# THE TRANSITION TO AIR BREATHING IN FISHES

## II. EFFECTS OF HYPOXIA ACCLIMATION ON THE BIMODAL GAS EXCHANGE OF *ANCISTRUS CHAGRESI* (LORICARIIDAE)

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#### SUMMARY

The armoured catfish, *Ancistrus chagresi*, is a facultative air breather and uses its stomach as an air-breathing organ (ABO). Comparisons of control fish and fish that had become acclimated to hypoxia and air breathing for 14–21 days were carried out to assess the effects of this treatment on bimodal (aerial and aquatic) gas exchange capacity. Hypoxia acclimation elicits physiological and biochemical changes that enable *A. chagresi* to increase $O_2$ utilization both by its gills in hypoxic water and by its ABO. Compared with control fish, hypoxia-acclimated *Ancistrus* have a higher blood–$O_2$ affinity and more haemoglobin (Hb) and can maintain a higher aquatic oxygen consumption rate ($\dot{V}O_2$) in hypoxic ($P_{w,O_2} = 5$–20 mmHg) water. They also have a 25 % larger ABO volume, are able to hold each air breath longer, and can reduce ABO $O_2$ partial pressure to a lower level. In both groups, respiratory $CO_2$-release occurs primarily through the gills. An air breath instantly causes tachycardia and a reduction in the frequency and amplitude of branchial ventilation. Their lower cardiac and gill ventilation rates in hypoxia and during air breathing suggest that hypoxia-acclimated fish are more adapted for hypoxia than are control fish. During the period an air breath is held in the ABO, hypoxia-acclimated fish exhibit more coordinated phase shifts in gill ventilation and cardiac rates. These may favour an initial phase of efficient aerial $O_2$ uptake from the ABO and transport through the body followed by a period of aquatic $CO_2$ release from the gills.

#### INTRODUCTION

The first paper in this series (Graham & Baird, 1982) reported differences in the levels of aquatic $O_2$ that elicited facultative air-breathing in two Panamanian loricariid catfish *Hypostomus* and *Ancistrus*. It also showed that both these species had significantly reduced air-breathing rates following 14–21 days of air breathing and acclimation to hypoxic (aquatic $O_2$ partial pressure, $P_{w,O_2} \leqslant 30$ mmHg) water. The present study investigates the physiological and biochemical bases for hypoxia acclimation in one of these, *Ancistrus chagresi* Eigenmann and Eigenmann, a species that uses its stomach as an air-breathing organ (ABO) and commences facultative air breathing when $P_{w,O_2}$ drops to or below 33 mmHg (Gee, 1976; Graham & Baird, 1982).

Although periodic confinement in hypoxic water may be a regular occurrence in the

life histories of most air-breathing fishes (Carter & Beadle, 1931; Kramer, Lindsey, Moodie & Stevens, 1978), surprisingly few studies have considered the extent to which, once facultative air-breathing is initiated, a species may become better adapted for aquatic hypoxia, a more proficient air breather, or both of these. Non-air-breathing fishes, for example, typically adapt to aquatic hypoxia by increasing either or both their total blood haemoglobin content and blood–$O_2$ affinity (Wood & Johansen, 1972; Powers, 1980). Would a facultative air breather therefore be expected to make similar adjustments in chronic hypoxia or would access to a rich supply of aerial $O_2$ obviate the necessity? Weber, Wood & Davis (1979) reported that 4–7 days of air breathing in hypoxia increased the Hb–$O_2$ affinity of two Brazilian loricariids (*Hypostomus* and *Pterygoplichthys*), but had a negative and no effect, respectively, on the total Hb of these species. Moreover, this same treatment did not affect either of these blood parameters in air-breathing Brazilian swamp eels (*Synbranchus*).

Hypoxia acclimation also appears to act differently on the physiological control of aerial respiration in some species. Neither hypoxia acclimation nor air-breathing history affected the $P_{w,O_2}$ air-breathing thresholds of *Ancistrus* or *Hypostomus* (Graham & Baird, 1982) or the mud minnow, *Umbra* (Gee, 1980). For Brazilian *Synbranchus*, however, Bicudo & Johansen (1979) reported that fish acclimated to hypoxia for 6 weeks switched to air breathing at a higher $P_{w,O_2}$ than did normoxic control fish.

This paper on *A. chagresi* examines physiological and biochemical adjustments that occur as a result of hypoxia acclimation and evaluates their combined effects on both the aerial and aquatic gas exchange capabilities and the control of respiration in this species.

### MATERIALS AND METHODS

Specimens of *A. chagresi* (2–200 g) were collected in the Rio Frijoles and Rio Frijolitos near Gamboa, Republic of Panama and returned to the Smithsonian Tropical Research Institute, Fort Amador, Panama where studies were carried out from 1974 to 1977. Subsequent work (1979–1982) was done with fish transported from Panama to La Jolla, California. Laboratory fish were maintained in low densities in darkened, aerated aquaria (25–27 °C) on natural photoperiods and were fed primarily on Tetramin flakes and green beans.

All experiments involved comparative testing of fish that had been acclimated to laboratory hypoxia ($P_{w,O_2} \leqslant 30$ mmHg) and air breathing for from 14–21 days (25–27 °C), and control fish that had been maintained in normoxic (130–150 mmHg) laboratory aquaria (25–27 °C) and did not breathe air for the same time or longer. In air-breathing tests, comparisons were made between hypoxia-acclimated fish and control fish that had recently (1–36 h) been induced by hypoxia to breathe air. Procedures for hypoxia acclimation were described by Graham & Baird (1982). In the present experiments, tank surfaces were partially covered with plastic sheeting to keep $P_{w,O_2} \leqslant 30$ mmHg and thus ensure regular air-breathing by all fish.

### Aquatic respiration

Control and hypoxia-acclimated fish were compared for the effect of $P_{w,O_2}$ on aquatic oxygen consumption rate ($\dot{V}O_2$), without access to an air phase, using a closed

respiratory system described by Graham (1973). Fish that had been starved for 24 h were placed in a darkened respirometer chamber and allowed 24 h for adjustment and recovery from handling prior to testing. Depending upon fish size, respirometer volumes ranged from 0·3–1·6 l. The respirometer was contained in a water bath (25 °C) and filtered, aerated water was continually pumped through it from a reservoir. During tests, the respirometer was closed, and because the same water was recirculated, ambient $O_2$ level steadily declined due to fish respiration.

The rate of $O_2$ decline (slope), when corrected for respirometer volume, fish mass (volume), and background respiration, indicated fish $\dot{V}O_2$. Penicillin was added to the reservoir to reduce background microbial respiration and blank respiration corrections were determined for each fish tested. An $O_2$ electrode (YSI Clark Type) connected to a millivolt recorder was used to monitor respirometer $P_{w,O_2}$. The electrode was initially calibrated in air and $N_2$ gas and standardized, before and after each run, against a Radiometer Blood Gas Analyser that was also calibrated at 25 °C.

