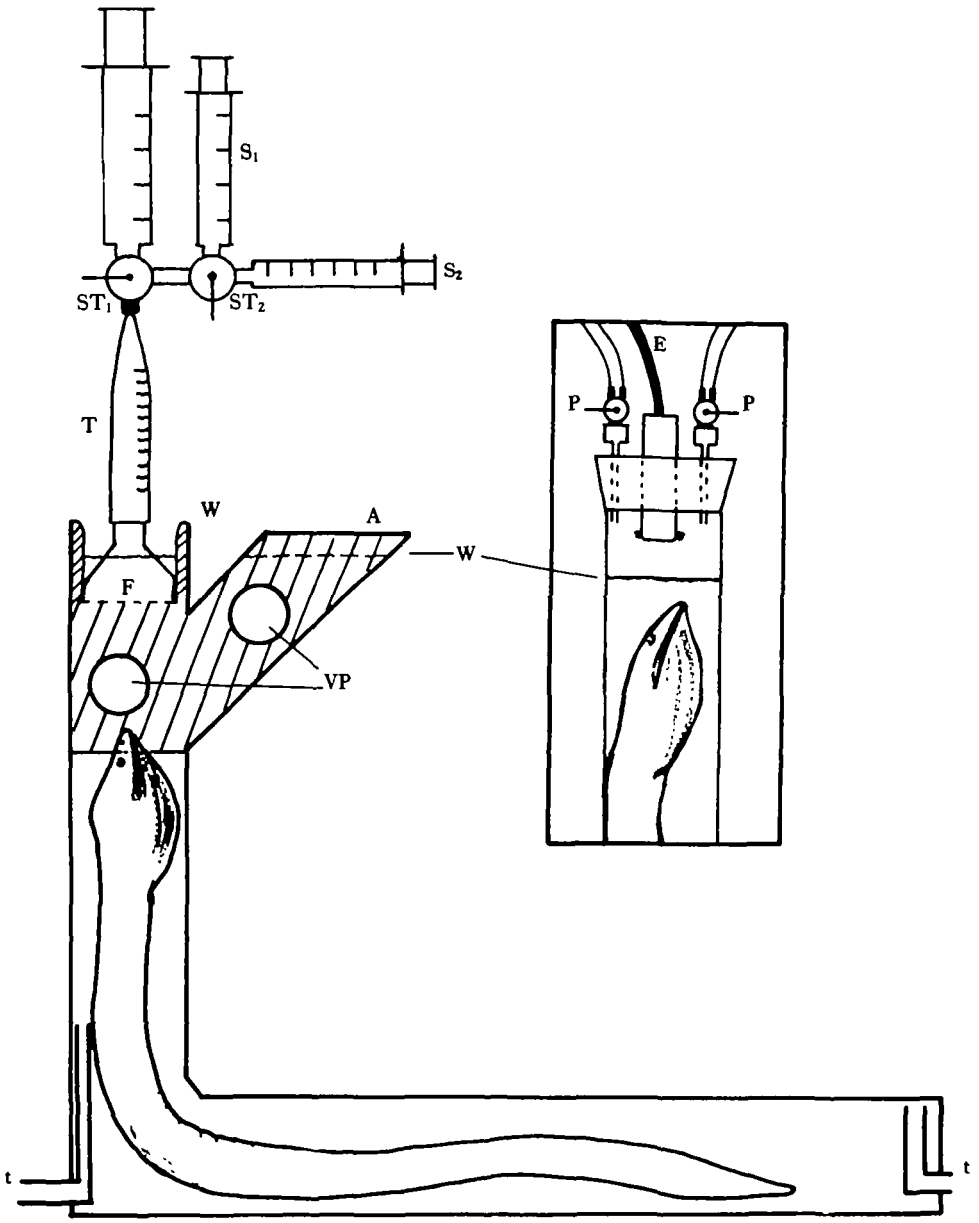

Fig. 1. The L-shaped respirometer used to measure the aerial and aquatic $\dot{V}_{O_2}$ of *Synbranchus marmoratus*. The Y-shaped extension on the vertical arm permitted capture of ascending expelled breaths for volume determination and analysis of respiratory gas contents while also allowing the fish direct access to atmospheric air (A). View ports (VP) in the side arm of the opaque 'Y' section permitted fish observation and visual confirmation that all released gas was captured. The gas collector was filled with water and tightly fitted into the vertical section of the 'Y' (cut away). Gas collected under the inverted funnel (F) was first pulled up into the graduated centrifuge tube (T) and then through stopcocks (ST_1, ST_2) to syringes 1 and 2 (S_1, S_2, see text). Inset shows position of stopper-mounted O_2 electrode (E) and air-flushing ports (P) in the vertical section during preliminary tests with a fixed air phase volume. W is respirometer water level. Water circulation is through glass tubes (t).

dropped to air-breathing threshold level, the fish would periodically ascend, take a gulp of air, and then sink to a resting position with all or most of its body in the horizontal section.

Aerial gas exchange measurements were made on fish that had been air-breathing for 12–96 h. In preliminary experiments fish were required to take successive breaths from an atmosphere in which the O_2 partial pressure was continuously recorded (Fig. 1 inset). At the beginning of each test period a 75–100 ml air phase was enclosed at the top of the vertical section by a rubber stopper. The YSI O_2 probe mounted in the stopper projected into the air phase. This was initially calibrated (25 °C) in air and N_2 gas, and a Scholander gas analyser was used to verify correspondence between the electrode reading and O_2 levels in the air phase. To ensure that air-phase O_2 remained close to saturation, the contents of this space were flushed completely with fresh air (using a syringe and Tygon hose connector) between every 3–4 air breaths. The vertical section was continuously observed so that the air release, stealthy ascent, and air gulp by the fish could be accurately recorded. The volume of an air breath was calculated from a measurement of the vertical displacement distance of the respirometer water surface following submergence of the fish with air in its ABO. Volume constants ($ml\,mm^{-1}$ displacement) were predetermined for each respirometer. Release of an air breath caused an abrupt step drop in the O_2 signal and was also confirmed by observation. The amount of O_2 utilized from the air breath was calculated from the magnitude of the change in the O_2 signal and the known air-breath and air-phase volumes. Utilization of O_2 could then be calculated and related to the duration of the air breath, or expressed as an instantaneous O_2 consumption rate ($\dot{V}_{O2}$). A correction factor for the rate of O_2 diffusion from the air phase into the hypoxic respirometer water was necessary and could be computed from the steady gradual decline in the air-phase O_2 record. Also, since *S. marmoratus* was observed routinely to exhale all gas from its ABO at the end of an air-breath, the calculated breath volume, when corrected for O_2 utilization, would indicate ABO volume and this could be related to fish body size.

