THE TRANSITION TO AIR BREATHING IN FISHES
IV. IMPACT OF BRANCHIAL SPECIALIZATIONS FOR AIR BREATHING ON THE AQUATIC RESPIRATORY MECHANISMS AND VENTILATORY COSTS OF THE SWAMP EEL SYNBRANCHUS MARMORATUS

BY JEFFREY B. GRAHAM, TROY A. BAIRD*
AND WIEランド STÖCKMANN†

Physiological Research Laboratory, Scripps Institution of Oceanography,
University of California, San Diego, La Jolla, CA 92093, USA

Accepted 14 October 1986

SUMMARY

The gills, adjacent buccopharyngeal epithelium, and skin of the swamp eel Synbranchus marmoratus (Bloch) function for both aerial and aquatic respiration. Aquatic cutaneous \( \text{O}_2 \) uptake occurs continuously at rates that, while dependent upon aquatic \( \text{O}_2 \) tension (\( P_{\text{wO}_2} \)), are in direct proportion to body surface area. Branchial aquatic \( \text{O}_2 \) uptake takes place during intermittent ventilation which occurs in proportion to body mass. Because of reductions in the body surface area to volume ratio that occur with growth, cutaneous oxygen uptake comprises a larger percentage of the total oxygen uptake of small fish and, to compensate, large fish ventilate more. The mass exponent for total rate of oxygen uptake (\( \dot{V}_{\text{O}_2} \)) (0.894 ± 0.145) is within the range predicted from the contributions of cutaneous \( \dot{V}_{\text{O}_2} \) (mass exponent 0.651 ± 0.167) and the number of minutes each hour that branchial ventilation occurs (0.378 ± 0.105). Hyperoxia increases cutaneous \( \dot{V}_{\text{O}_2} \) and reduces branchial ventilation. Total \( \dot{V}_{\text{O}_2} \) was also reduced in hyperoxia and calculations relating this to the reduction in ventilation time yield ventilatory cost estimates that increase with body size and that are high compared to those of other fish when the large component of cutaneous respiration in this species is considered. Large ventilatory costs reflect gill and branchial apparatus specialization for aerial respiration. Accessory cutaneous respiration and intermittent aquatic ventilation reduce these costs, and intermittent gill use in aquatic breathing, which is the exact analogue of the pattern for branchial respiratory use during air breathing, seems to optimize aquatic \( \text{O}_2 \) uptake with minimal ventilatory cost.

*Present address: Department of Zoology, University of British Columbia, Vancouver, British Columbia, V6T 2A9, Canada.
†Present address: Institut für Anatomie und Zytobiologie der Justus-Leibig-Universität Giessen, 6300 Giessen, Federal Republic of Germany.

Key words: air breathing, gas exchange, cutaneous respiration, body size, Synbranchus, optimization of respiratory function.
INTRODUCTION

Most fish that breathe air while in water utilize a specialized organ (e.g. stomach, intestine, gas bladder, lung, labyrinth) to hold air (Johansen, 1970; Randall, Burggren, Farrell & Haswell, 1981; Graham & Baird, 1982). Because their aerial O₂ supply is sequestered, these fish can concurrently ventilate their gills sufficiently to sustain normal branchial functions of CO₂ release, ion balance and the extrusion of nitrogenous wastes (Cameron & Wood, 1978; Stiffler, Graham, Dickson & Stöckmann, 1986). Moreover, depending upon aquatic O₂ tension (PwO₂), the gills may potentially supplement respiration with some O₂ uptake from water (Johansen, 1970; Graham, 1983).

Exceptions to this bimodal respiratory pattern occur among fish that use their gills, the adjacent buccopharyngeal epithelium or both as an air-breathing organ. Many amphibious species do this (Graham, 1976) as do some aquatic air-breathers such as the electric eel, *Electrophorus*, an obligate air-breather (Johansen, Lenfant, Schmidt-Nielsen & Petersen, 1968), and the facultatively air-breathing knifefish *Hypopomus* (Carter & Beadle, 1931). Most of the tropical swamp eels (Synbranchidae) also breathe air in this manner while in hypoxic water, during terrestrial excursions and when confined to moist burrows during the dry season (Carter & Beadle, 1931; Liem, 1967; Rosen & Greenwood, 1976; Lüling, 1980; Graham & Baird, 1984; Graham, 1985).

Involvement of gills in air breathing poses a suite of physiological problems. Even if gills are not the primary aerial exchange surface, retention of air in the brancial chamber precludes aquatic ventilation and thus bimodal respiration. If auxiliary exchange surfaces (e.g. skin) are not active, CO₂ release, ion balance and nitrogen excretion may all be affected (Stiffler et al. 1986). Moreover, large differences in the density and O₂ content of air and water (Holeton, 1980) suggest that simultaneous optimal design for aquatic and aerial respiration is unlikely. Thus, the respiratory physiology of these fishes may reflect an evolutionary trade-off in the optimization of branchial morphology for gas exchange in both air and water.

The objectives of this paper are to examine what effects air breathing has had on the aquatic respiratory mechanisms of the neotropical swamp eel *Synbranchus marmoratus*. Synbranchid specializations for air breathing have received considerable attention (reviewed by Graham & Baird, 1984), but there have been few studies of aquatic respiration in this group, and the impact upon aquatic respiration of branchial modifications for air breathing is largely unknown. The Asian swamp eel, *Monopterus*, for example, has a reduced gill area on branchial arches I–III and no gill lamella on arch IV (Rosen & Greenwood, 1976) and is an obligate air-breather (Liem, 1967). In contrast, *S. marmoratus* still has four lamellate gill arches (Rosen & Greenwood, 1976) and is a facultative air-breather (Graham & Baird, 1984). The capability of synbranchids to carry out aquatic and aerial cutaneous respiration is well documented (Liem, 1967, 1981; Bicudo & Johansen, 1979), but this has not been quantified for *S. marmoratus*. Of more fundamental importance, however, is our present lack of understanding of what the adaptive significance of a largely
Aquatic respiration of Synbranchus may be a diffusion-limited process such as cutaneous respiration (Feder & Burggren, 1985) may be for the Synbranchidae, which have a well-developed air-breathing capability and often occur in waters that have limited utility both for aquatic branchial and cutaneous respiration (Graham, 1985).