The effect of $P_{w,O_2}$ on the $\dot{V}O_2$ of both experimental groups was evaluated by comparing relative $\dot{V}O_2$ (corrected slope) over 10 mmHg ranges of $P_{w,O_2}$ (i.e. ambient $O_2$ in the respirometer). Tests were started in normoxic water and continued until a fish had extracted nearly all $O_2$ from the system ($P_{w,O_2}$ 5–15 mmHg). At least two runs, each on successive days, were made on all fish. In the later stages of each run, when $P_{w,O_2}$ was very low, the fish was observed at regular intervals to be sure it did not succumb to hypoxia. Neither $P_{w,CO_2}$ nor pH were regulated in the respirometer and during the tests $P_{w,CO_2}$ increased (from 1–6 mmHg) while pH usually dropped from 7·8 to 6·5.

### Blood analyses

Blood haemoglobin (Hb) content, $O_2$ affinity, and total red cell triphosphate–Hb ratios (P/Hb) were compared in control and hypoxia-acclimated *Ancistrus*. Blood samples, taken by cardiac puncture, were withdrawn into tuberculin syringes that were flushed with heparin and dried prior to use. Total Hb was estimated for each sample using the cyanmethaemoglobin conversion procedure (Sigma Chemical Co.).

The blood mixing method (Edwards & Martin, 1966) was used to determine $O_2$ dissociation curves. Pooled blood samples (6–10 ml, 3–5 fish) were equilibrated for 1 h with humidified air or $N_2$ at 25 °C in 50 ml stoppered flask tonometers. Using a 1 ml glass syringe, volumes of blood were mixed without bubble contamination in proportions that resulted in 95, 75, 40, and 10 % $O_2$ saturation levels. After the blood had been mixed in the syringe, $PO_2$ and pH at 25 °C was determined using the Radiometer Analyser. Tonometered blood was used within 2 h and replicate determinations at $P_{40}$ were carried out at the end of all tests to verify that large changes in blood affinity had not taken place in the time course of equilibration.

Previous investigations (Bartlett, 1978, 1980; Johansen, Mangum & Lykkeboe, 1978; Weber, Wood & Davis, 1979) established that loricariid red cells contain both adenosine (ATP) and guanosine (GTP) triphosphate. In the present work, only total red cell triphosphate levels were estimated for control and hypoxia-acclimated fish using the Sigma ATP test kit which is a non-specific indicator for all red cell triphosphates (Bartlett, 1978). Pooled blood samples (4–6 fish) were also used in these tests.

*Aerial gas exchange*

The volume and the $O_2$ and $CO_2$ contents of gas expelled by air-breathing *Ancistrus* were measured in order to determine if fish that had been air breathing for 14–21 days could hold more air, utilize more of the $O_2$ in a breath, and perhaps release more $CO_2$ aerially than could control fish that were recently (1–36 h) induced to breathe air. The average duration of air breaths was also compared for the two groups.

Gee (1976) determined the relationship between body length and *in vivo* stomach volume for *Ancistrus*. In the present tests, a gas collector similar to that used by Gee (1976) was employed. Individual fish were placed in a hypoxic, partially covered, 10 l aquarium (25 °C) that contained an inverted, water-filled funnel attached to a graduated pipette (Fig. 1). By positioning the funnel over the fish, expired air bubbles could be trapped. The water-filled syringe at the top of the pipette was used to withdraw the gas quickly into the pipette and to determine volume. The sample was then pulled up and into the side syringe for analyses of $O_2$ and $CO_2$ content. Gases were originally analysed with a Scholander gas analyser, although it was later determined that the Radiometer $O_2$ and $CO_2$ electrodes could be used if carefully and regularly calibrated. Previous work (Graham & Baird, 1982) had established that air-breathing rate is affected by $P_{w,O_2}$, which was therefore monitored using the YSI $O_2$ electrode during all gas-release studies. By bubbling small amounts of $CO_2$ and $N_2$ into the aquarium it was possible to alter the respiratory gas content of the water and thus determine the relationship between $P_{w,O_2}$ and $P_{w,CO_2}$ and expired gas content.

The aquarium and gas collector were placed behind a visual blind and dimly backlighted so that the fish and the sampler could be continually observed. This was necessary to verify that all gas expelled was in fact collected by the funnel. Also, for accuracy, the sample had to be processed immediately upon capture. Calibration experiments were done using small bubbles of air and gas mixtures of known $O_2$ and $N_2$ content which were released at the bottom of the aquarium and collected in the funnel. These tests showed that significant contamination of the sample could be avoided if it was processed quickly (15 s). The potential for the diffusion of $O_2$ in or out of the expelled gas was low because of the usually low diffusion gradient between expired gas and $P_{w,O_2}$ (see Results). $CO_2$, however, was more difficult to work with because of its higher solubility in water and the larger diffusion gradients that existed in some tests (see Results). The dead space of the side-arm syringe contained hypoxic water and this was expelled through the stopcock as soon as the sample was pulled in and the syringe disconnected. A dark plastic apron attached to the ventral side of the funnel (Fig. 1) increased the effective area of the sampler and made it possible to collect bubbles released near corners of the tank. The apron also darkened the area below the collector and aided in keeping the fish in a fixed position between air breaths. Suspending the sampler by its top syringe made it possible to swing the apparatus in the event the fish changed position slightly (Fig. 1).

*Gill ventilation and cardiac response during air breathing*

Hypoxia-acclimated fish and normoxic fish that had been recently induced to breathe air were compared for the relationship between heart-beat and gill ventilation
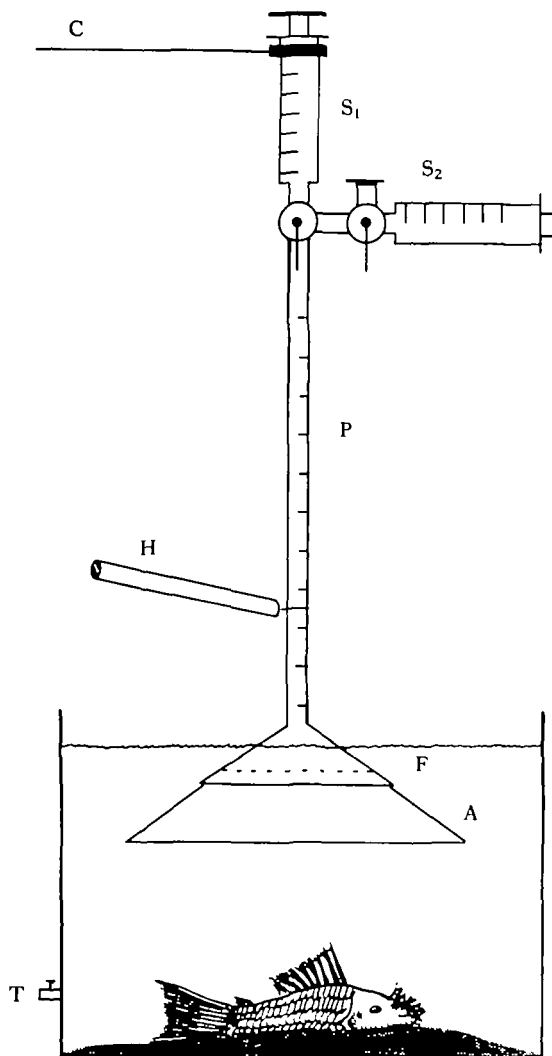
Fig. 1. Collector used to capture gas exhaled by *Ancistrus chagresi*. The top syringe ($S_1$) was used to pull bubbles trapped in the funnel (F) up into the graduated pipette (P) to read the volume and then to the level of the side syringe ($S_2$) for gas analysis. Stopcocks prevented leakage. Depending on fish size, different combination of pipette volumes and funnels were used. The collector was suspended by a clamp (C) and pivoted using the handle (H) in order to keep it above the fish. A black plastic apron (A) darkened the area below the funnel which helped to keep the fish in position. Vertical distance from the fish to the funnel neck was usually 10–12 cm. Swimming movements by the fish during air breathing kept aquarium water mixed. The sampling tube (T) was used to remove water for $O_2$ and $CO_2$ determinations (25 °C).