Analysis of expelled breaths

Procedures described above did not permit separate analyses of each air breath for both CO_2 and O_2 content and necessitated both an indirect measurement of ABO volume and a correction for O_2 diffusion from air to water. To eliminate these limitations and to examine the effects of Pw_{CO2} on aerial gas exchange, the respirometer was fitted with a Y-shaped side arm (Fig. 1) that allowed an air-breathing fish regular access to atmospheric air. Aided by favourable back lighting of the open water surface, fish in the respirometer quickly learned how to surface for an air breath. Also, since fish typically released air breaths from below the surface and always several minutes prior to surfacing for another gulp, expelled gas bubbles invariably ascended under the collection apparatus mounted over the vertical section of the tube. A gas collector similar to that described by Graham (1983) was fitted into the vertical section. This consisted of a funnel filled with hypoxic water and a graduated centrifuge tube that fitted tightly into the neck of the section (Fig. 1). A 20 gauge blunt needle mounted flush with the inner apex of the tube permitted attachment of a stopcock and syringe assembly. Using the large vertical syringe (Fig. 1) expelled gas was immediately (15 s)

pulled into the tube for a volume determination, then raised to the level of the stopcocks and withdrawn sequentially into syringes 1 and 2. Hypoxic water contacted gas in the centrifuge tube, funnel, and in stopcock 1, but mercury was used to fill the dead spaces of syringes S_1 and S_2 and stopcock 2 (Fig. 1). Gas taken into syringe S_2 was analysed for O_2 and CO_2 content with the Scholander and Radiometer O_2 and CO_2 electrodes calibrated at 25 °C. The fish was observed continually to time both air breaths and the inter-air-breath intervals and to verify that all released gas was captured. Gas diffusion between water and an expelled breath necessitated rapid processing of a breath (Graham, 1983). A mean hourly aerial $\dot{V}_{O_2}$ (STPD) was calculated from O_2 uptake and utilization data recorded for a series (usually covering 1 h or longer) of air breaths. Measurements of Pw_{O_2} and Pw_{CO_2} were made at regular intervals during each test in order to examine their effects on breath duration and gas exchange. In some tests a 5 % CO_2: 95 % N_2 gas mixture was initially bubbled into respirometer water to determine the combined effects of aquatic hypoxia and hypercapnia on aerial respiration.

Aquatic gas exchange

Respirometer tests without an air phase were conducted to determine how progressive aquatic hypoxia affected the aquatic $\dot{V}_{O_2}$ of *S. marmoratus*. Procedures (Graham, 1983) were as follows: the respirometer was closed and the rate of O_2 decline was monitored using the YSI electrode mounted in the flow of the system. Antibiotics (furacin and penicillin) were initially added to the reservoir to reduce background microbial respiration, and blank respiration corrections were made for each fish. Instantaneous $\dot{V}_{O_2}$ estimates were made for each 10 mmHg range of ambient Pw_{O_2}. Tests were repeated on successive days for all fish and runs were continued down to a Pw_{O_2} of 15 mmHg or to the point where a fish repeatedly ascended the vertical section 'to search for air'. The effect of Pw_{O_2} on aquatic ventilation rate was determined by counting the ventilations of fish ($N = 15$) held in a clear respirometer tube positioned behind a blind and dimly back lighted.

RESULTS

Hypoxia acclimation, Hb, and air-breathing threshold

Hypoxia acclimation does not significantly ($P > 0{\cdot}05$, t tests) change either the mean blood Hb content or the mean Pw_{O_2} air-breathing threshold of *S. marmoratus* (Table 1A, B). Tests of the effect of body size revealed that larger fish initiate air breathing at a higher Pw_{O_2} than do smaller fish. The threshold Pw_{O_2} (Table 2) of 16 fish with a mean body weight of 151·8 g is 32·7 mmHg. This is not significantly different from that of control fish in Table 1B (39·9 mmHg) but is significantly less ($P < 0{\cdot}05$, t test) than the mean threshold (68·6 mmHg) of seven larger *S. marmoratus* (Table 2). The least squares regression equation relating the Pw_{O_2} air-breathing threshold (Y) to body weight (X) is:

$$Y = 26{\cdot}47 + 0{\cdot}048\ X,$$

and Fig. 2 shows the air-breathing threshold range calculated for 6–850 g fish. The 95 % confidence limits around the slope of the above equation do not cross zero

$(0{\cdot}048 \pm 0{\cdot}035)$ and the correlation coefficient for body size and threshold is 0·582 $(N = 49, P < 0{\cdot}05)$.

Aquatic respiration

At or above a Pw_{O_2} of 120 mmHg, the mean aquatic $\dot{V}_{O_2}$ of *S. marmoratus* (10–790 g) is $26{\cdot}4 \pm 5{\cdot}4$ ml kg^{-1} h^{-1} ($\bar{x} \pm 95$ % confidence limits, $N = 84$, 25 °C). The $\dot{V}_{O_2}$ of fish denied access to air steadily decreases in progressive hypoxia in the pattern of a metabolic O_2 conformer (Fig. 2 and Discussion). A least squares regression analysis reveals a significant correlation between mean aquatic $\dot{V}_{O_2}$ (within each 10 mmHg increment of Pw_{O_2}) and Pw_{O_2} from 30–150 mmHg ($r = 0{\cdot}90$, $N = 12$, $P < 0{\cdot}05$), and the slope of the regression equation is significantly different from zero $(0{\cdot}179 \pm 0{\cdot}061)$.