Initial studies of the aquatic respiration of *S. marmoratus* revealed that periods of ventilation were interspersed with periods of apnoea. Although apnoeic intervals have been observed for other fishes (Smith, Duiker & Cooke, 1983), our observations suggested these were longer in *S. marmoratus*, and that they were affected by ambient conditions and related to body size. All synbranchids have a constricted branchial aperture and observations of *S. marmoratus* suggested that more work may be required to expel ventilatory water through this small exit. One consequence of branchial specializations for aerial respiration may therefore be a relative increase in the energetic cost for aquatic ventilation compared with that for other fishes, and possibly because of this a greater dependence upon accessory respiratory surfaces.

In this paper we investigate the hypothesis that, as a result of specializations for air breathing, the cost of branchial aquatic ventilation for *S. marmoratus* is relatively high and that intermittent ventilation together with accessory cutaneous respiration are adaptations that partially offset this cost. In addition to estimating ventilatory costs our experiments determined how the percentage contribution of the rates of cutaneous and branchial oxygen consumption (\(V_{O_2}\)) to total \(V_{O_2}\) changed with body size and with \(P_{W_{O_2}}\). Because body surface area to volume ratio diminishes with size, the potential contribution of cutaneous respiration should decline in larger fish which should ventilate more. To examine this, we quantified the body-mass scaling relationships of both cutaneous and branchial \(V_{O_2}\) and factors directly related to these two respiratory modes; total body surface area and the amount of time that branchial ventilation occurred. We also compared the mass-scaling functions for total aerial and aquatic \(V_{O_2}\).

**Materials and Methods**

*Synbranchus marmoratus* weighing from 0.5 to 900 g were collected by hand net and live-baited minnow traps in the Burungu 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 and aerated aquaria (25–27°C) on a natural photoperiod and fed beef liver, frozen mackerel or live goldfish at least once each week.

**Respirometry**

Flow-through respirometry was used to measure the cutaneous, branchial and total \(V_{O_2}\) of *S. marmoratus* ranging, after growth in captivity, from 10 to 930 g. Also determined was how the intermittency of branchial ventilation varied with body size and was affected by \(P_{W_{O_2}}\). Fish that had been starved for 48 h were placed in a flow-through respirometer and allowed 24 h to recover from handling prior to tests. Depending upon body size, fish-holding chambers ranged from 0.3 to 4 litres. Water
was pumped via Tygon tubes from a reservoir through the holding chamber and back at a constant rate using a voltage-controlled oscillating pump and a flow meter (Fig. 1). Respirometer temperatures were 25–26°C, reservoir water was aerated and filtered, and the antibiotic Furacin was added to minimize background microbial respiration.

YSI (Yellow Springs Instrument, Model 54) O2 electrodes, mounted in special Lucite housings that permitted easy bubble clearance and calibration without disturbing the fish, were placed in both the inflow and outflow tubes of the fish chamber (Fig. 1) to obtain continuous data on O2 consumption. A Radiometer O2 electrode was also used to measure PO2 in samples withdrawn from the reservoir, fish chamber and outflow line. Before each study both the YSI and Radiometer electrodes were calibrated with N2 and air at prevailing atmospheric pressure and 25°C.

Signals from the YSI probes were fed into a two-channel recorder. During periods of apnoea the inflow and outflow O2 signals were parallel (Fig. 2), with the difference between them reflecting the magnitude of cutaneous respiration. However, because of the different millivolt outputs and recorder settings inherent to each electrode circuit, this information could not be taken directly from the chart. Rather, it was necessary, at regular intervals during apnoeic periods, reciprocally to transfer each electrode to the other position. In this manner each electrode acted as its own control (i.e. inflow PO2 − outflow PO2 = ΔPO2). Additionally, ΔPO2 could be determined from Radiometer measurements of reservoir and outflow PO2. When the ΔPO2 is combined
Fig. 2. Simultaneous ventilation and $E_1/E_2$ $O_2$ traces from an experiment with a 550-g Synbranchus marmoratus in normoxia (A) and hyperoxia (B) (25°C). Because $O_2$ and ventilation records were made at different chart speeds, reference time marks are used for orientation. Note differences in rate and amplitude of ventilation and in the time lapse before a decline in $O_2$ is recorded. The quantity of $O_2$ extracted during the ventilation period is indicated by the black area. Vertical $O_2$ scales differ in A and B, and step changes in $E_1$ and $E_2$ signals reflect reciprocal electrode transfers (R) done for calibration and cutaneous $V_{O_2}$ estimation.
with the known total water O₂ content and flow rate through the system, an estimate of cutaneous \(\dot{V}_{O_2}\) is obtained.

The onset of fish ventilation was registered by a downward excursion of the outflow O₂ signal (Fig. 2). The magnitude of this deflection was a function of both ventilation intensity and rate of water flow. For the entire ventilation period, the total area of displacement from the outflow baseline, when combined with total water O₂ and flow rate, yielded an estimate of the volume of O₂ taken up by the gills. Direct observations and gill-ventilation recordings (Fig. 2 and see below) verified that these signal excursions occurred only during intermittent branchial ventilation.

Continuous records lasting from 3 to 21 h permitted estimation of the mean duration of eupnoeic and apnoeic periods, the percentage of total time that gill ventilation occurred, the cumulative and mean hourly total \(\dot{V}_{O_2}\), the rates of branchial and cutaneous respiration and their percentage contribution to total \(\dot{V}_{O_2}\).

All these determinations were initially made while fish were exposed to normoxic (near air-saturated, 135–150 mmHg, 1 mmHg = 133.3 Pa) water. A comparative data set was obtained for most fish while they were subjected to hyperoxic (180–220 mmHg) water. Hyperoxia was established by bubbling the discharge from a Wöstoff gas-mixing pump (88% air, 12%O₂) into the reservoir. To maintain a stable O₂ tension in the system, it was necessary to cover the reservoir and minimize surface disturbance (i.e. aeration) from the outflow return (Fig. 1).