rates during regular cycles of air-breathing activity. Electrode pairs (hooked, 0·07 mm diameter, stainless steel) were implanted around the pericardium and adjacent to the hypobranchial muscle of lightly anaesthetized (MS 222) fish using procedures described by Roberts & Graham (1979). Following recovery from anaesthesia, the fish was placed in a small (4 l) darkened aquarium (24–26 °C). A Faraday cage covered the tank and the electrode leads were connected to a Gilson recorder. No signal

preamplification was made but in some cases an impedance converter was used t(
obtain clearer signals. Records were made over 12–72 h following electrode implanta-
tion. $N_2$ was gently bubbled into the aquarium to achieve and maintain desired levels
of hypoxia; $P_{w,CO_2}$ and $P_{w,O_2}$ were measured regularly.

<div align="center">RESULTS</div>

<div align="center">*Aquatic respiration in hypoxia*</div>

Oxygen consumption dependence curves (Fig. 2) compare the effect of $P_{w,O_2}$ on the
aquatic $\dot{V}O_2$ of control ($n = 7$) and hypoxia-acclimated ($n = 9$) *Ancistrus* of equal
body size. From 140 down to 100 mmHg, the $\dot{V}O_2$ of both groups is similar with a
mean of $84.0 \pm 4$ ml kg h$^{-1}$ ($\bar{x} \pm 95\%$ conf. int., $n = 35$, mean wt 26 g, range 3–99 g).
Variable respiration rates occurred between 100 and 50 mmHg $P_{w,O_2}$, and, below
40 mmHg $P_{w,O_2}$ the $\dot{V}O_2$ of both groups decreased rapidly with that of the acclimated
fish remaining higher relative to the control. Acclimated fish had a low level of
respiration at 5 mmHg but the $\dot{V}O_2$ of the control group became nearly zero at
10 mmHg $P_{w,O_2}$. In addition, mean rates of the two groups are significantly different
($P < 0.05$, $t$ test) at 10 and 20 mmHg. Since neither group of fish had access to air
inside the respirometer the measured $\dot{V}O_2$ is assumed to be the maximum attainable
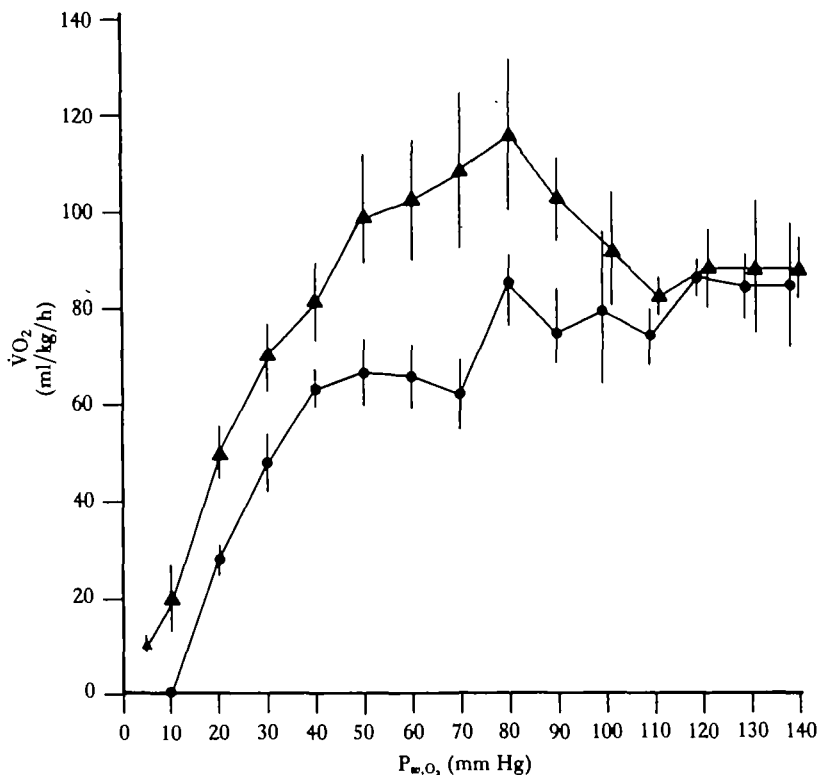by aquatic uptake at each level of hypoxia.



Fig. 2. Mean aquatic oxygen consumption rates of control (●) and hypoxia-acclimated (▲) groups
of *Ancistrus chagresi* exposed to progressive hypoxia without access to air. Lines are ±1s.E. (25 °C).

### Changes in blood properties

Hypoxia-acclimated *Ancistrus* have a significantly higher blood Hb content, a significantly lower amount of total red cell phosphate ($P < 0.05$, $t$ test, both comparisons), and a higher Hb–$O_2$ affinity ($P_{50}$, estimated from dissociation curves, Fig. 3) than do control fish (Table 1). Fig. 3 compares $O_2$ dissociation curves and molar phosphate–Hb ratios of the two groups. To enable visualization of the impact of the Hb increase on the $O_2$-carrying capacity of hypoxia-acclimated fish blood, the Y axis of Fig. 3 is expressed as total $O_2$ content rather than percent saturation (Wood & Johansen, 1972). Oxygen content was calculated assuming an $O_2$-combining power of $1.39$ ml$O_2$ gHb as determined for loricariids and other air-breathing fishes (Johansen *et al.* 1978). The pH of tonometered blood is similar to other values reported for loricariids (Weber *et al.* 1979; Wood, Weber & Davis, 1979).
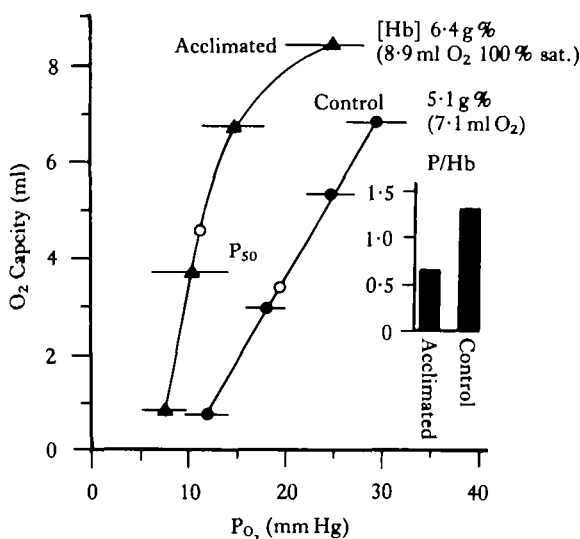


Fig. 3. Oxygen dissociation curves of control (●) and hypoxia-acclimated (▲) *Ancistrus chagresi* (25 °C). Curves are fitted by eye through mean values for $P_{10}$, $P_{40}$, $P_{75}$ and $P_{95}$; horizontal bars indicate the range of each estimate ($n$). $O_2$ saturation values (Y axis) are expressed as total $O_2$ content (see Results) to show effect of increased Hb. Estimated $O_2$ capacity, the Hb content, P–Hb ratios, and $P_{50}$ values are also indicated for each group.