The aquatic ventilation rate of 15 *S. marmoratus* (76–360 g) is $27{\cdot}9 \pm 3{\cdot}1$ ventilations min^{-1} ($\bar{x}$+95 % confidence limits, range 17–39, $N = 133$, 25 °C). Larger fish tend to have lower rates but the negative correlation is not significant ($P > 0{\cdot}05$). In normoxic water, *S. marmoratus* does not ventilate its gills continuously and periods of apnoea regularly occur (see Discussion and Heisler, 1982). Fish exposed to progressive hypoxia ventilate their gills both slightly faster and for a greater amount of time (i.e. more each hour) than in normoxia. However, mean ventilation rates did not change even with Pw_{O_2} values as low as 5–15 mmHg. Ventilation rates are also not different in control and hypoxia-acclimated *S. marmoratus*.

Table 1A. *Haemoglobin concentrations (g%) of control and 14–28 day hypoxia-acclimated ($Pw_{O_2} \leqslant 20$ mmHg)* Synbranchus marmoratus

Group	Control	Hypoxia-acclimated
Hb	10·7 ± 1·54 (12)	11·6 ± 1·81 (7)

Table 1B. *Air-breathing threshold (Pw_{O_2}) and comparative body size data for control and 6–10 week (42–70 days) hypoxia-acclimated* Synbranchus marmoratus

Pw_{O_2} (mmHg)	39·9 ± 11·6 (19)	34·7 ± 7·6 (30)
Body weight (g)	91·8 ± 63·2 (5)	99·9 ± 24·4 (10)
Range	40–152	64–150

Values are $\bar{x} \pm 95$ % confidence limits, (N). Weight range of both test groups 125–450 g. T = 25 °C.

Table 2. *The O_2 air-breathing threshold of two size groupings of* Synbranchus marmoratus

Group	Weight (g)	Pw_{O_2} (mmHg)
I	151·8 ± 63·0 (16)	32·7 ± 4·7 (33)
Range	6–380	8·0–90·0
II	722·9 ± 85·4 (7)	68·6 ± 19·94 (16)
Range	630–850	23·0–127·0

Values for both weight and Pw_{O_2} are $\bar{x} \pm 95$ % confidence limits, (N). T = 25 °C.

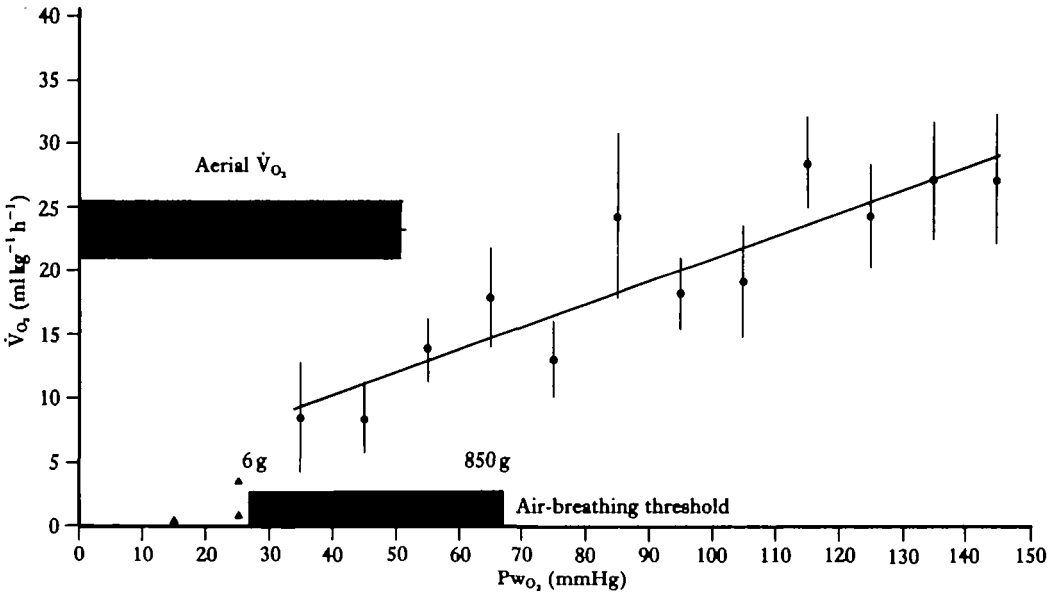

Fig. 2. Aquatic $\dot{V}_{O_2}$ (●) of *Synbranchus marmoratus* exposed to progressive hypoxia without access to air. Vertical lines are ±1 S.E. for $N \geq 3$. Regression equation: Aquatic $\dot{V}_{O_2} = 3{\cdot}022 + 0{\cdot}179\ (Pw_{O_2})$, $r = 0{\cdot}901$, $N = 12$, total observations = 214. Data for one fish at $Pw_{O_2} < 30$ mmHg are shown (▲). Mean instantaneous aerial $\dot{V}_{O_2}$ (±95 % confidence limits, $N = 137$) and air-breathing threshold of fish weighing 6–850 g (see text) are also shown in relation to Pw_{O_2} (25 °C).

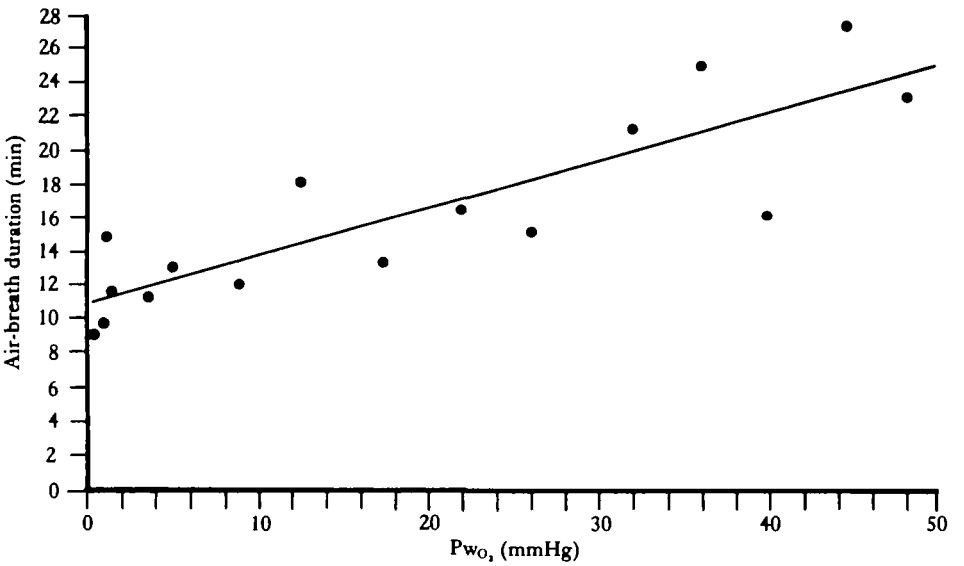

Fig. 3. Mean air-breath durations of individual *Synbranchus marmoratus* in relation to Pw_{O_2} (25 ± 1 °C). Line is least squares regression: Duration = 11·05+0·272 (Pw_{O_2}), $r = 0{\cdot}899$, $N = 16$.