**Partitioned respirometry**

A partitioned respirometer chamber (Fig. 1) similar to that described by Sacca & Burggren (1982) was used to separate and quantify the branchial and cutaneous components of respiration in *S. marmoratus*. Respirometer chambers ranged in volume from 1 to 3 litres. Each contained two in-series rubber collars set 1 cm apart. The collars sealed against the fish's body on the inside and were made secure by flanges, sealed with 'O' rings, and held in place by bolts. Dental dam and closed cell Neoprene were used for collars, and initial tests showed that both materials were impermeable to gas diffusion over the range of differential partial pressures used in these tests (500 mmHg). Before the fish was mounted, a round hole about 20% smaller than the body diameter of the fish to be studied (180–930 g) was cut in each collar. These were then mounted in the chamber and the fish was introduced head first and, as it crawled forward, it passed through both collars thus establishing a double barrier that isolated the head and branchial apparatus in one chamber from the remainder of the body in the other. Double collars reduced the chances of leakage which, if it occurred, could be easily detected by a step-change in the compartamental O₂ signal. The partitioned-chamber experiments ran for 4–12 h and, once a fish was in the correct position for a set of observations, the length of its body in each chamber was measured (for a later mass correction) and spacers were placed behind its tail to prevent excessive position changes. This maintained approximately the same proportions of the body in each chamber for the tests. Also, care had to be taken to balance the open-system head pressures in each chamber to avoid pushing the fish
Aquatic respiration of Synbranchus past the dams. No correction was made for the body surface area isolated between the two collars.

After placement of a fish in the partitioned chamber, flow-through respirometry techniques were applied. Conditions within each chamber could be modified independently either by using separate reservoirs or by closing the inflow and outflow loops of each, due to fish and background respiration, led to a decline in $P_{WO_2}$. (Whenever closed-system respirometry was done it was necessary within a few hours, to do a blank run in order to correct for background microbial respiration.) A valve and stopcock in the roof of the head chamber permitted addition of an air or mixed-gas phase for aerial respiration (Fig. 1).

**Ventilation**

Impedance records of branchial ventilation were made for several fish (180–960 g) to verify direct correspondence between the periodic change in outflow $O_2$ signal and aquatic ventilation (Fig. 2). Selected fish were anaesthetized in chilled water (6–10°C for 20 min) and a single strand of 36-gauge enamel-coated stainless steel wire was inserted via a 23-gauge needle into the connective tissue on either side of the opercular valve. Each electrode was secured by three self-tying loops, and the two wires were wound together and tied to the body with three subcutaneous anchor stitches. The still unconscious fish was then placed in the respirometer tube, and the electrodes were passed out under the rubber stopper at the head end. The electrodes were connected to a UFI (Model 2991) impedance converter which was connected to a millivolt recorder. The fish was allowed 24 h to recover before simultaneous impedance and $\dot{V}_{O_2}$ data were taken.

**Body surface area**

The body surface areas of 18 fish (1–535 g, total length 11–70 cm) were estimated using gravimetric and geometric techniques. In the gravimetric procedure, fish were tightly wrapped in smooth aluminium foil which was cut to conform to all body contours. The foil was then removed from the fish, blotted dry and weighed. Surface area was calculated using predetermined unit area constants of foil mass. Geometric surface area estimates involved measuring the length of four body regions (head, anterior trunk, posterior trunk and tail) and the body circumferences at three positions marking the limits of each region (gill opening, vent and trunk midpoint). Head surface area was estimated using the equation for the area of a cone of head length, and a base equal to body circumference at the gill opening. The two trunk areas were estimated assuming they were both cylinders with a circumference equal to the median of the anterior and posterior circumferences of each. Tail area was computed from the equation for the area of a triangle with a base equal to body circumference at the vent. The sum of the separately determined head, tail and two trunk area estimates equalled total surface area. Surface area estimates were made on dead fish that had been frozen but thawed prior to measurement. Examination of an unfrozen, dead specimen confirmed the absence of any effects of freezing on surface area–mass relationships, and it was initially determined that the length and body
mass relationships of all fish studied were in general agreement with that found for live fish collected in Panama (J. B. Graham, unpublished data).

All body mass-related functions were evaluated using the power growth equation which, when logarithmically transformed, yields a linear equation amenable to regression analysis.

RESULTS

Scaling relationships

Power equations relating the body mass of *S. marmoratus* to its total, cutaneous and branchial \( \dot{V}_O_2 \) (25°C), its body surface area and the min h\(^{-1}\) (25°C) that eupnoea occurred are presented in Table 1. The total \( \dot{V}_O_2 \) of this species has a mass exponent (±95%) of 0.894 ± 0.145 which is not significantly different from 1.0 (Fig. 3A). Replicate surface area estimates varied by less than 10% (mean difference 5.4 ± 2.5%) and data from the two methods were combined to obtain the mass—surface area regression (Fig. 4). The mass exponents for skin surface area (0.618) and cutaneous \( \dot{V}_O_2 \) (0.651) do not differ significantly.

Cutaneous respiration in *S. marmoratus* occurs continuously at rates determined by \( \dot{P}wO_2 \) (see below) and body size (Fig. 3B). The cutaneous \( \dot{V}_O_2 \) mass exponent is significantly \((P < 0.05)\) less than that for total \( \dot{V}_O_2 \) (Table 1; Fig. 3A,B), indicating that the relative amount of the total \( \dot{V}_O_2 \) sustainable by cutaneous respiration declines with increasing body mass. Tests with the partitioned respirometer revealed that the estimated skin respiration rate of a fish was proportional to the area of its skin that was in the posterior chamber, which is consistent with our observations that no skin areas appear specialized for gas exchange. Examination of 50 semi-thin sections of skin taken from a 260 g (57 cm) glutaraldehyde-fixed specimen revealed that most blood vessels (arterioles, venules and capillaries) are situated 15—20 \( \mu m \) beneath the basal lamina (Fig. 5). The distance between these vessels and the skin surface ranges from 80 to 105 \( \mu m \), and an average of seven vessels occurred across the 450 \( \mu m \) breadth of each section.