Table 1. *Red cell haemoglobin (Hb) and phosphate (P) concentrations, molar P–Hb ratios, and $P_{50}$ estimates for control and hypoxia–acclimated* Ancistrus chagresi

Mean blood haematocrits (Hmct) are also indicated. Concentration values are mean ± 95 percent confidence intervals ($n$), $P_{50}$ values are estimated from Fig. 3.

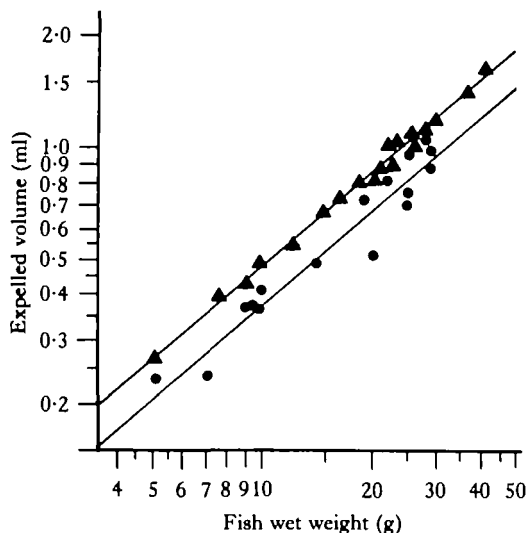|  | Control | Hypoxia–acclimated |
|---|---|---|
| Hb (g%) | $5.1 \pm 0.28$ (34) | $6.4 \pm 0.42$ (23) |
| Hmct (%) | $23.4 \pm 3.95$ (7) | $31.2 \pm 2.39$ (10) |
| P ($\mu$mol/100 ml) | $101.8 \pm 8.8$ (6) | $63.2 \pm 17.4$ (7) |
| P/Hb | 1.32 | 0.65 |
| $P_{50}$ (mmHg) | 19.8 | 10.2 |
| pH | 7.44 | 7.38 |

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Fig. 4. Relationship between mean expelled ABO gas volume and body weight in control (●) and hypoxia-acclimated (▲) *Ancistrus chagresi* (25 °C). Least squares regressions: control fish, log $y = -1\cdot27 + 0\cdot85 \log x$, $r = 0\cdot99$, $n = 16$; hypoxia-acclimated fish, log $y = -1\cdot16 + 0\cdot84 \log x$, $r = 0\cdot99$, $n = 19$. Total air breaths examined: control 112; hypoxia 168.

## Aerial gas exchange

### Expired gas volume

Fig. 4. illustrates the relationship between the volume of gas expelled from the ABO and body weight in *Ancistrus* and shows that the volume released by hypoxia-acclimated fish is about 25 % greater than that of control fish recently induced to breathe air. The slopes of the two lines in Fig. 4 are not different but covariance analysis revealed that the mean gas release volumes (i.e. line intercepts) of the two groups differ significantly $F_{2,31} = 12\cdot3, P < 0\cdot05$). Expired gas volume was nearly constant in all fish tested. It was not affected by $P_{w,O_2}$ and, in most series of repeated determinations, the 95 % confidence intervals of the mean volume estimate were usually within 10 % of the mean. Also, volumes collected from control fish fit perfect-ly on the fish length–*in vivo* ABO volume curve of Gee (1976), confirming this organ to be completely emptied upon each expiration.

### Expired gas content

The $O_2$ partial pressure of an expired breath ($P_{EO_2}$) is inversely proportional to the time it is held (Fig. 5). Both groups of fish usually held an air breath for longer than 2 min during which time, assuming complete expiration of the previous breath (see above), $P_{EO_2}$ dropped from 155 (air value) to below 75 mmHg. Compared to control

Fig. 5. Relationship between mean $P_{EO_2}$ measured in an expelled air breath (air $O_2 = 155$ mmHg) and air-breath duration in control and hypoxia-acclimated *Ancistrus chagresi* (25 °C). The mean $P_{EO_2}$, $\pm 95$ % confidence intervals and ranges are indicated for all durations where the number of air-breaths examined was $\geqslant 4$. For $n < 4$, the mean and data points are shown. Dashed lines show probable drop in ABO $O_2$ content during the first 2 min of the air breath. Least squares regressions for $O_2$ decline from 2 min to the longest duration observed are: control fish, log $y = 1\cdot92 - 0\cdot36 \log x$, $r = -0\cdot82$, $n = 12$; hypoxia fish, log $y = 2\cdot00 - 0\cdot56 \log x$, $r = -0\cdot78$, $n = 22$.
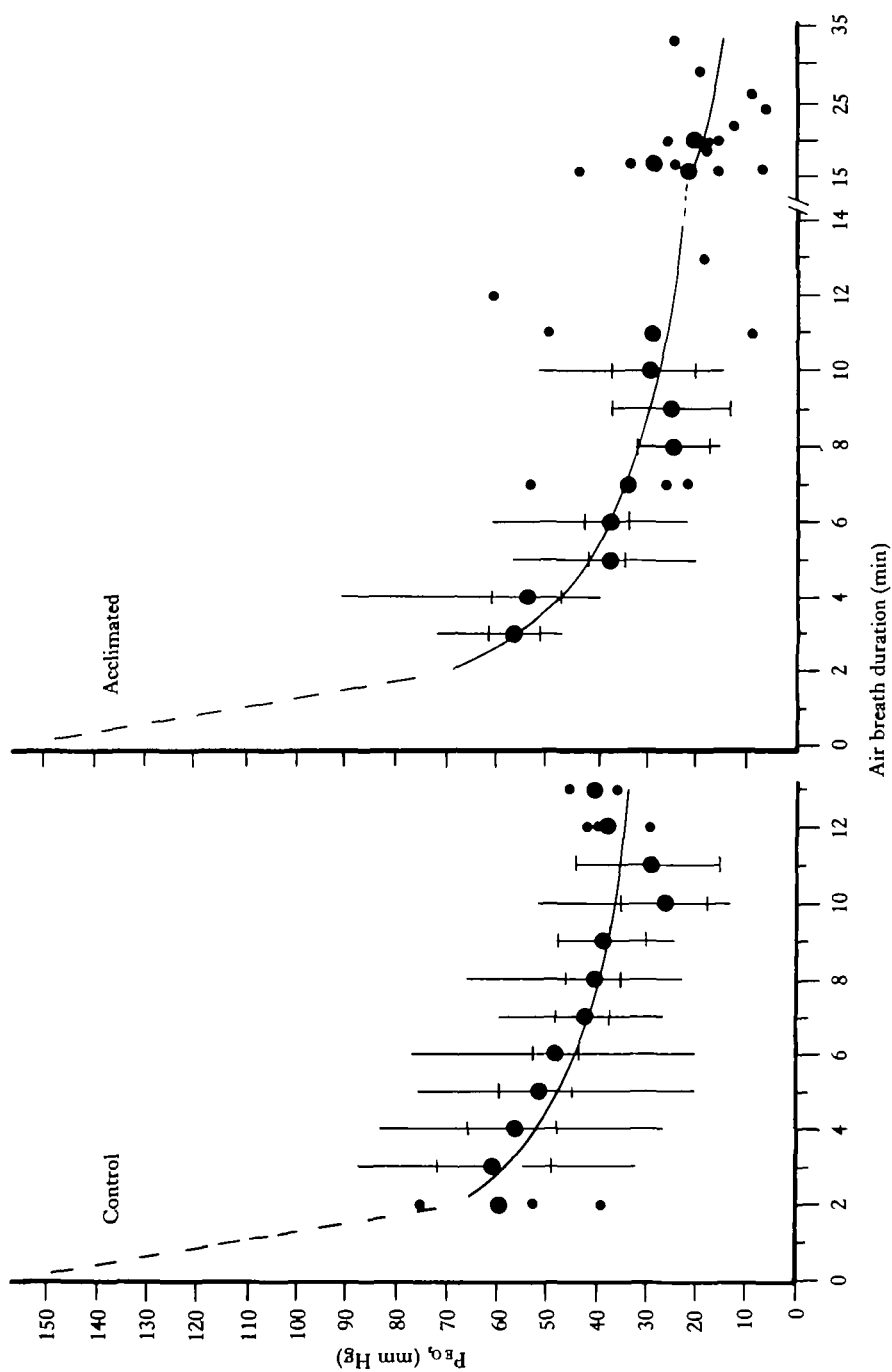
Fig. 5