Aerial gas exchange

Air-breath duration and the O_2 content, and volume of expelled air breaths were determined for 17 *S. marmoratus* (58–760 g). Over the Pw_{O_2} range 0–20 mmHg, average air-breath duration is 15·7 ± 2·1 min ($\bar{x}$ ± 95 % confidence limits, range 2–53, $N = 137$, 25 °C). The inter-air-breath interval of this fish, that is the time between the release of an air breath and the gulping of the next one, averaged 15·1 ± 2·7 min ($\bar{x}$ ± 95 % confidence limits, range 1–42, $N = 117$). Although both mean air-breath duration and mean inter-breath interval are similar, no correlation exists between them (also see Heisler, 1982) nor does breath duration relate to body size. Further the inter-air-breath interval is not correlated with O_2 utilization on either the preceding or succeeding air breaths, with fish body size, or with Pw_{O_2}. Decreasing Pw_{O_2},

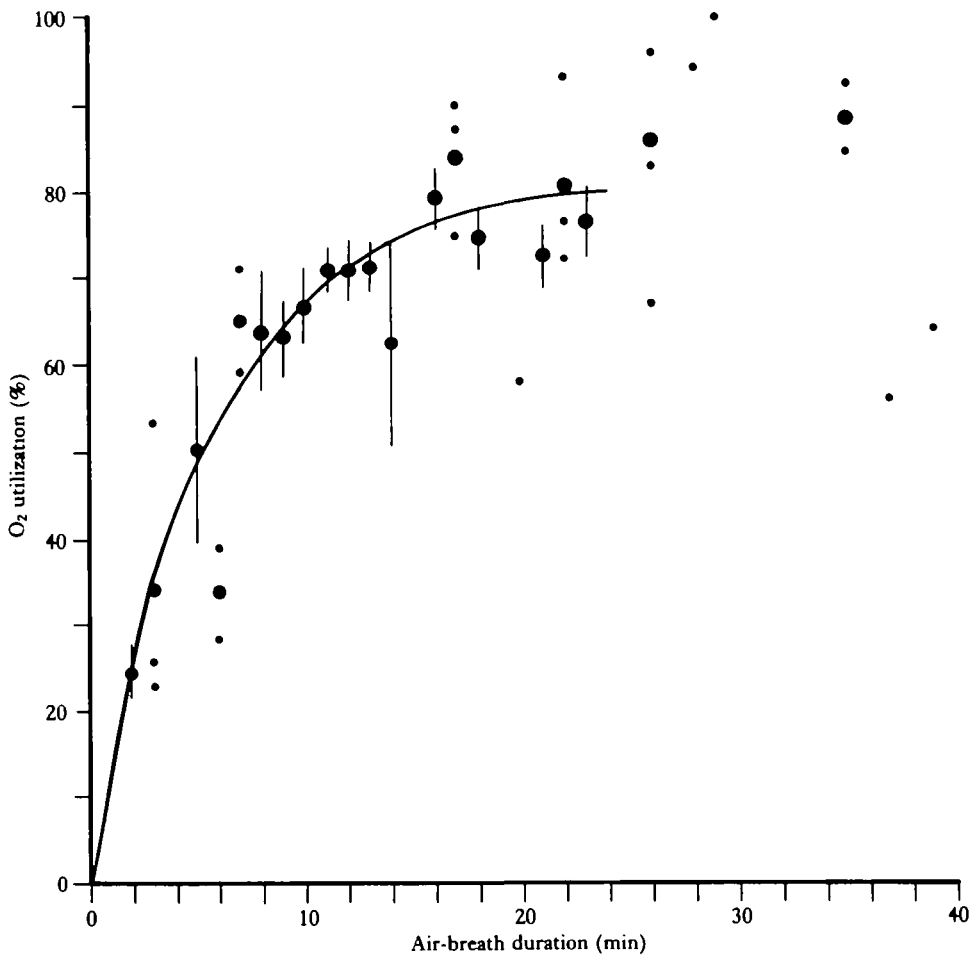

Fig. 4. Relationship between air-breath duration (rounded to minutes) and O_2 utilization for *Synbranchus marmoratus* (25 °C). Mean O_2 utilization ±95% confidence limits indicated for all durations where four or more breaths were examined. For $N < 4$, mean (large dot) and each data point (small dot) are shown. Line fitted by eye.

however, has a significant negative effect on air-breath duration (Fig. 3) with shorter but more frequent air breaths occurring in progressive hypoxia.

Mean aerial O_2 utilization correlates with air-breath duration ($r = 0{\cdot}80$, $P < 0{\cdot}05$, $N = 22$); however, the relationship (Fig. 4) is not linear and the rate of O_2 uptake is much greater from short air breaths than from longer ones. This is similar to results obtained from fish with cannulated ABOs (Johansen, 1966; Bicudo & Johansen, 1979). Fig. 4 reveals that at mean air-breath duration (15·7 min) O_2 utilization approaches 80 %. In a few cases utilization is nearly 100 % whereas in others it is relatively low. Although variability in O_2 utilization (and duration) was evident for individual fish, no relationship between utilization and body size was found.

The mean aerial $\dot{V}_{O_2}$ of *S. marmoratus* (Fig. 2), estimated only for the time air breaths were held and thus not including the inter-breath interval, is $23{\cdot}3 \pm 2{\cdot}2$ ml kg^{-1} h^{-1} ($\bar{x} \pm 95$ % confidence limits, range 6·9–55·7, $N = 137$, 25 °C). This is not significantly different from aquatic $\dot{V}_{O_2}$ in normoxic water (26·4 ml kg^{-1} h^{-1}) and is not significantly affected by Pw_{O_2} which ranged from 0·5 to 51·4 mmHg in these tests (Fig. 2).