Branchial \( O_2 \) uptake by *S. marmoratus* occurs only during intermittent ventilatory bouts which typically lasted from 2 to 23 min, but varied with body size and also varied intrinsically over long periods (Fig. 6). General characteristics of the apnoea and eupnoea cycles have been summarized by combining data for several fish (Table 2). The mean durations of eupnoea and apnoea were 7.4 and 16.6 min in normoxic water and 6.0 and 22.3 min in hyperoxic water. Table 2 shows that mean eupnoea and apnoea in hyperoxia and normoxia did not differ very much; however, this is an artefact of the pooling of data for a large size range. It will be shown below that in hyperoxia all fish had significantly reduced ventilation time and longer periods of apnoea. In general, the durations of successive eupnoeic and apnoeic periods were not correlated, and neither was related to body mass.

Ventilation rate during eupnoea ranged between 10 and 22 strokes min\(^{-1}\). This varied intrinsically among fish and was not significantly correlated with body mass.
Table 1. Power equations relating body mass (W) to surface area and five respiratory parameters of Synbranchus marmoratus (25°C)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Equation</th>
<th>95% range of weight exponent</th>
<th>N</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Total $\dot{V}_O_2$</td>
<td>$\text{ml h}^{-1} = 0.028W^{0.894}$</td>
<td>0.749–1.039</td>
<td>28</td>
<td>0.93</td>
</tr>
<tr>
<td>(2) Body surface area</td>
<td>$\text{cm}^2 = 13.076W^{0.618}$</td>
<td>0.604–0.632</td>
<td>18</td>
<td>1.00</td>
</tr>
<tr>
<td>(3) Cutaneous $\dot{V}_O_2$</td>
<td>$\text{ml h}^{-1} = 0.046W^{0.651}$</td>
<td>0.484–0.818</td>
<td>29</td>
<td>0.84</td>
</tr>
<tr>
<td>(4) Hourly gill ventilation</td>
<td>$\text{min h}^{-1} = 2.515W^{0.378}$</td>
<td>0.273–0.483</td>
<td>36</td>
<td>0.78</td>
</tr>
<tr>
<td>(5) Branchial $\dot{V}_O_2$</td>
<td>$\text{ml h}^{-1} = 0.090W^{0.802}$</td>
<td>0.657–0.947</td>
<td>24</td>
<td>0.91</td>
</tr>
<tr>
<td>(6) Time-corrected branchial $\dot{V}_O_2$</td>
<td>$\text{ml h}^{-1} = 0.005W^{1.180}$</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All exponents are significantly different from zero.

* Instantaneous rate.

† Combination of equations 4 and 5.
(see also Graham & Baird, 1984). Rates were always fastest at the beginning of a eupnoeic period and slowest just before it ended (Fig. 2).

As could be expected on the basis of the scaling of cutaneous $\dot{V}_O_2$, the total number of minutes each hour that *S. marmoratus* ventilates its gills significantly increases with body mass (Fig. 3C). Eupnoeic periods were of variable duration and, in order to make comparisons, branchial uptakes were corrected to instantaneous hourly rates. The mass exponent of the instantaneous hourly branchial $\dot{V}_O_2$ is 0.802 (equation 5, Table 1; Fig. 3D). The hourly branchial $\dot{V}_O_2$ of *S. marmoratus*, however, depends upon the number of minutes the gills are ventilated. We estimated this (the time-corrected branchial $\dot{V}_O_2$, equation 6, Table 1; Fig. 3D) by correcting
Aquatic respiration of Synbranchus

Fig. 4. Relationship between skin surface area and body mass for Synbranchus marmoratus. Power equation and statistics are given in Table 1.

Table 2. Mean eupnoeic and apnoeic intervals (min) of Synbranchus marmoratus (58–790 g) during exposure to normoxia (135–150 mmHg) and hyperoxia (180–220 mmHg) at 25°C

<table>
<thead>
<tr>
<th></th>
<th>Normoxia</th>
<th>Hyperoxia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Eupnoea</td>
<td>Apnoea</td>
</tr>
<tr>
<td>Total fish tested</td>
<td>38</td>
<td>38</td>
</tr>
<tr>
<td>Grand mean ± S.E. (min)</td>
<td>7·4 ± 0·2</td>
<td>*16·6 ± 0·6</td>
</tr>
<tr>
<td>Range (min)</td>
<td>2–23</td>
<td>3–46</td>
</tr>
<tr>
<td>Total observations</td>
<td>420</td>
<td>409</td>
</tr>
<tr>
<td>Range (min)</td>
<td>1–35</td>
<td>1–133</td>
</tr>
</tbody>
</table>

Asterisks indicate significant differences between adjacent means.

Values determined from equation 5 with the time factor derived from equation 4 and solving for the intercept of the resulting linear equation which has a slope of 1·180 (0·378 + 0·802). The accuracy of this estimation is revealed by good agreement between the empirically determined regression line for total $\dot{V}_O_2$ and a line predicting total $\dot{V}_O_2$ based on the completely independent estimates of time-corrected branchial $\dot{V}_O_2$ summed with values for the empirically determined cutaneous $\dot{V}_O_2$ (Fig. 7).

Effects of hyperoxia on respiratory partitioning

Hyperoxia effects were examined for three size groups of S. marmoratus. The percentage of total $\dot{V}_O_2$ gained by gills in normoxia ranged from 30 to 81% (Table 3)
with larger fish (Group III) having the highest mean value. In hyperoxia the percentage contribution to total $V_O_2$ by this organ decreased as all three groups used more cutaneous $O_2$ uptake. In both normoxia and hyperoxia the percentage of time of gill use and the percentage of total $V_O_2$ gained by the gills remained correlated.

Fig. 5. Cross-section of epaxial mid-body skin from a 260-g (57 cm) *Synbranchus marmoratus*. $c$, capillary; $p$, pigment; $l$, lymphocyte; $b$, basal lamina; $eg$, collagen bundle; $cv$, central vacuole of a serous gland; $g$, goblet cell. Scale bar, 100 $\mu$m.
Aquatic respiration of Synbranchus

(Fig. 8). Fish in hyperoxia also ventilated their gills for significantly fewer minutes each hour (Table 3) and had longer periods of apnoea (up to 275 min). Relative to their mean total \( \dot{V}_{O_2} \) in normoxia, the mean total \( \dot{V}_{O_2} \) of fish in each group became reduced in hyperoxia. Variability among fishes and the influence of the range of body mass within each group obscures the magnitude of the hyperoxia effect on the reduction of mean \( \dot{V}_{O_2} \). For this reason, data on the mean percentage reduction of \( \dot{V}_{O_2} \) for each fish in each group provide a preferable index (Table 3). All fish tested in groups II and III had a decreased \( \dot{V}_{O_2} \) in hyperoxia. Group I showed the greatest variability of response; from 11 fish tested in hyperoxia, the \( \dot{V}_{O_2} \) of two actually increased slightly (data for these are not included in Table 3) and did not change in two others.