fish (Table 2A), hypoxia-acclimated fish had a significantly ($P < 0.05$, $t$ test) lower average $P_{EO_2}$ (and therefore a larger $O_2$ extraction 80 vs 71 %) and a longer average air-breath duration. Only 27 % of the breaths discharged by control fish had a $P_{EO_2}$ of or less than 35 mmHg compared to 54 % of the breaths released by acclimated fish (Table 2B). Fig. 5 shows that control fish seldom had a $P_{EO_2}$ as low as 15 mmHg but, in some acclimated fish, $P_{EO_2}$ was below 10 mmHg. Also, a greater percentage of the air breaths of hypoxia-acclimated fish were held for 10 min or longer (32 vs 13 %, Table 2B). In neither group was a relationship found between $P_{EO_2}$ and $P_{w,O_2}$, nor did they differ in regard to mean $P_{ECO_2}$ (Table 2A). The amount of expired $CO_2$ is very low (respiratory exchange ratios $< 0.1$, Table 2A), indicating that most $CO_2$ is released aquatically. $P_{w,CO_2}$ directly affected $P_{ECO_2}$. Blood probably reached an equilibrium state with $P_{w,CO_2}$ and the fish used $HCO_3^-$ buffering to conserve blood pH (Wood *et al.* 1979). Tests with mixed gas bubbles released under the funnel showed that $CO_2$ was also acquired from water when the diffusion gradient was high.

*Estimation of inspired air volume*

To calculate aerial $\dot{V}O_2$, inspired air volume must be known but, because $O_2$ removed by the air-breathing organ is not replaced by an equivalent volume of $CO_2$ (Table 2A), expired gas volume is always less than inspired. If the proportions of the

Table 2A. *Mean duration (min) and respiratory gas contents ($P_{EO_2}$, $P_{ECO_2}$) of gas released from the stomachs of control* (n = 6) *and hypoxia–acclimated* (n = 7) *Ancistrus chagresi*

Values are mean ± 95 % confidence intervals, T = 25 °C. $CO_2$ values were determined for gas expired by fish in $P_{w,CO_2}$ 1–3 mmHg. RE (respiratory exchange ratio) = $P_{ECO_2}/155 - P_{EO_2}$.

| Group | Control | Hypoxia–acclimated |
|---|---|---|
| Duration and content | | |
| total breaths sampled | 140 | 105 |
| duration (min) | $7.24 \pm 1.36$ | $8.49 \pm 1.17$ |
| $P_{EO_2}$ | $45.69 \pm 2.4$ | $30.93 \pm 3.10$ |
| $O_2$ Extraction (%) | 70.5 | 80.0 |
| $P_{ECO_2}$ | $1.49 \pm 0.37$ | $1.49 \pm 0.37$ |
| RE | 0.014 | 0.012 |

Table 2B. *Contrasts of control and hypoxia–acclimated* Ancistrus *for the percentile distributions of air breaths $P_{EO_2} \leqslant 45$ mmHg and with durations of 10 min or longer*

| Percentile distribution of $P_{EO_2}$ | n (%) | n (%) |
|---|---|---|
| $P_{EO_2} \leqslant 45$ mmHg | 84 (60.0) | 80 (76.2) |
| $P_{EO_2} \leqslant 35$ mmHg | 38 (27.1) | 57 (54.3) |
| $P_{EO_2} \leqslant 25$ mmHg | 10 (16.4) | 44 (41.9) |
| $P_{EO_2} \leqslant 15$ mmHg | 2 (1.43) | 15 (14.3) |
| $P_{EO_2} \leqslant 5$ mmHg | 0 | 2 (1.9) |
| Duration: Time held $\geqslant 10$ min | 18 (12.9) | 34 (32.4) |
| Time held $\geqslant 11$ min | 9 ( 6.4) | 23 (21.9) |
| Time held $\geqslant 12$ min | 5 ( 3.6) | 21 (20.0) |
| Time held $\geqslant 13$ min | 2 ( 1.4) | 20 (19.0) |
| Time held $\geqslant 14$ min | 0 ( 0) | 19 (18.0) |

$P_2$ and $CO_2$ in expired gas are measured and if it can be assumed that the amounts of $N_2$ and inert gases (79 % of air) in the breath did not change, then inspired volume could be calculated by algebraic substitution. For example, a 25 g hypoxia-acclimated fish would expel about 1·02 ml of gas (Fig. 4) that would on the average (Table 2A) contain about 4·2 % $O_2$ (21 % × 31/155 mmHg) and 0·2 % $CO_2$ (0·03 × 1·49/0·22). Assuming that $N_2$ and inert gases constitute the balance of the expelled sample, then their volume would be 0·975 ml (1·020 − [1·020 × 0·044]). If this volume originally constituted 79 % of the inspired volume, then inspired volume was 1·23 ml, 20 % greater than the expired volume. For a 25 g control fish with an expired volume of 0·82 ml (Fig. 4) and average gas contents of 6·2 % $O_2$ and 0·2 % $CO_2$ (Table 2A), inspired volume is 0·97 ml (18 % larger). The difference between expired and inspired volume would depend on expired gas $O_2$ and $CO_2$ levels. In theory it could not exceed 21 % and would be more difficult to calculate for fish in high $P_{w,CO_2}$ owing to the effect of this variable on $P_{E\,CO_2}$.