Each ABO volume estimate for *S. marmoratus*, either measured directly or calculated from a water displacement volume, was corrected for O_2 utilization and plotted against body weight (Fig. 5). Volumes determined for each fish were tightly grouped around the mean and no correlations were found between ABO volume and Pw_{O_2}, air-breath duration, or inter-air-breath duration. The 95 % confidence limits around the exponent relating ABO volume to body weight ($0{\cdot}737 \pm 0{\cdot}088$) in Fig. 5

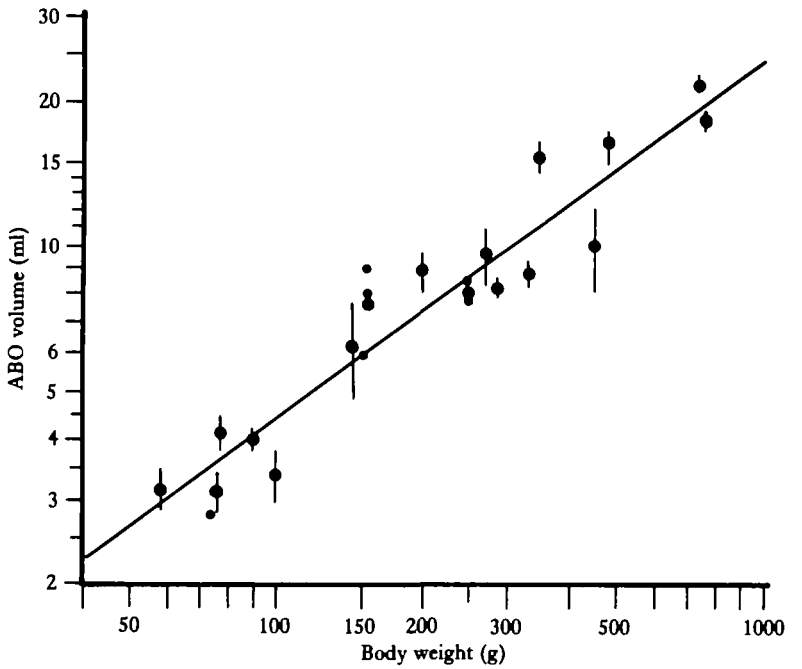

Fig. 5. Relationship between air-breathing organ (ABO) volume and body weight for *Synbranchus marmoratus*. Mean ±95 % confidence limits (vertical lines) indicated for all $N \geq 4$ data. For $N < 4$ mean and each data point are shown. Regression equation: log ABO volume = −0·825+0·737 (log body weight), $r = 0{\cdot}958$, $N = 17$; slope 95 % confidence limits are ±0·088.

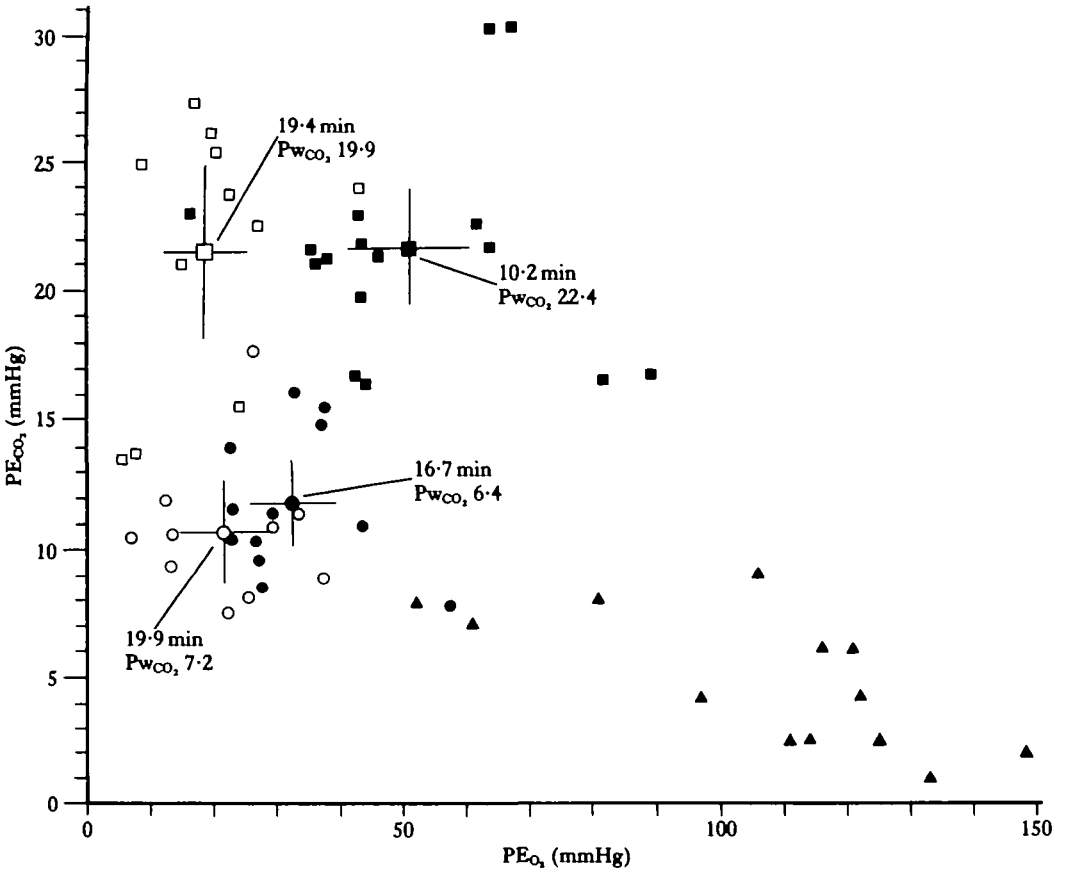

Fig. 6. PE_{CO_2}–PE_{O_2} values determined for 49 breaths released by five *Synbranchus marmoratus* (25 °C). Breaths were combined into four groups on the basis of mean duration and the Pw_{CO_2} in the respirometer (see text and Table 3): Short duration-low CO_2 (●) $N = 12$; long duration-low CO_2 (○) $N = 10$; short duration-high CO_2 (■) $N = 16$; long duration-high CO_2 (□) $N = 11$. Corresponding oversized symbols show mean PE_{CO_2}–PE_{O_2} (±95 % confidence limits) for each group and numbers are mean duration (min) and Pw_{CO_2}. Also shown are 13 PE_{CO_2}–PE_{O_2} values reported for *S. marmoratus* (▲) from Brazil by Bicudo & Johansen (1979) (25 °C).

do not overlap 1·0, indicating that the rate of increase in ABO volume occurs disproportionately with respect to body size in *S. marmoratus*.

