

THE TRANSITION TO AIR BREATHING IN FISHES

I. ENVIRONMENTAL EFFECTS ON THE FACULTATIVE AIR BREATHING OF *ANCISTRUS CHAGRESI* AND *HYPOSTOMUS PLECOSTOMUS* (LORICARIIDAE)

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SUMMARY

In response to progressive aquatic hypoxia, the armoured loricariid catfishes *Ancistrus chagresi* and *Hypostomus plecostomus* become facultative air-breathers and utilize their stomachs as accessory air-breathing organs. *Hypostomus* initiates air breathing at a higher aquatic O_2 tension (P_{w,O_2}) than does *Ancistrus* (60 v. 33 mmHg). Once begun, the air-breathing frequencies of both species increase with decreasing P_{w,O_2} ; the frequency of *Ancistrus*, however, is greater than and increases more with hypoxia than does that of *Hypostomus*, which appears to be a more efficient air breather.

Hypoxia acclimation reduces the air-breathing rate of both species. A larger rate reduction occurs in *Ancistrus*, which, however, continues to require more frequent breaths than *Hypostomus*. Hypoxia acclimation does not affect the air-breathing threshold of either species, suggesting that external O_2 receptors initiate facultative air breathing.

In progressive aquatic hypercapnia *Ancistrus* has a lower air-breathing CO_2 threshold (8.7 mmHg) than *Hypostomus* (12.8 mmHg). However, in some tests, individual fish of both species did not initiate air breathing even at P_{w,CO_2} as high as 21 mmHg. Also, air breathing evoked by hypercapnia was short-lived; both species quickly compensated for this gas and resumed exclusively aquatic respiration within a few hours of exposure. Thus, CO_2 is not an important regulator of air breathing in these species. Between 25 and 35 °C, the P_{w,O_2} air breathing threshold of *Ancistrus* is temperature-independent, but air-breathing frequency increases with temperature.

Ancistrus and *Hypostomus* do not breathe air in normoxic (air-saturated) water; their air-breathing responses are evoked by environmental hypoxia. This is fundamentally different from other fish species that breathe air in normoxia in order to meet heightened metabolic demands. Also, the facultative air-breathing adaptations of *Ancistrus* and *Hypostomus* differ in scope and magnitude from those utilized by species that breathe air in normoxia and adapt to hypoxia by increasing air-breathing rate.

INTRODUCTION

Some fishes become air-breathers when environmental factors act to reduce the suitability of ambient water for aquatic respiration (Carter & Beadle, 1931; Johansen, 1970; Graham, Rosenblatt & Gans, 1978; Kramer *et al.* 1978). Numerous investigations have determined threshold levels of aquatic O₂ (and, in some cases, CO₂) that initiate facultative air breathing (Hughes & Singh, 1970*a, b*; Gee, 1976, 1980; Jordan, 1976; Bicudo & Johansen, 1979). Also, the role of temperature both in initiating air breathing and in affecting air-breathing frequency has been demonstrated (Johansen, Hanson & Lenfant, 1970; Rahn *et al.* 1971; Gee, 1980).

Our knowledge of the extent to which environmental factors control air breathing, however, remains incomplete for most species. Few studies (Bicudo & Johansen, 1979; Gee, 1980) have focused attention on the facultative air-breather's ability to acclimate to environmental conditions that initiate air breathing. It seems likely that through adaptive mechanisms a species may be able to improve its air-breathing capability and thus modify air-breathing frequency and also change its air-breathing threshold.