### Effects of hypoxia acclimation on bimodal respiration

The impact of hypoxia acclimation on total respiration can be seen in Table 3, which compares the effect of $P_{w,O_2}$ on the bimodal and total respiration rates of control and hypoxia-acclimated *Ancistrus*. For both groups the combined influences of different air-breathing frequencies (from Graham & Baird, 1982), ABO volumes (Fig. 4) and $O_2$ extraction percentages (Table 2A) on aerial respiration rate have been computed for a 25 g fish. (Inspired volumes were calculated above. Note that for both groups inspired volume and $O_2$ extraction do not change with $P_{w,O_2}$ [see above].) Also included in Table 3 are the maximum aquatic respiration rates for a 25 g fish at each $P_{w,O_2}$ (Fig. 2). In both control and acclimated fish, aerial $O_2$ uptake increases and aquatic respiration decreases as $P_{w,O_2}$ is lowered. At 30 mmHg a control fish has a

Table 3. *Estimated bimodal and total respiration rates, as a function of* $P_{w,\,O_2}$ *in 25 g control and hypoxia–acclimated* Ancistrus chagresi *(T = 25 ℃)*

Calculations are based on air–breathing frequency data (Graham & Baird, 1982) and data compiled in this study. For ease of comparison, total respiration rates (ml $O_2$/h) are used rather than weight–specific rates (ml/kg/h).

| | Control | | | | Hypoxia–acclimated | | |
|---|---|---|---|---|---|---|---|
| $P_{w,\,O_2}$ (mmHg) | 10 | 20 | 30 | | 10 | 20 | 30 |
| Air–breathing frequency (No/h) | 13 | 11 | 9 | | 11 | 8 | 5 |
| Inspired ABO volume (ml) | 0·97 | 0·97 | 0·97 | | 1·23 | 1·23 | 1·23 |
| Inspired $O_2$ (ml) (21 % of above) | 0·204 | 0·204 | 0·204 | (21 %) | 0·258 | 0·258 | 0·258 |
| $O_2$ extraction in ABO (ml) (71 % of above) | 0·145 | 0·145 | 0·145 | (80 %) | 0·206 | 0·206 | 0·206 |
| Aerial respiration (ml/h) | 1·89 | 1·60 | 1·31 | | 2·27 | 1·65 | 1·03 |
| Maximum aquatic respiration (ml/h) | 0 | 0·70 | 1·20 | | 0·50 | 1·28 | 1·75 |
| Total potential respiration (ml/h) | 1·89 | 2·30 | 2·51 | | 2·77 | 2·93 | 2·78 |
| Routine level | 2·10 | 2·10 | 2·10 | | 2·10 | 2·10 | 2·10 |
| 'Excess' consumption (ml/h) | −0·21 | +0·20 | +0·31 | | 0·67 | +0·83 | +0·68 |
| Level of supplementary respiration required for routine (% max. aquatic respiration) | — | 71 | 74 | | 0 | 35 | 61 |

greater aerial respiration rate than an acclimated fish because of a higher air-breathing
frequency (breaths/h × ml/breath = ml/h). But, at 10 mmHg its aerial $O_2$ uptake is
below that of an acclimated fish; it also has about zero aquatic consumption capacity at
10 mmHg (Fig. 2), thus its total potential respiration would fall below routine. From
30–10 mmHg $P_{w,O_2}$, the aerial uptake calculated for a hypoxia-acclimated fish increases
more than two-fold and the level of supplementary aquatic respiration needed to sustain
routine consumption is very low. This shows that a control *Ancistrus* may be initially
incapable of surviving severe hypoxia but, through gradual hypoxia-acclimation, this
fish can utilize aerial respiration more effectively and minimise its use of aquatic respira-
tion, even though this respiratory mode has also become more proficient.

### Gill ventilation and heart-rate changes during air breathing

When *Ancistrus* takes an air breath its heart rate immediately rises (tachycardia)
and the frequency and amplitude of its gill ventilation abruptly drops. During the
time air is held, heart rate gradually declines and aquatic ventilation slowly climbs to
'pre-air breath' levels (Fig. 6). Table 4A compares hypoxia-acclimated and control
fish for their mean heart and gill ventilation frequencies and the magnitude of the
shifts occurring just after an air breath is taken and immediately prior to the next one.
Hypoxia-acclimated *Ancistrus* have significantly lower ($P < 0.05$, $t$ test) mean 'pre-
breath' heart and opercular-beat frequencies than control fish. Just after an air breath,
hypoxia-acclimated and control fish show similar relative drops in opercular
frequency ($-21\%$), but the hypoxia group has a much larger tachycardia ($32\%$).

Fig. 6 compares the pattern of mean heart and gill ventilation-frequency shifts
observed for the two groups in the course of one air-breath cycle. An air breath by a
hypoxia-acclimated fish results in nearly equivalent shifts in the ratios of heart and gill
ventilation frequencies before and after the breath is taken (Table 4B). By contrast,
the pre-breath ratio of gill ventilation to heart rate in control fish is 1·32 (Table 4B)
but after the air breath is taken, heart rate exceeds gill ventilation by only 1·13 times.
These comparisons indicate that hypoxia acclimation enables *Ancistrus* to reduce both
cardiac and gill-ventilatory activity in hypoxic water. Also, complimentary shifts in
heart and gill ventilation frequency ratios with an air breath suggest that hypoxia-
acclimated fish may be able to integrate the aerial and aquatic phases of bimodal
respiration more smoothly.

Table 4. A. Pre– and post–air breath heart beat and gill ventilation frequencies of four
control and five hypoxia–acclimated Ancistrus chagresi

Values are mean events/min ± 95 % confidence intervals. The percentage change in frequency (%), the number
of air–breath records examined (n), and phase–change frequency (B) ratios are indicated (T = 24–26 °C).

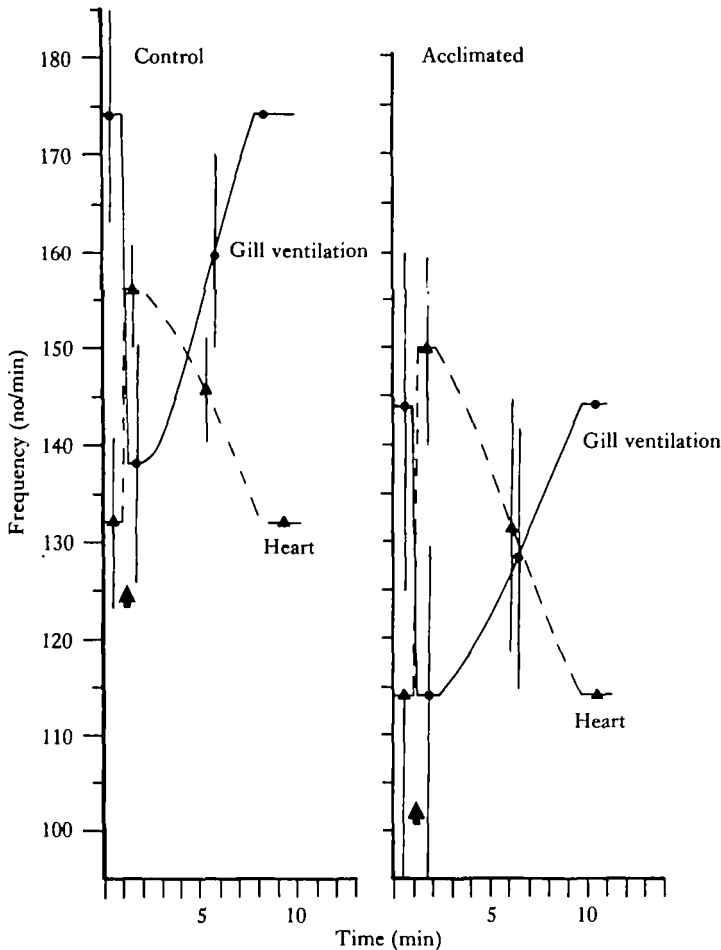|  | Control | | | | Hypoxia–acclimated | | | |
|  | Pre–breath | Post–breath | % | n | Pre–breath | Post–breath | % | n |
|---|---|---|---|---|---|---|---|---|
| A. Frequency | | | | | | | | |
| Heart beat | 132 ± 9 | 156 ± 6 | +18 | 34 | 114 ± 15 | 150 ± 9 | +32 | 52 |
| Gill ventilation | 174 ± 11 | 138 ± 12 | −21 | 27 | 144 ± 17 | 144 ± 16 | −21 | 52 |
| B. Frequency ratios | | | | | | | | |
| Heart/gill ventilation | | 1·13 | | | | 1·32 | | |
| Gill ventilation/heart | 1·32 | | | | 1·26 | | | |