Partial pressures of O_2 and CO_2 in released breaths

Expelled air-breaths from five *S. marmoratus* ($N = 49$, Table 3, Fig. 6) were examined to learn the effects of air-breath duration and Pw_{CO_2} on expired breath O_2 and CO_2 partial pressures (PE_{O_2}, PE_{CO_2}). Mean values for four groupings of air breaths, separated on the basis of relative duration (i.e. shorter or longer than mean duration) and Pw_{CO_2} conditions in the respirometer, are contrasted in a PE_{CO_2}–PE_{O_2} diagram (Fig. 6) and in Table 3. Fig. 6 reveals a correspondence between Pw_{CO_2} and PE_{CO_2} and the expected inverse relationship for breath duration and PE_{O_2}. Also shown are 13 mean breath PE_{CO_2}–PE_{O_2} points (typical duration 5 min, reported for Brazilian *S. marmoratus* by Bicudo & Johansen (1979). Because of their longer breat

Table 3. *Duration, PE_{CO_2} and PE_{O_2} (mmHg) of air breaths released by four groups of* Synbranchus marmoratus *exposed to different ambient Pw_{O_2} and Pw_{CO_2} levels and holding air breaths for different time periods at 25 °C*

Air-breath grouping		Aquatic conditions		Air-breath variables						
Duration	Pw_{CO_2}	Pw_{O_2}	Pw_{CO_2}	*N*	Duration	PE_{CO_2}	*N* $PE_{CO_2} > Pw_{CO_2}$	PE_{O_2}	% utilization	RE
Short	low	9·88(6·34)	6·43(0·55)	12	10·70(0·62)	11·80(1·72)	12	32·54 (6·80)	79·0(4·4)	0·03(0·02)
Long	low	1·21(0·87)	7·19(0·97)	10	19·90(5·41)	10·64(2·02)	10	22·12 (7·36)	85·7(4·3)	0·04(0·02)
Short	high	10·12(5·71)	22·44(2·34)	16	10·19(0·48)	21·69(2·23)	7	51·30(10·02)	66·9(6·5)	0·02(0·01)*
Long	high	0·54(0·29)	19·90(3·92)	11	19·36(3·58)	21·63(3·36)	8	19·35 (7·19)	87·5(4·7)	0·02(0·01)†

Number of breaths in which $PE_{CO_2} > Pw_{CO_2}$ are indicated as air-breath O_2 utilization and RE data.
* RE calculated for 7 of 16 breaths; † 8 of 11. *N* is total air breaths examined, all values are $\bar{x}$ (95 % confidence limits), times are in min.

duration, breaths from fish in the present study have both a lower mean O_2 content and a higher mean CO_2 content than those studied by Bicudo & Johansen (1979).

Table 3 summarizes data for aquatic O_2 and CO_2 conditions and for breath duration and both PE_{CO_2} and PE_{O_2} in the four groups of data shown in Fig. 6. Moderately good agreement exists between each group's duration and O_2 utilization relationships (Table 3) and that shown in Fig. 4. Depending upon both Pw_{CO_2} and the time an air-breath was held, PE_{CO_2} did not always exceed Pw_{CO_2}. Table 3 shows that PE_{CO_2} is greater than Pw_{CO_2} in all breaths released by fish in ambient Pw_{CO_2} values of 5–10 mmHg irrespective of breath duration. In a higher Pw_{CO_2} range, PE_{CO_2} is above Pw_{CO_2} in only 7 of 16 (44 %) breaths held 8–12 min (Table 3). For breaths held 14–35 min, the number in which PE_{CO_2} exceeds Pw_{CO_2} increases significantly (Chi square contingency = 6·8, $P < 0{\cdot}05$) to 8 of 11 (73 %, Table 3). The aerial respiratory exchange ratio (RE, Table 3) of *S. marmoratus* is very low. This value was computed for each group using only positive values (i.e. $PE_{CO_2} > Pw_{CO_2}$) and each PE_{CO_2} was corrected for both Pw_{CO_2} and the P_{CO_2} of inspired air prior to calculation.

DISCUSSION

Metabolic conformity in progressive hypoxia

Typically, fishes are metabolic O_2 regulators and thus can maintain $\dot{V}_{O_2}$ down to a critical Pw_{O_2} (Ultsch, Jackson & Moalli, 1981). Documented instances of metabolic O_2 conformity among lower vertebrates are rare and most data showing this response are considered equivocal (Ultsch *et al.* 1981). Our study shows that Panamanian *S. marmoratus* is a metabolic O_2 conformer when exposed to progressive aquatic hypoxia without access to air. The air-breathing threshold of this species varies directly with body size (see below) and, for the weight range of fish examined (6–850 g), the percentage reduction in aquatic $\dot{V}_{O_2}$ at threshold extends from 43 % (850 g) to 72 % (6 g) of mean aquatic $\dot{V}_{O_2}$ measured above 120 mmHg Pw_{O_2} (Fig. 2). These reductions are greater than in the armoured catfish *Ancistrus* which regulated aquatic $\dot{V}_{O_2}$ nearly down to its air-breathing threshold (33 mmHg, Graham, 1983). Additional studies (J. B. Graham & T. A. Baird, in preparation) have shown that the energetic cost of gill ventilation is higher in *S. marmoratus* than in other fishes. Metabolic O_2 conformity with hypoxia may therefore be an energy-saving mechanism utilized by this fish until, at a combination of Pw_{O_2} and reduced relative $\dot{V}_{O_2}$ determined by body size, air breathing must be initiated (Fig. 2).

Our finding of O_2 conformity is different from that of Bicudo & Johansen (1979), who determined that Brazilian *S. marmoratus* regulate $\dot{V}_{O_2}$ down to 30–50 mmHg Pw_{O_2}. Also in contrast are our observations that the gill ventilation rate of *S. marmoratus* remained constant in progressive hypoxia. Possible bases for these and other physiological differences between Panamanian and South American populations of *S. marmoratus* are discussed below.