The question of whether or not a facultatively air-breathing fish can reduce the influence of ambient conditions assumes added significance in view of the effects of aquatic O₂ and CO₂ on air breathing. In many species, external O₂ and CO₂ sensors have been implicated in the control of air-breathing behaviour, aquatic ventilation frequency, and the partitioning of O₂ uptake between air and water (Johansen, 1970; Hughes & Singh, 1970*b*). Moreover, the operation of bimodal respiratory mechanisms in most aquatic air-breathing species ensures intimate contact with ambient water (Graham, 1976; Hughes, 1976). This is because even though aerial respiration meets O₂ requirements, gill ventilation is needed by these species in order to excrete respiratory CO₂, regulate acid-base status, and maintain ion balance (Hughes & Singh, 1970*b*; Johansen, 1970; Hughes, 1976; Burggren, 1979).

This paper examines the role of aquatic O₂ and CO₂ partial pressures (tensions) in regulating the facultative air-breathing responses of two species of Panamanian armoured catfish, *Ancistrus chagresi* Eigenmann and Eigenmann and *Hypostomus (Plecostomus) plecostomus* (L.). The effects of acute and chronic exposure to aquatic hypoxia and hypercapnia on the air-breathing threshold and air-breathing frequency of both species are compared. In addition, the effect of temperature on the hypoxia air-breathing threshold and air-breathing rate was determined for *A. chagresi*. Finally synchronous air-breathing is documented for *H. plecostomus*. A previous investigation (Kramer & Graham, 1976) demonstrated this behaviour in *Ancistrus*, but failed to detect it in *Hypostomus*.

Ancistrus chagresi and *Hypostomus plecostomus* are members of the family Loricariidae, the largest and most diversified family of South American catfish (Lowe-McConnell, 1975). Loricariids are benthic, flowing-water species, that have a ventral sucker mouth used for grasping substrate as well as grazing on algae and detritus (Gradwell, 1971; Lowe-McConnell, 1975). In Panama, *Ancistrus* and *Hypostomus* occur sympatrically with other loricariids in small streams. These two species use their stomachs as air-breathing organs and breathe air in response to aquatic hypoxia (Gee,

1976). However, the extent to which these species differ with regard to their sensitivities to air-breathing stimuli is not known. Thus, a comparative study that determined specific differences in physiological and behavioural adaptations for facultative air-breathing may also indicate ways in which *Ancistrus* and *Hypostomus* reduce competitive interactions and are thus ecologically separated in their natural environment.

MATERIALS AND METHODS

Collection and maintenance of fishes

Hypostomus and *Ancistrus* ranging in mass from 9 to 300 g were collected by seine from the Frijoles River near Gamboa, Republic of Panama and transported to holding tanks at the Smithsonian Tropical Research Institute (STRI), Fort Amador, Panama. Experiments were conducted at STRI from 1975–77. Subsequent work (1979–80) was done with fish transported from Panama to the Physiological Research Laboratory at Scripps Institution of Oceanography, La Jolla, California.

Laboratory fish were maintained in low densities in darkened aerated aquaria (25–27 °C) with a photoperiod that varied seasonally. Fish were regularly fed canned spinach and green beans.

Small groups of both species (5–10 fish in 60 l aquaria) were acclimated to hypoxia ($P_{w,O_2} = 15\text{--}40$ mmHg) for 14–21 days in aquaria (25–27 °C) that were filtered but not aerated. Without aeration, fish respiration removed O_2 from the water faster than it was replaced by diffusion from air, resulting in hypoxic conditions. Pump filtration and fish activity kept water in the tank well mixed, although tanks had to be cleaned regularly to prevent toxic conditions. A temperature-compensated YSI oxygen electrode/thermistor probe was used to monitor O_2 and temperature in all aquaria and water samples were routinely withdrawn to measure P_{w,CO_2} and pH using a Radiometer Blood Gas Analyzer.

Determination of facultative air-breathing responses

General experimental procedures. The facultative air-breathing threshold and air-breathing frequency responses to controlled changes in aquatic O_2 and CO_2 were determined for groups of *Ancistrus* and *Hypostomus*. Air-breathing threshold is defined as the mean of the aquatic gas tensions measured at the instant each individual in the group breathed air for the first time in the test. Following the onset of air breathing (i.e. beyond threshold conditions), air-breathing frequencies were determined by counting the total air breaths taken by the group during a set period of time (20–60 min) and correcting this to a mean number of breaths per fish each hour. In each test, air-breathing threshold could only be measured once, whereas in most experiments air-breathing frequency was measured at several different time intervals (up to 72 h) following the onset of air breathing.