![Graph showing long-term ventilation records](image)

Fig. 6. Summaries of the long-term ventilation records of three *Synbranchus marmoratus* showing how the hourly percentage of gill ventilation differs with body size and also with time for each fish. Values are the mean ± s.e. (N) percentage of an hour that ventilation occurred.
Quantitative effects of hyperoxia on branchial and cutaneous \( \text{O}_2 \) uptake are examined by comparison (Table 4) of the unit rates of these processes in groups I–III. The minute rates of branchial \( \text{O}_2 \) uptake are not different in normoxia and hyperoxia; however, the rates of larger fish are higher, suggesting the presence of an increased gill diffusivity. The equation for total \( \dot{V}_{\text{O}_2} \) in hyperoxia, \( \text{ml h}^{-1} = 0.045W^{0.794} \) \( (r = 0.84, N = 21) \), has a lower slope than in normoxia \( (0.894) \), but variability in the data are such that neither the intercept nor the slope differences between the hyperoxia and normoxia equations are significant. The rates of hourly \( \text{O}_2 \) uptake per square centimetre of skin are not different among the three size groups (Table 3) and increase by similar amounts in hyperoxia. The equation relating the cutaneous \( \dot{V}_{\text{O}_2} \) of fish in hyperoxia to body mass is \( \text{ml h}^{-1} = 0.072W^{0.636} \) \( (r = 0.86, N = 18) \). This mass exponent is not significantly different from that for

![Graph](image)

Fig. 7. Comparison of the empirically determined body mass–total \( \dot{V}_{\text{O}_2} \) regression line for *Synbranchus marmoratus* (thick solid line) with a mass–total \( \dot{V}_{\text{O}_2} \) estimate derived from the sum of the separately measured cutaneous \( \dot{V}_{\text{O}_2} \) (line over area with vertical bars) and the time-corrected branchial \( \dot{V}_{\text{O}_2} \) (line over area with open squares). The latter was calculated by combining equations (Table 1) relating mass to intermittent ventilation and to instantaneous branchial \( \dot{V}_{\text{O}_2} \).
Aquatic respiration of Synbranchus

Table 3. Relative effects of aquatic hyperoxia (180–222 mmHg) on the partitioning of aquatic respiration between gills and skin, the amount of ventilation, and the total $\dot{V}_O_2$ of groups I–III of Synbranchus marmoratus (25°C)

<table>
<thead>
<tr>
<th>Group</th>
<th>Total $\dot{V}_O_2$ via gills</th>
<th>Gill ventilation (mm h⁻¹)</th>
<th>Total $\dot{V}_O_2$ (ml kg⁻¹ h⁻¹)</th>
<th>% Reduction of total $\dot{V}_O_2$ in hyperoxia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Reduction</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>% Total $\dot{V}_O_2$ via gills</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Normoxia</td>
<td>Hyperoxia</td>
<td>Normoxia</td>
<td>Hyperoxia</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>Range</td>
<td>Range</td>
<td>Range</td>
</tr>
<tr>
<td>I</td>
<td>121 ± 11 (9)</td>
<td>361 ± 32 (10)</td>
<td>780 ± 74 (5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>81–163</td>
<td>210–470</td>
<td>550–930</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>47.4 ± 5.3 (7)</td>
<td>30–71</td>
<td>59.3 ± 4.6 (10)</td>
<td>55±81</td>
</tr>
<tr>
<td></td>
<td>3–33</td>
<td>35–83</td>
<td>50–9 ± 5.0 (8)</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>361 ± 32 (10)</td>
<td>10–43</td>
<td>26.8 ± 2.5 (11)</td>
<td>30–42</td>
</tr>
<tr>
<td></td>
<td>10–43</td>
<td>16–46</td>
<td>15–4 ± 2.0 (11)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.6 ± 1.7 (9)</td>
<td>1–16</td>
<td>14.5 ± 2.5 (11)</td>
<td>16–24</td>
</tr>
<tr>
<td></td>
<td>15–0–40–1</td>
<td>6–29</td>
<td>15–9–24–3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.6–36.2</td>
<td></td>
<td>11.9–24–3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>17.8 ± 2.9 (9)</td>
<td></td>
<td>16.1 ± 1.5 (8)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10.7 ± 1.9 (4)</td>
<td></td>
<td>7.4–15.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>17.5 ± 5.4 (9)</td>
<td></td>
<td>11.2 ± 3.0 (8)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>13.1 ± 2.8 (4)</td>
<td></td>
<td>9–21</td>
<td></td>
</tr>
</tbody>
</table>

Mean mass (g) ± s.e. (N) are given and the range of each group is indicated below the group number.

Values are mean ± s.e. (number of fish) and range.

* A significant difference ($P<0.05$, t-test) between adjacent values.

†% cutaneous = 100−% gills.

‡Two of 11 fish in group I had a higher (<15%) $\bar{\dot{V}}_O_2$ in hyperoxia and these data are not included. Two of the remaining nine showed no decrease in $\dot{V}_O_2$.

Cutaneous $\dot{V}_O_2$ in normoxia (0.651, equation 3, Table 1); however, as expected, the intercept is significantly ($P<0.05$, t-test) higher (i.e. 0.046 vs 0.072).