Air-breathing threshold

The effect of body size on the air-breathing threshold of *S. marmoratus* may be due to factors such as the scaling of ABO (respiratory chamber) volume (Fig. 5) which

becomes relatively smaller in larger fish. Also important are the smaller body-surface area to volume ratio of larger fish which would limit cutaneous O_2 uptake, especially in hypoxia (Heisler, 1982), and the energetic cost of ventilation which is relatively high for *S. marmoratus* and increases with body size (J. B. Graham & T. A. Baird, in preparation).

Our experiments demonstrated no effect of hypoxia acclimation on the Pw_{O_2} air-breathing threshold of *S. marmoratus* and thus do not verify the results of Bicudo & Johansen (1979) with Brazilian fish. These workers did not specify their procedures for threshold determination but reported that five fish (mean weight 123·8 g, range 76–190) 'chronically' adapted to hypoxia (25 °C) for 6 weeks commenced air breathing at a significantly higher Pw_{O_2} than did control fish. Our tests were done using fish of similar size (Table 1) acclimated to hypoxia and regularly air breathing for 6–10 weeks. Thus unknown differences in experimental protocol probably account for the different results (see below). However, our finding of no effect of hypoxia on the air-breathing threshold of *S. marmoratus* is similar to results for other species (Gee, 1980; Graham & Baird, 1982) and suggests that similar mechanisms (i.e. ambient O_2 sensors, a reduced aquatic $\dot{V}_{O_2}$ in hypoxia, and possibly limited cutaneous O_2 uptake) trigger air breathing in this fish (Heisler, 1982).

Aerial gas exchange

Compared with air-breathing data for Brazilian fish (Bicudo & Johansen, 1979), *S. marmoratus* from Panama held air-breaths longer (15·7 *vs* 5–10 min) and had correspondingly higher average O_2 utilizations (80 *vs* 40–50 %). Bicudo & Johansen (1979, p. 61) reported a 2–3 ml ABO volume for a 150 g fish but did not indicate sample size or the variability around this estimate. Since our Fig. 5 shows a 150 g fish to have about a 6 ml ABO volume, it would seem that the ABO of Brazilian fish is smaller. However, we calculated ABO volume from the body weight (74–211 g) and tidal volume data given by Bicudo & Johansen (1979, Table 2) and obtained values of 2·9–10·8 ml which, when plotted with body weight, agree closely with our data in Fig. 5. The aerial RE of Brazilian fish was found to be about 0·1 by Bicudo & Johansen (1979) and the PE_{CO_2}–PE_{O_2} data points reported by them are in line (Fig. 6) with values in the present study. Bicudo & Johansen (1979) did not specify respirometer Pw_{CO_2} in their tests and slight differences between their RE estimate and our lower values (Table 3) may reflect the absence of a PE_{CO_2}–Pw_{CO_2} correction factor.

Analyses of expelled breaths indicate that aerial CO_2 release by *S. marmoratus* has no effect on O_2 uptake and is a passive process directly related to breath duration and Pw_{CO_2}. The positive effect of Pw_{CO_2} necessitated use of a Pw_{CO_2} correction factor prior to calculation of aerial RE (see above and Results). This suggests that a steady state equilibrium condition for CO_2 exists between *S. marmoratus* and its ambient water and that this establishes, during the time air is held, a CO_2 diffusion gradient from fish tissue to gas in the ABO. Depending upon gradient steepness and breath duration, a net outward flux of CO_2 may occur. However, since little exhalant CO_2 is released aerially (Table 3), *S. marmoratus* must either accumulate respiratory CO_2 in its tissues during an air-breath and then flush this gas by aquatic ventilation during the inter-air-breath interval, or it must release CO_2 simultaneously while air breathing, utilizing an aquatic extra-branchial exchange surface such as the skin (Heisler, 1982).

Air breathing and Pw_{O2}

Air-breath duration is affected by Pw_{O2} (Fig. 3) but the aerial $\dot{V}_{O2}$ of *S. marmoratus* remained independent of this factor (Fig. 2). The average durations of both the air-breaths (15·7 min) and the inter-air-breath intervals (15·1 min) of this fish are much longer that has been observed for other species (Gee, 1976; Kramer & Graham, 1976; Graham & Baird, 1982). Because of its long inter-breath interval the air-breathing frequency (i.e. the number of complete breath and inter-breath cycles that occur hourly) of *S. marmoratus* is much less than for either *Ancistrus* or *Hypostomus*. By combining the linear equation for air-breath duration and Pw_{O2} determined for this fish (Fig. 3) with its mean inter-breath interval, we obtain an estimated air-breathing frequency of about 4 breaths h^{-1} at a Pw_{O2} of 0 mmHg and 2 breaths h^{-1} at 30 mmHg. These frequencies are similar to some observed by Bicudo & Johansen (1979, Fig. 5) but are much lower and change less with Pw_{O2} than those of hypoxia-acclimated *Ancistrus* (13 breaths h^{-1} at 0 mmHg, *vs* 5 at 30 mmHg) and *Hypostomus* (13 at 0 mmHg *vs* 8 at 30 mmHg) (Graham & Baird, 1982). A negative relationship between air-breathing frequency and Pw_{O2} can be attributed to reductions in air-breathing effectiveness caused by the greater diffusive loss of aerially-obtained O_2 in more hypoxic water (Graham & Baird, 1982; Graham, 1983). This problem, common to many air-breathing fishes, stems from the 'series' circulation between ABO and gills and the need to ventilate gills in hypoxic water (for N_2 and CO_2 release and ion balance) which leads to transbranchial O_2 loss (Johansen, 1970; Johansen, Mangum & Lykkeboe, 1978). The above comparison shows a smaller effect of Pw_{O2} on the air-breathing frequency of *Synbranchus* than for either *Ancistrus* or *Hypostomus*, which is to be expected since the former holds air over its gills and cannot simultaneously ventilate water while air breathing. Nevertheless, these calculations do suggest that some aerial O_2 is lost by *S. marmoratus* and this probably occurs through its scaleless skin. Studies in progress show that the skin of this fish is active in aquatic O_2 uptake and that some cutaneous O_2 loss could occur during air breathing, particularly if a cutaneous pathway for CO_2 release is used (Heisler, 1982).