Most tests were designed to compare the responses of two groups of fishes simultaneously. For this purpose, two aquaria (40–60 l) were set beside each other with a visual barrier placed between them. They were backlighted and positioned for easy observation in a darkened room (Panama) or from behind a blind (La Jolla).

Experiments were begun with 15–30 min of observation of fish in normoxic

($P_{w,O_2} = 130\text{--}150$ mmHg) water to verify that no air breathing was occurring. The fish were then continuously observed during exposure to gradual changes in aquatic O_2 or CO_2 tensions (or both) produced by bubbling compressed gases either directly into the two aquaria or into a central mixing tank from which water was pumped to and from the two aquaria. (Hypoxia was produced with N_2 gas; hypercapnia with either 5 or 10 % CO_2 in air; and simultaneous hypoxia and hypercapnia with 5 % CO_2 in 95 % N_2 .) In all tests, gas was delivered until air-breathing threshold was reached or it was judged that there would be no response. In P_{w,O_2} threshold determinations, N_2 was bubbled continuously into the tanks at slow rates that reduced O_2 tension by about 0.7 mmHg/min. Because P_{w,CO_2} thresholds were not very much above ambient, (~ 2 mmHg) it was necessary to deliver CO_2 gas very slowly (average tension increase 0.2 torr/min).

An O_2 electrode positioned in the flow of a pump-siphon system that circulated water between the two tanks was used to monitor P_{w,O_2} during all tests. Water from this system was also periodically withdrawn, through a sampling port, for determination of P_{w,CO_2} , P_{w,O_2} , and pH using a Radiometer Blood Gas Analyzer that had been standardized with known gas mixtures and buffer solutions at the experimental temperature.

Comparisons within and between species. Simultaneous observations of groups of *Hypostomus* and *Ancistrus* were made to compare the separate effects of aquatic hypoxia and hypercapnia and the combined effects of these two factors on the air-breathing threshold and frequency of each species. Air-breathing frequency determinations in hypoxia were made during both daylight and darkness and at a variety of fish activity levels up to 72 h beyond threshold determination. By contrast, the effect of CO_2 on air breathing had usually diminished within 6 h of threshold determination.

For each species, groups of fish held in normoxic water were simultaneously compared with groups of hypoxia-acclimated (14–21 days) fish to determine the effect of hypoxia on air-breathing threshold and frequency. All these comparisons were made at 24–27 °C using groups that had a similar mean body size and contained equal numbers of individuals (5–6).

The effect of water temperature (15–35 °C) on the P_{w,O_2} air-breathing threshold of *Ancistrus* was determined as was the effect of temperature (20–30 °C) on air-breathing frequency. Tests were done with a group ($n = 3\text{--}5$) of fish in a 20 l aquarium that was placed in a water bath. Threshold responses were determined only once each day but frequencies were measured several times. Experimental temperature was not changed more than 5 °C in 24 h and the fish were allowed 6–12 h to adapt to the new temperature before tests were begun. The oxygen electrode was calibrated at each test temperature prior to testing.

Air-breathing records of isolated groups ($n = 5$) of *Hypostomus* and *Ancistrus* were analysed for air-breathing synchrony. Records (20–40 min duration; and all made at $P_{w,O_2} < 32$ mmHg) were divided into 1 min intervals and each interval was scored on the basis of the number of breaths it contained (i.e. 0, 1, 2, . . . , n). (When fish averaged air-breathing rates above 8/h, records were analysed using 30 s intervals.) The variance to mean ratio of the resultant frequency distribution was then calculated and tested for its difference from a random distribution (i.e. $S^2/\bar{X} = 1$) with a t test (Kramer & Graham, 1976).