Estimation of ventilatory cost

With an analysis similar to that of Steffensen (1985), we used the reduction in total $\dot{V}_O_2$ in hyperoxia to estimate the aquatic ventilation costs of S. marmoratus (Table 5). Using the mean mass of fish in each group, an expected total $\dot{V}_O_2$ was first calculated (equation 1, Table 1) from which the reduction in the number of ml of $O_2$ used in hyperoxia was estimated from the percentage reduction data (Table 3). This decline was then divided by the observed number of minutes of reduced ventilation each hour (Table 3) to yield an estimated minute cost of ventilation. Multiplication of this by an expected hourly ventilation time for each fish (equation 4, Table 1) yields an estimated hourly ventilation cost. Table 5 expresses these costs relative to total $\dot{V}_O_2$ and the percentage of total $\dot{V}_O_2$ gained by gills. The minute costs of ventilation are greater by a factor of 3 in group III compared to group I. Also, the $O_2$
Fig. 8. Relationships between the percentage of time that gill ventilation occurs and the percentage of total $V_O_2$ that is contributed by the gills of Synbranchus marmoratus in normoxia (O) and hyperoxia (●). Equation for the significant regression is: percentage total $V_O_2 = 13.1 + 1.18$ (percentage time gill use), $r = 0.84$ ($N = 31$). The line passing through zero is hand drawn.

DISCUSSION

Allometric scaling of $V_O_2$

Aquatic oxygen uptake by Synbranchus marmoratus occurs continuously across a scaleless skin and intermittently at branchial surfaces during eupnoea. The mass exponent for cutaneous $V_O_2$ does not differ significantly from that for body surface area (Table 1), which means that with growth cutaneous $V_O_2$ remains directly proportional to skin surface area. This is verified by comparison (Table 4) of the unit rates of cutaneous oxygen uptake of size groups I–III. The absence of a change in the relative ability of the skin of larger S. marmoratus to absorb O$_2$ is different from the situation in most aquatic amphibians. For this group Ulsch (1973) observed that an increased skin thickness together with relative reductions in cutaneous blood flow
Aquatic respiration of Synbranchus

Table 4. Unit branchial and cutaneous oxygen uptake rates of three size groups of Synbranchus marmoratus in normoxia and hyperoxia

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean mass (g)</th>
<th>Normoxia (135–150 mmHg)</th>
<th>Hyperoxia (180–222 mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Branchial $\dot{V}_{O_2}$ (ml O$_2$ min$^{-1}$ of ventilation)</td>
<td>Cutaneous $\dot{V}_{O_2}$ (ml O$_2$ h$^{-1}$ cm$^{-2}$ skin area × 1000)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0·064 ± 0·008 (6)</td>
<td>0·058 ± 0·011 (7)</td>
</tr>
<tr>
<td>I</td>
<td>110 ± 11 (8)</td>
<td>0·05–0·10</td>
<td>0·02–0·10</td>
</tr>
<tr>
<td></td>
<td>80–163</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0·128 ± 0·007 (4)</td>
<td>0·156 ± 0·005 (3)</td>
</tr>
<tr>
<td>II</td>
<td>300 ± 26 (8)</td>
<td>0·11–0·15</td>
<td>0·15–0·17</td>
</tr>
<tr>
<td></td>
<td>210–470</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0·184 ± 0·027 (3)</td>
<td>0·185 ± 0·021 (3)</td>
</tr>
<tr>
<td>III</td>
<td>713 ± 113 (3)</td>
<td>0·15–0·24</td>
<td>0·14–0·21</td>
</tr>
<tr>
<td></td>
<td>550–930</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ml O$_2$ per unit time ± s.e., (N), range.

* A significant difference ($P < 0·05$, t-test) between adjacent values. (Values within each group were pooled.)

Note that due to variation in the individuals and total number of fish comprising each data set, the mean mass for each group does not always agree with values in Table 3.

and capillary density and perhaps a decline in relative respiratory surface area can reduce the skin exchange capacity of larger forms. The uniform increase in cutaneous $\dot{V}_{O_2}$ among groups I–III (Table 4) is further verified by the nearly identical mass exponents in hyperoxia (0·656) and normoxia (0·651). Rather than control of gas exchange, the higher cutaneous $\dot{V}_{O_2}$ in hyperoxia may simply reflect increased O$_2$ diffusion. Studies to determine if $S. marmoratus$ reciprocally modulates cutaneous and branchial gas exchange in response to ambient conditions are now in progress.

The mass exponent for cutaneous $\dot{V}_{O_2}$ (0·651) is significantly lower than for total $\dot{V}_{O_2}$ (0·894). Thus, as body size increases, cutaneous $\dot{V}_{O_2}$ contributes a steadily declining percentage of total $\dot{V}_{O_2}$ (Fig. 3B). A 100-g fish obtains 62% of its total O$_2$ via the skin, but, in a 1000-g fish, the skin will contribute only 31%. Since gills (and adjacent epithelia) and skin are the principal respiratory surfaces available to $S. marmoratus$, the difference in the mass exponents for total and cutaneous $\dot{V}_{O_2}$ should reflect the amount by which branchial $\dot{V}_{O_2}$ would have to be increased in order to sustain total $\dot{V}_{O_2}$; when the 95% confidence intervals of each are taken into consideration this is the case (Table 1; Fig. 7).

The eupnoeic–apnoeic cycle described here for $S. marmoratus$ greatly exceeds that of most fishes and is also slightly longer than that of $Anguilla australis$ (Smith et al. 1983). In normoxic water (20°C) this fish ventilates for an average of 3·6 min,
Table 5. *Estimation of the minute and relative total costs of gill ventilation for three size groups of Synbranchus marmoratus*

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean mass (g)</th>
<th>Total $V_O_2$ (ml h(^{-1}))</th>
<th>Reduction in hyperoxia (%)</th>
<th>$\dot{V}_O_2$ decrease in hyperoxia (ml $O_2$)</th>
<th>Reduced ventilation time (min)</th>
<th>Minute ventilation cost (ml $O_2$ min(^{-1}))</th>
<th>Ventilation time in normoxia (min h(^{-1}))</th>
<th>Hourly ventilation cost (ml $O_2$ h(^{-1}))</th>
<th>Relative cost (% of total $V_O_2$)</th>
<th>% Total $\dot{V}_O_2$ via gills</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>121</td>
<td>2.04</td>
<td>17.5</td>
<td>0.36</td>
<td>12.2</td>
<td>0.029</td>
<td>15.9</td>
<td>0.46</td>
<td>23</td>
<td>49</td>
</tr>
<tr>
<td>II</td>
<td>361</td>
<td>5.41</td>
<td>11.2</td>
<td>0.61</td>
<td>12.3</td>
<td>0.050</td>
<td>23.1</td>
<td>1.16</td>
<td>21</td>
<td>61</td>
</tr>
<tr>
<td>III</td>
<td>780</td>
<td>10.78</td>
<td>13.1</td>
<td>1.41</td>
<td>13.1</td>
<td>0.108</td>
<td>30.1</td>
<td>3.24</td>
<td>30</td>
<td>68</td>
</tr>
</tbody>
</table>