Blood Hb concentration, hypoxia and respiration

Our finding that hypoxia acclimation had no effect on the Hb concentration of *S. marmoratus* agrees with results obtained by Weber *et al.* (1979), who acclimated Brazilian fish to hypoxia for 4–7 days. These workers also found that hypoxia exposure did not alter the Hb-O_2 affinity (indexed by erythrocyte phosphate-Hb ratio) of their study fish. Together these observations indicate that a prolonged transition period is not required to develop air-breathing proficiency in *S. marmoratus* which, regardless of its history of exposure to hypoxia, retains the physiological capacity to make a complete and abrupt transition to aerial respiration. This is unlike the gradual hypoxia-acclimation response of *Ancistrus* which is initially intolerant of severe hypoxia (Graham, 1983), but consistent with the natural respiratory requirements imposed on *S. marmoratus* by terrestrial excursions and by conditions that occur in some of its habitats at night when environmental O_2 demand can result in the rapid onset of severe hypoxia (Johansen, 1970; Kramer *et al.* 1978).

Compared to most other air-breathing fishes *S. marmoratus* has a high blood Hb content (Johansen, 1970; Johansen *et al.* 1978). Combining data from several studies (Lenfant & Johansen, 1972; Johansen *et al.* 1978; Weber *et al.* 1979), we calculate that the South American population of *S. marmoratus* has a mean Hb of $13{\cdot}8 \pm 1{\cdot}0$ g% ($\bar{x} \pm 95$ % confidence limits). This is significantly higher than the overall mean (combined hypoxia and control fish) of Panamanian *S. marmoratus* ($11{\cdot}1 \pm 1{\cdot}6$ g%) and may reflect genetic isolation and different environmental selection pressures in the two regions (see below). The high Hb of *S. marmoratus* probably plays a role in blood buffering (Johansen *et al.* 1978; Heisler, 1982) since this fish accumulates CO_2 (Table 3) in its tissues and often occurs in habitats that are acidic and subject to large thermal fluctuations (Kramer *et al.* 1978).

Physiological differences between South American and Panamanian populations of Synbranchus marmoratus

Respiratory physiology studies with *S. marmoratus* have been mostly done with fish from South America (Carter & Beadle, 1931; Johansen, 1966, 1970; Lenfant & Johansen, 1972; Johansen *et al.* 1978; Bicudo & Johansen, 1979; Weber *et al.* 1979; Heisler, 1982). Our study of Panamanian *S. marmoratus* reveals several physiological differences between fish from these two areas. A second species of synbranchid eel, *Ophisternon aenigmaticum*, occurs sympatrically with *S. marmoratus* throughout Brazil and northern South America, but not in Panama (Rosen & Greenwood, 1976). Since *S. marmoratus* and *Ophisternon* are very similar in appearance the possibility cannot be discounted that some results reported for South American *S. marmoratus* may have actually been obtained from *O. aenigmaticum*. (Another species, *S. madierae* occurs in eastern Bolivia and western Brazil, but physiological studies of fish from this region have probably not been conducted.) The 'slow' *vs* 'fast' response times for arterial P_{CO_2} and pH noted for specimens by Heisler (1982, Fig. 5) may be the result of studying different species.

Although additional studies are needed, differences between the aquatic respiratory patterns, air-breathing thresholds, and air-breath durations observed by Bicudo & Johansen (1979) and those reported in the present study seem to be caused by experimental procedures. Bicudo & Johansen (1979, Table 1) found the aquatic $\dot{V}_{O_2}$ of their fish to vary following different exposure times in hypoxia. Also, these workers only acclimated fish in the respirometer for 4–6 h prior to $\dot{V}_{O_2}$ measurements. The mean $\dot{V}_{O_2}$ of their control group (normoxia, 25 °C, Bicudo & Johansen, 1979, Table 1) was $39{\cdot}8 \pm 8{\cdot}0$ ml kg^{-1} h^{-1} ($\bar{x} \pm 95$ % confidence limits), which is significantly higher than our value for Panamanian fish at or above 120 mmHg ($26{\cdot}4 \pm 5{\cdot}4$ ml kg^{-1} h^{-1}, 25 °C, Fig. 2). Unsettled fish may have a higher $\dot{V}_{O_2}$; this would affect O_2 storage and in turn alter metabolic responses to hypoxia, giving the appearance of $\dot{V}_{O_2}$ regulation (Ultsch *et al.* 1981). Bicudo & Johansen did not specify the conditions under which they observed ventilation, and their graph (1979, Fig. 5) showing a steady rise in aquatic ventilation rate in increasing hypoxia presents no statistical information. The maximum rate they observed, about 32 ventilations min^{-1}, is slightly higher than our mean value of 28. J. B. Graham & T. A. Baird (in preparation) have observed that hyperoxia greatly reduces aquatic ventilation in *S. marmoratus* and in normoxia this fish uses a pattern of intermittent aquatic branchial respiration in which, depending upon both body size

and P_{WO_2}, periods of apnoea are regularly interspersed between intervals of aquatic ventilation. We conclude that factors related to handling stress, the use of differently sized fish, and observation periods of insufficient duration to allow correction for regular periods of apnoea in high P_{WO_2} may have all contributed to the different findings for ventilation. Our preliminary studies demonstrated that shorter duration air breaths commonly occurred immediately after the induction of air breathing in hypoxia. A rapid (1 h) onset of hypoxia also affected air breathing and experiments conducted too soon after handling altered both air-breathing threshold and $\dot{V}_{O_2}$.

The higher Hb concentration of South American fish may reflect genetic differences that have evolved through isolation and different environmental selection pressures and could also be further investigated. Our literature review indicates that neither fish body-size differences nor methodological factors account for the recorded Hb differences. In addition, the proportionally shifted haematocrits in these two populations (Panama 40%, this study; South America 47%, above cited Hb references and Heisler, 1982) indicate the Hb differences between them are real. In contrast to Panama the broad expanse of the Amazon Basin may contribute to the regular (annual) occurrence of extreme seasonal conditions (e.g. the dry season) that are uniformly severe in all habitats occupied by *S. marmoratus* (Kramer *et al.* 1978; Heisler, 1982) in South America and this may have intensified selection for respiratory adaptations such as a higher Hb.

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