Table 1. Threshold levels (mmHg) of O_2 and CO_2 eliciting air breathing in *Ancistrus chagresi* and *Hypostomus plecostomus* at 25–27 °C

(Separate and combined effects of aquatic hypoxia and hypercapnia are compared. Values are mean gas tension, standard error and sample size. Asterisks indicate that the intra- or inter-specific mean differences observed for different treatments are significant ($P < 0.05$) as determined by t or Mann Whitney U tests. Daggers indicate incomplete air-breathing responses (see text for details). In hypoxia tests $P_{w,CO_2} \leq 2$ mmHg; in hypercapnic tests $P_{w,O_2} \geq 120$ mmHg.)

Hypoxic water			Hypoxic and hypercapnic water					Hypercapnic water				
P_{w,O_2}	S.E.	N	P_{w,O_2}	S.E.	N	P_{w,CO_2}	S.E.	N	P_{w,CO_2}	S.E.	N	
<i>Ancistrus chagresi</i>												
32.7	0.7	95	*	64.0	3.0	20	10.7	0.7	20	8.7†	0.7	28
<i>Hypostomus plecostomus</i>												
60.4	3.3	34	*	78.5	3.8	20	8.2	0.7	20	12.8†	1.4	13

RESULTS

Air-breathing threshold

All experimental fish initiated air breathing in response to progressive aquatic hypoxia (Table 1) but *Hypostomus* began air breathing at a significantly higher P_{w,O_2} than *Ancistrus* (60.4 *v* 32.7 mmHg). However, the threshold P_{w,CO_2} for air breathing was lower in *Ancistrus* than in *Hypostomus*. The higher sensitivity of *Ancistrus* to hypercapnia is further substantiated by specific differences in the number of fish that did not initiate air breathing in response to this gas at P_{w,CO_2} as high as 21 mmHg (Table 1). Four of six replicate tests with *Ancistrus* were complete (i.e. all five fish in the group took an initial air breath) and in each of the two incomplete tests only one fish did not respond (thus 28 of 30 potential hypercapnia responses occurred). By contrast, all six replicate tests with *Hypostomus* were incomplete (only 13 of 30 potential responses occurred).

Complete air-breathing responses were observed in all combined hypoxia and hypercapnia tests (Table 1). These tests elicited air breathing at a P_{w,CO_2} threshold similar to that determined separately for CO_2 , however, the corresponding P_{w,O_2} thresholds of both species were raised significantly, with that of *Hypostomus* remaining higher than *Ancistrus*.

Neither acclimation to hypoxia nor repeated and prolonged exposure to hypercapnia changed the P_{w,O_2} or P_{w,CO_2} thresholds of either species. Also comparisons of differently sized fish in each group indicated no size-related threshold differences (mass range of fish tested 19–125 g). In all hypercapnia tests, a change in P_{w,CO_2} from 2 (air equilibration) to 21 mmHg corresponded to a pH drop from 8.2 to 6.5 in the experimental aquaria.

The air-breathing threshold P_{w,O_2} determined in this study for *Ancistrus* (33 mmHg) agrees with that found by Gee (1976, 35 mmHg), but the value determined here for *Hypostomus* (60 mmHg) is much higher than determined by Gee (1976, 21 mmHg). Gee did not indicate the level of variability associated with his threshold determinations and in our experiments only one value (18 mmHg) was near the mean he reported. Thus, the very large difference in the two results is probably due to the effects of