Data are from Tables 1 and 3.
Aquatic respiration of Synbranchus 101

followed by 12 min of apnoea. The respiratory cycles of individual Anguilla, like those of S. marmoratus, varied considerably. However, unlike the swamp eel, A. australis seems locked into a standard breathing cycle of 15·5 min; Smith et al. (1983) reported that hypoxia ($P_{O_2} = 80$ mmHg) increased its ventilatory period to 7·6 min and reduced mean apnoeic periods to 7·9 min (15·5 min). Our observations are similar in that aquatic hypoxia increases ventilation time and shortens apnoeic intervals for S. marmoratus (Graham & Baird, 1984); however, discrete short-term breathing cycles are not evident in normoxia (Fig. 6) or hypoxia (J. B. Graham, unpublished results).

The mass exponent for instantaneous branchial $V_O_2$ ($0·802$) implies that body size does not markedly affect the branchial exchange capacity (i.e. either or both the total surface area and $O_2$ permeability of the gills and adjacent tissues) of S. marmoratus. This is at odds with data showing an increase in the minute rate of branchial $O_2$ absorption in larger fish (Table 4) and is currently under study. Estimation of the branchial contribution to total $V_O_2$ in this species must take into account both instantaneous branchial $O_2$ uptake rate and the time that ventilation occurs; the quantity obtained by combining the regression equations for these two factors, when added to the cutaneous $V_O_2$ calculated from the regression equation, provides an independent estimate of total $V_O_2$ that is in good agreement with values predicted by the empirically determined regression (Fig. 7). Thus, compensation for the relative decline in the cutaneous $V_O_2$ of larger S. marmoratus occurs through increased use of gill ventilation and may also involve an increased branchial diffusivity (Table 4).

**Energetic cost of gill ventilation**

The mass exponent of the total $V_O_2$ of S. marmoratus ($0·894$) approaches 1·0 and is higher than values ($0·6–0·75$) typically found for the ‘resting’ metabolism–mass exponent of many (but not all) vertebrates (Bartholomew, 1982). An intermittent gill ventilation and a somewhat ‘elevated’ mass exponent for total $V_O_2$ suggest the presence, even in ‘resting’ S. marmoratus, of an allometric (i.e. not scaling in direct proportion to mass) component of metabolic activity attributable to branchial ventilation. Our experiments contrasting the $V_O_2$ of groups I–III in hyperoxic and normoxic waters illustrate how the costs resulting from gill ventilation change with body size. If aquatic hyperoxia had no effect other than to reduce ventilation time, then ventilatory costs could be solely ascribed to the decline in total $V_O_2$. However, the interactions between ventilation and several factors that affect $V_O_2$ have been well documented. These include the effect of ventilation on heart stroke volume and frequency (Jones, 1971; Farrell, 1978; Hipkins & Smith, 1983; Roberts & Graham, 1985) and on the circulating concentration of $CO_2$, which, in turn, directly affects aerobic metabolism (Dejours, 1973, 1975; Hughes, 1973). Thus our estimates provide a relative indication of how total metabolic costs related to gill ventilation are affected by body mass.

For most fish, estimates of ventilatory costs are generally about 10% of total oxygen uptake (Hughes, 1973), with a range of 0·5–43% (Roberts, 1975; Holeton, 1980). The ventilatory costs for S. marmoratus lie within this range, despite a
dependence upon cutaneous respiration; thus its costs are higher than those of other species. For example, Table 5 shows that a 121-g fish invests an estimated 23% of its total oxygen uptake to power a respiratory pump that only delivers 49% of its total O$_2$ requirement. Stated another way, fish in groups I–III invest almost half the O$_2$ they take up each hour through their gills on gill ventilation itself. The relative costs for gill ventilation increase in larger $S$. marmoratus because of more gill use and higher minute costs of branchial ventilation (Table 5). Mechanical and morphological features may contribute to the latter. With a greater minute rate of branchial O$_2$ absorption (Table 4), larger fish would need to ventilate more water each minute, and a higher ventilation volume may reduce pump efficiency and raise V$\dot{O}_2$. We do not know how the gill surface and permeability change with body size.

**Ventilatory costs and air-breathing specialization**

Compared to species that use aquatic respiration exclusively, air-breathing fish typically have reduced gill surface areas and a more limited capacity for aquatic respiration (Randall *et al.* 1981). This is seen among synbranchids which all have a vascular epithelial lining throughout most of the mouth and branchial chamber. Some members of this family also lack lamellae on branchial arch IV and have suprapharyngeal pouches (expanded vascular areas for air contact, Rosen & Greenwood, 1976). Moreover, the mouth and buccal chamber of these fish are large, and the pharyngeal chamber contains longitudinal branchiostegal folds that permit expansion needed to hold a large air-breath volume (0·02–0·05 ml g$^{-1}$, Graham & Baird, 1984). However, the ventral, narrow opercular slit of $S$. marmoratus, which prevents air escape from the branchial chamber and also keeps burrow mud and debris from entering this space, may impede water outflow.

Although $S$. marmoratus has a full set of gills and lacks pharyngeal pouches, several lines of evidence suggest that the degree of branchial specializations for air breathing it does possess have, in fact, increased the level of effort required for aquatic ventilation. Most air-breathing fishes are capable of cutaneous respiration (Feder & Burggren, 1985) but, because the cutaneous $\dot{V}_O_2$ of this fish constitutes such a significant portion of its total $\dot{V}_O_2$, it can be argued that a particularly efficacious cutaneous respiration, which also allows adoption of intermittent gill ventilation, co-evolved as an energy-saving mechanism. The 'reluctance' of this fish to ventilate is suggested by the hyperoxia experiments. Because O$_2$ diffuses faster, most fish reduce ventilation in hyperoxia (Dejours, 1975), which is how $S$. marmoratus responded. However, our data (Table 3; Figs 2, 8) show that its minute rate of branchial oxygen rate did not change and less O$_2$ was actually taken up by the gills. This fish, in effect, deferred the greater percentage of its respiration from the gills, which required ventilation, to the skin, where uptake was passive.