Fig. 6. Changes in mean gill ventilation and heart-beat frequencies during the air-breathing period of control and hypoxia-acclimated *Ancistrus chagresi*. Arrows show the time an air breath is taken. Subsequent rate changes reflect the longer mean duration of an air breath in hypoxia-acclimated fish (Table 2). Vertical lines are 95 % confidence intervals. Symbols, heart beat (▲), gill ventilation (●).

## DISCUSSION

Hypoxia-acclimation results in a suite of adaptive changes that enhance both the aerial and aquatic respiration capacities of *Ancistrus* in hypoxic water. An acclimated fish gulps a greater volume of air and, on average, holds it longer than control fish. It also seems able to reduce $P_{EO_2}$ to a lower level, although this is not regularly done. Moreover, hypoxia-acclimated fish have about a 25 % greater maximum capacity for aquatic respiration over the $P_{w,O_2}$ range ($\leqslant 33$ mmHg) where facultative air-breathing is required. This discussion examines the way these adaptations may contribute to the separate aquatic and aerial modes of respiration as well as to the total gas exchange of *Ancistrus*.

*Aerial gas exchange*

*The ABO*

In their landmark study, Carter & Beadle (1931) determined that an air-breath expired by *Ancistrus anisitsi* contained 13·8 % $O_2$ and no $CO_2$ ($n = 2$). The present study with *A. chagresi* found only slight amounts of $CO_2$ and discharged-breath $O_2$ content averaged between 5 and 6 % $O_2$. Carter & Beadle (1931) also described respiratory adaptations in the stomach of *Ancistrus*. This organ has thin compliant walls that contain layers of smooth muscle which, through contraction, serve to expel its contents. The inner surface of the stomach consists of a single layer of cuboidal epithelial cells which is invested by a uniformly-distributed and dense capillary bed. Blood supply is by the coeliac artery and drainage is through the interrenal vein. The ABO of *A. chagresi* is morphologically similar to that of *A. anisitsi*. *In vivo* inspection revealed no differences in the tributary vessel sizes or in the capillary densities of the stomachs of hypoxia-acclimated and control fish. However, histological comparisons were not made. The increase in ABO volume following hypoxia-acclimation may result from stretching caused by repeated air breathing or growth in the organ.

*Hb content and $O_2$ affinity*

In *Ancistrus*, as in most other teleostean air breathers, $O_2$ rich blood that drains the ABO is mixed with other venous blood and must circulate through the gills before reaching systemic circulation (Carter & Beadle, 1931; Johansen, 1970). The increased Hb content and Hb–$O_2$ affinity of hypoxia-acclimated fish minimize mixing and gill exposure effects. An increased $O_2$ capacity will offset, to some extent, the mixing of $O_2$-rich ABO blood with $O_2$-poor venous blood and the increased affinity may ensure complete $O_2$ saturation (Johanson *et al*. 1978). A higher $O_2$ affinity would also favour $O_2$ retention and lessen transbranchial $O_2$ loss (see below). The lower $P_{EO_2}$ in some air breaths from hypoxia-acclimated fish (10 mmHg vs 21 mmHg, mean of six lowest values for each group, Fig. 5) correlates with their higher Hb–$O_2$ affinity (Table 1). However, since $O_2$ depletion from the ABO only rarely proceeded to this point (Fig. 5, Table 2) the primary function of an increased Hb–$O_2$ affinity is probably not for the capture of aerial $O_2$.

*Ventilatory and cardiac coordination*

The significance to aerial $O_2$ transport of the cyclic shifts in heart beat and gill ventilation during each air breath cannot be quantitatively assessed without information on cardiac output and relative blood flow through gills and the ABO, together with ventilation volume at different times in the air-breath interval. It would also be desirable to know the extent to which branchial blood flow can be controlled autonomically. Measurements of this type would be difficult on fish of the size used in this study but could probably be done on slightly larger specimens (Farrell, 1978). Other work has shown that air-breathing tachycardia occurs in most air-breathing fish as well as other air-breathing vertebrates (Heatwole & Seymour, 1976; Singh, 1976; Randall, Burggren, Farrell & Haswell, 1981). It has also been shown that increased cardiac output to the ABO occurs just after inspiration (Singh, 1976; Farrell, 1978). Surprisingly few

▸udies have examined the coordination of cardiac and opercular activity during air breathing in fishes. Singh & Hughes (1973) found that *Anabas* completely stopped gill ventilation during periods when it exclusively utilized $O_2$ in its ABO and when it was exposed in air or to hypercapnic water. During bimodal respiration, gill ventilation was constant and unaffected by air-breathing events even though heart rate cycled. This is unlike the phase shifts in cardiac and gill ventilatory activity that occur with air breathing in *Ancistrus*.

### Respiratory control

This study provides insight into the factors controlling air breathing in *Ancistrus*. The mean $P_{w,O_2}$ air-breathing threshold of this species (33 mmHg) is unaffected by hypoxia-acclimation (Graham & Baird, 1982). The aquatic oxygen consumption-dependence curve (Fig. 2) of hypoxia-acclimated fish shows that they can, through increased gill ventilation, maintain routine $\dot{V}O_2$ down to nearly 40 mmHg $P_{w,O_2}$. Below 40 mmHg, however, the $\dot{V}O_2$ of both experimental groups drops precipitously. Thus air breathing is initiated soon after maximum aquatic $\dot{V}O_2$ drops below routine. Even though hypoxia-acclimated fish are more effective air breathers (Table 3) their air-breathing frequency remains inversely related to $P_{w,O_2}$ (Graham & Baird, 1982). This, together with the longer average time an air breath was held by acclimated fish (Fig. 5), suggests that $P_{w,O_2}$ exerts continued indirect control over air breathing through its effect on aquatic $\dot{V}O_2$ and the potential for aerial $O_2$ loss through the gills.