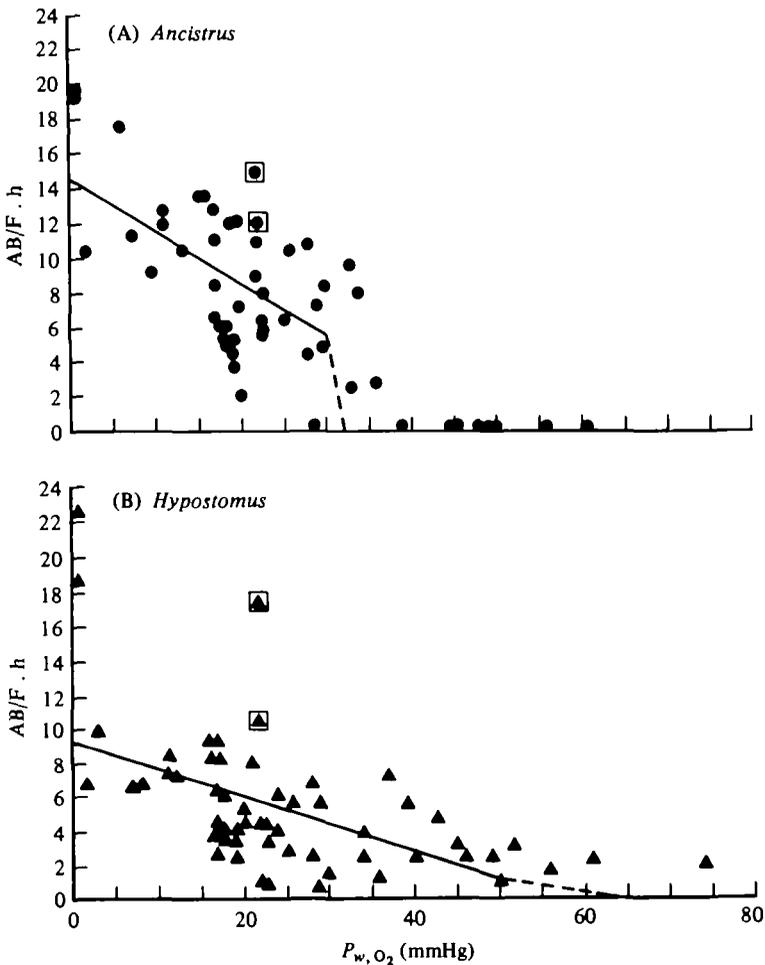


Fig. 1. Air-breathing frequencies (AB/F.h) determined for groups of *Ancistrus chagresi* (A) and *Hypostomus plecostomus* (B) in relation to P_{w,O_2} (25–27 °C). Solid lines are least squares regressions: *Ancistrus*, $y = -0.30 (P_{w,O_2}) + 14.4$, $r = -0.79$, $n = 53$; *Hypostomus*, $y = -0.16 (P_{w,O_2}) + 9.2$, $r = -0.56$, $n = 54$. Dashed line connects calculated line to mean air-breathing threshold determined for each species. Frequencies determined during feeding (squares) were not used in regression calculations.

different experimental protocols (e.g. the control of extraneous disturbances, the time between handling and testing, test group size, gas delivery rate) on air-breathing threshold in *Hypostomus*. Our experience indicates that disturbance suppresses normal air-breathing behaviour in these species.

Air-breathing frequencies

Air-breathing frequencies of both *Ancistrus* and *Hypostomus* changed with spontaneous fish activity and thus varied considerably (range 0.5–23 breaths/h) in different observation periods (Fig. 1). Comparison of dark and daylight frequencies revealed no circadian changes and within-group comparisons of small and large fish showed no effect of fish size on air breathing. Preliminary observations indicate that feeding can