While certain local and temporal conditions and behaviour patterns may occasionally favour cutaneous respiration, this mode cannot function effectively at all times and in all habitats occupied by this species (Graham, 1985). When $S$. marmoratus is experimentally exposed to progressive hypoxia without access to air, it responds first by reducing its apnoeic intervals, then by lowering its ventilatory frequency, and
finally it stops ventilation entirely. Unlike most fish (Hughes, 1973), it is an O₂ conformer in hypoxia (Graham & Baird, 1984), suggesting that gill ventilation is both costly and not particularly efficacious at low PwO₂. In natural conditions, *S. marmoratus* responds to hypoxia by breathing air, and larger fish initiate this at a higher PwO₂ than do smaller ones (Graham & Baird, 1984), which is consistent with the finding of higher ventilatory costs and lower relative cutaneous VQ in the former. Further suggestion that this species avoids ventilation comes from the frequent impression that fish in the respirometers 'learned' to position themselves to take advantage of water flow patterns to ventilate passively both gills and skin. Reversing water flow in the respirometer (Fig. 1), for example, often initiated an uncharacteristically long eupnoea. Finally we have observed that *S. marmoratus* will also commence air breathing when it is stressed by handling, a sudden change in aquarium conditions or illness.

Support for conclusions about the ventilatory costs of *S. marmoratus* also comes from differences in the slopes of the mass regressions for the total VO₂ of fish (1) in normoxia, (2) in hyperoxia and (3) breathing air in hypoxia (N = 14, 25°C, Graham & Baird, 1984). Fish in hyperoxia ventilated their gills less than in normoxia, and, correspondingly, the regression line for this group has a lower, although not significantly different, slope (0.794 vs 0.894). Fish that are air breathing in hypoxic water do not ventilate their gills at all, even when they are not holding air (Roberts & Graham, 1985, see below). The power equation for the air-breathers is mlO₂ h⁻¹ = 0.034+W₀^0.815 (r = 0.90). This mass exponent is smaller but not significantly different from that in normoxia (Table 1).

Measurements of VO₂ are often too variable to resolve small metabolic effects of a particular factor (Bartholomew, 1982; Graham, 1983). Our hyperoxia experiments and the contrast with air breathers affirm that reduced energy consumption, particularly for larger *S. marmoratus*, results from a decrease in gill ventilation. Why then do larger fish not breathe air more or less continuously, even in normoxic water? Numerous ecological, physiological and behavioural factors have doubtless been at play in establishing the selective balances that determine the extent and nature of the dependence upon air breathing in a species. The potential energetic savings to be realized by a larger *S. marmoratus* that adopts continuous air breathing must not outweigh the constraints (such as a limited mobility, the needs for more and regular surface access and proximity to the water surface, and the energetic costs of ascent) that are probably imposed by obligatory aerial respiration. However, the complete spectrum of respiratory specialization (from solely aquatic to obligatory air breathing) is seen among the Synbranchidae, and species of *Monopterus* have made the evolutionary progression from facultative to obligatory air breathing. Numerous considerations, extending from habitat water quality (e.g. stagnation and sediment load) to juvenile and adult life histories – including maximum body size of a species, and even to subtleties such as the possibility that aquatic ventilation or air breathing may alert either or both potential predators and prey are probably of selective importance in this transition.
Interrelationships between air breathing, intermittent aquatic ventilation and the control of respiration

Recent studies of *S. marmoratus* indicate that its respiratory control and gas exchange mechanisms are extremely similar, whether it is breathing aerially or aquatically. First, during air breathing *S. marmoratus* holds a breath for an average of 15 min, expels it, and waits an average of 15 min before taking another one, all without ventilating its gills [the inter-(air)-breath interval, Graham & Baird, 1984; Roberts & Graham, 1985]. This is, of course, very similar to aquatic ventilation, where an average of 7 min of ventilation alternates with 17 min of apnoea (Table 2). Similarity also extends to cardiac action. Both the period when air is held and aquatic eupnoea are characterized by tachycardia; bradycardia prevails both during the inter-air-breath interval and in aquatic eupnoea (Johansen, 1966; Roberts & Graham, 1985; J. B. Graham, unpublished results). With both a high blood haemoglobin concentration and high haemoglobin–O₂ affinity (Graham & Baird, 1984; Graham, 1985), *S. marmoratus* could, during the course of a short period of holding air or aquatic ventilation, fully saturate its haemoglobin and accumulate a large blood O₂ store. Once this was done ventilation could cease or air could be expelled, and cardiac activity lowered. During the subsequent interval, circulating O₂ supply would be steadily reduced and reach the level that again elicited eupnoea or the taking of an air-breath. This respiratory specialization has the effect of maximizing branchial O₂ uptake while minimizing ventilatory cost. The skin of *S. marmoratus*, in addition to its supplementary respiratory functions, including CO₂ extrusion (Graham & Baird, 1984), has also evolved the capacity for ion balance (Stiffler et al. 1986) which, owing to intermittent ventilation and air breathing, cannot always take place in the gills.

This work was partially supported by the Smithsonian Tropical Research Institute. Major funding was through NSF DEB 79-12235. Appreciation is expressed to G. de Alba, L. Chin, K. Dickson, W. Lowell, J. Roberts, I. Rubinoff, R. W. Rubinoff and N. Smith for advice and technical assistance. We thank Dr Donald Kramer for critically evaluating a draft of this paper. Mrs Heidi Snipes provided assistance with the histological analysis. The gracious cooperation of the Department of Natural and Renewable Resources (RENARE) of the Republic of Panama in permitting field work and exportation of fish to the USA is also acknowledged.

REFERENCES


**Aquatic respiration of Synbranchus**