### Aquatic respiration

### Hb and Hb–$O_2$ affinity

Even though it can use the $O_2$-rich aerial medium, *Ancistrus* makes respiratory adjustments for aquatic hypoxia like those seen in non air-breathing fish (Wood & Johansen, 1972; Powers, 1980). An increased Hb–$O_2$ affinity improves the effectiveness of aquatic $O_2$ uptake by acclimated fish and is consistent with the demonstrated left-shift of their oxygen consumption-dependence curves (Fig. 2). In addition to extending the lower $P_{w,O_2}$ limits for aquatic respiration, an increased Hb–$O_2$ affinity also lowers the effective limit for the transbranchial loss of $O_2$ ; in control fish, $O_2$-saturated blood circulating through the gills would unload $O_2$ to water at $P_{w,O_2} \leqslant$ 29 mmHg. In hypoxia-acclimated fish this would begin at $P_{w,O_2} \leqslant 23$ mmHg (Fig. 3).

### P–Hb ratio

Erythrocytic triphosphates are allosteric modulators of Hb–$O_2$ affinity and usually decrease in concentration and thus raise $O_2$ affinity in fish exposed to hypoxic water (Wood & Johansen, 1972). Affinity increases are often accompanied by a rise in total Hb (Powers, 1980) as shown here for *Ancistrus*. The combined effect of a rise in Hb and a drop in phosphate is to diminish further the molar P–Hb ratio, an index of relative $O_2$ affinity.

In this work with *Ancistrus*, the nearly 10 mmHg left-shift of $P_{50}$ was accompanied by a drop in intracellular P/Hb from 1·32 to 0·65 (Table 1). These ratios and the magnitude of their change are larger than reported for a South American air-breathing armoured catfish (*Hypostomus* sp.) by Weber *et al.* (1979). These workers tested fish

that had been acclimated to hypoxia for only 4–7 days and correlated a drop in $P_{50}$ from 17·9 to 13·3 mmHg (pH = 7·65) with a 50 % reduction in red cell phosphate. However, they also found a significant drop in Hb; thus P/Hb was reduced only from 0·47 to 0·28. With another hypoxia-acclimated air-breathing loricariid (*Pterygoplicht-hys*), Weber *et al.* (1979) found a significant drop in total phosphate (34 %) but no change in Hb as P/Hb shifted from 0·57 to 0·40. Red cell triphosphate levels can be changed quickly, whereas more time is needed to produce more erythrocytes which raise haematocrit and Hb (Powers, 1980; J. B. Graham, in preparation). With only 4–7 days of hypoxia acclimation these two South American loricariids were able to reduce triphosphate levels but probably did not complete an erythropoietic response like that of *Ancistrus*.

*Ventilation and CO₂ release*

Only small amounts of respiratory $CO_2$ are discharged aerially by *Ancistrus* (Table 2) and because thick scales cover most of the body of this fish it is concluded that $CO_2$ release must occur aquatically through the gills. Survival in chronic hypoxia would therefore depend upon the ability of *Ancistrus* to balance the use of its gills for aquatic $CO_2$ release with the need to prevent the transbranchial loss of $O_2$ gained from the ABO. Cyclic changes in gill ventilation rate that occur during each air breath period suggest the manner in which this may be done. Some branchial $CO_2$ exchange probably occurs at all times, but the rate of this process seems likely to be highest during the latter phase of each air breath cycle when, together with the progressive depletion of $O_2$ from blood and the ABO, gill ventilation increases.

REFERENCES

BARTLETT, G. R. (1978). Water-soluble phosphates of fish red cells. *Can. J. Zool.* 56, 870–877.

BARTLETT, G. R. (1980). Phosphate compounds in vertebrate red blood cells. *Am. Zool.* 20, 103–114.

BICUDO, J. E. P. W. & JOHANSEN, K. (1979). Respiratory gas exchange in the air breathing fish, *Synbranchus marmoratus*. *Environ. Biol. Fish.* 4, 55–64.

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

EDWARDS, M. J. & MARTIN, R. J. (1966). Mixing technique for the oxygen–hemoglobin equilibrium and Bohr effect. *J. appl. Physiol.* 21, 1898–1902.

FARRELL, A. P. (1978). Cardiovascular events associated with air breathing in two teleosts, *Hoplerythrinus unitaeniatus* and *Arapaima gigas*. *Can. J. Zool.* 56, 953–958.

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

GEE, J. H. (1980). Respiratory patterns and antipredator responses in the central mudminnow, *Umbra limi*, a continuous, facultative, air-breathing fish. *Can. J. Zool.* 58, 819–827.

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

GRAHAM, J. B. & BAIRD, T. A. (1982). The transition to air breathing in fishes: I. Environmental effects on the facultative air breathing of *Ancistrus chagresi* and *Hypostomus plecostomus* (Loricariidae). *J. exp. Biol.* **96**, 53–67.

HEATWOLE, H. & SEYMOUR, R. (1976). Respiration of marine snakes. In *Respiration of Amphibious Vertebrates*, (ed. G. M. Hughes), pp. 375–389. London: Academic Press.

JOHANSEN, K. (1970). Air breathing in fishes. In *Fish Physiology*, Vol. IV, (ed. W. S. Hoar & D. J. Randall), pp. 361–411. New York: Academic Press.

JOHANSEN, K., MANGUM, C. P. & LYKKEBOE, G. (1978). Respiratory properties of the blood of Amazon fishes. *Can J. Zool.* **56**, 898–906.

KRAMER, D. L., LINDSEY, C. C., MOODIE, G. E. E. & STEVENS, E. D. (1978). The fishes and the aquatic environment of the central Amazon basin, with particular reference to respiratory patterns. *Can. J. Zool.* **56**, 717–729.

POWERS, D. A. (1980). Molecular ecology of teleost fish hemoglobins: Strategies for adapting to changing environments. *Am. Zool.* **20**, 139–162.

RANDALL, D. J., BURGGREN, W. W., FARRELL, A. P. & HASWELL, M. S. (1981). *The Evolution of Air Breathing in Vertebrates*. Cambridge: Cambridge University Press.

ROBERTS, J. L. & GRAHAM, J. B. (1979). Effect of swimming speed on the excess temperatures and activities of heart and red and white muscles in the mackerel, *Scomber japonicus*. *Fish. Bull.* **76**, 861–867.

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

SINGH, B. N. & HUGHES, G. M. (1973). Cardiac and respiratory responses in the climbing perch *Anabas testudineus*. *J. comp. Physiol.* **84**, 205–226.

WEBER, R. E., WOOD, S. C. & DAVIS, B. J. (1979). Acclimation to hypoxic water in facultative air-breathing fish: Blood oxygen affinity and allosteric effectors. *Comp. Biochem Physiol.* **62**A, 125–129.

WOOD, S. C. & JOHANSEN, K. (1972). Adaptation to hypoxia by increased $HbO_2$ affinity and decreased red cell ATP concentration. *Nature, Lond.* **237**, 278–279.

WOOD, S. C., WEBER, R. E. & DAVIS, B. J. (1979). Effects of air-breathing on acid-base balance in the catfish, *Hypostomus* sp. *Comp. Biochem. Physiol.* **62**A, 185–187.