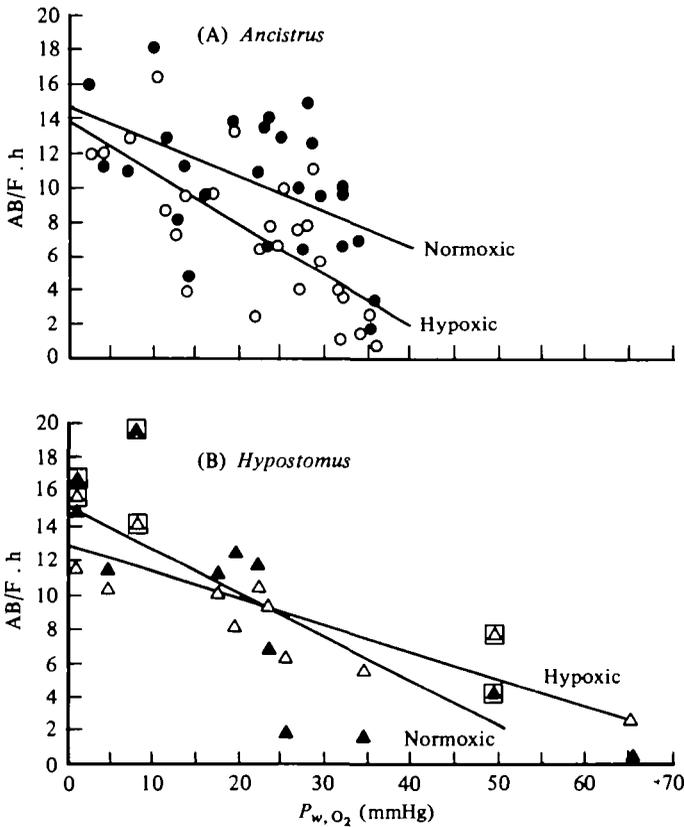


Fig. 2. (A) Air-breathing frequency in relation to P_{w,O_2} of hypoxic (○) and normoxic (●) water acclimated groups of *Ancistrus chagresi* (25 °C). Least squares regressions: hypoxic fish, $y = -0.30 (P_{w,O_2}) + 13.8$, $r = -0.72$, $n = 26$; normoxic fish, $y = -0.20 (P_{w,O_2}) + 14.6$, $r = -0.50$, $n = 26$. (B) Air-breathing frequency in relation to P_{w,O_2} of hypoxic (△) and normoxic (▲) water acclimated groups of *Hypostomus plecostomus* (25 °C). Least-squares regressions: hypoxic fish, $y = -0.16 (P_{w,O_2}) + 12.6$, $r = -0.85$, $n = 12$; normoxic fish, $y = -0.26 (P_{w,O_2}) + 15$, $r = -0.82$, $n = 12$. Frequencies determined during feeding (squares) were included in regression calculations.

increase air breathing (Figs. 1, 2); in addition, the four very high rates at 1 mmHg P_{w,O_2} (Fig. 1) were measured with some decaying uneaten food in the aquaria. This may have resulted in the release of substances that interfered with aquatic respiration and necessitated additional air breaths. Fig. 1 shows that the frequencies of both species are inversely related to P_{w,O_2} , and that, although *Ancistrus* commenced air-breathing at a lower P_{w,O_2} , its frequency was generally higher and increased more with hypoxia than did that of *Hypostomus*. In 76 paired air-breathing frequency comparisons made at or above threshold P_{w,O_2} for both species, *Ancistrus* had a higher rate 67 times compared to 5 for *Hypostomus*, with 4 ties. A chi-square single classification analysis of these results reveals a significant difference in air-breathing frequency (χ^2 , 1 degree of freedom (D.F.) = 42.8, $P < 0.05$).

Following acclimation to hypoxia, the air-breathing frequency of *Ancistrus* remained inversely proportional to P_{w,O_2} , but was reduced compared to that of fish from nor-

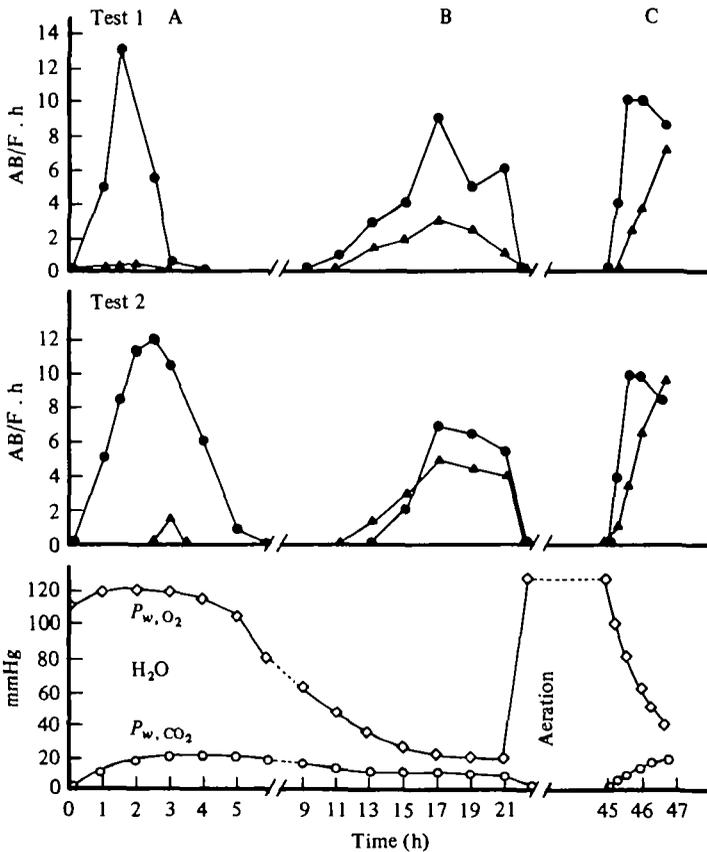


Fig. 3. Air-breathing frequencies of groups of *Ancistrus chagresi* (●) and *Hypostomus plecostomus* (▲) during two 48 h tests in which fish were exposed to progressive hypercapnia while in normoxic water (A), followed by the gradual onset of hypoxia while in hypercapnic water (B) and, following 24 h in aerated water, abrupt exposure to simultaneous hypoxia and hypercapnia (C). P_{w,O_2} and P_{w,CO_2} values during each phase are shown in the bottom panel.

moxic water (Fig. 2A). In 26 paired air-breathing rate comparisons of hypoxia and normoxia acclimated *Ancistrus*, hypoxic fish had a lower frequency in 22 tests versus only 2 for normoxic fish, with 2 ties (single classification χ^2 , 1 D.F. = 11.1, $P < 0.05$). By contrast, hypoxia acclimation did not significantly alter the P_{w,O_2} -air-breathing frequency of *Hypostomus* except at a P_{w,O_2} less than 24 mmHg (Fig. 2B), where hypoxia-acclimated fish consistently ($n = 7$) had a lower air-breathing rate than fish from normoxia. The opposite, however, was true from 24 to 65 mmHg ($n = 5$).

Fig. 3 summarizes two 48 h experiments contrasting the responses of *Hypostomus* and *Ancistrus* to progressive hypercapnia while in normoxic water, followed by the gradual onset of hypoxia while in hypercapnic water, and then to abrupt, simultaneous hypercapnia and hypoxia. Above threshold levels of hypercapnia, *Ancistrus* had a higher air-breathing frequency than *Hypostomus* (Fig. 3A), which is consistent with the observed interspecific threshold differences (Table 1), but no correlation between P_{w,CO_2} and air-breathing frequency was found for either species. Moreover, both species gradually compensated for hypercapnia and stopped all air-breathing

Table 2. Mean air-breathing frequencies (breaths/fish h^{-1}) of *Ancistrus chagresi* and *Hypostomus plecostomus* compared in hypoxic water and in combined hypoxic and hypercapnic water at 25–27 °C

(Rates were measured only when aquatic conditions had been stable for 12–24 h. Values are mean frequency \pm 95% confidence intervals, the range observed, and (N). Asterisk indicates intraspecific frequency differences are significant [$P < 0.05$] by t test. Mean (\pm S.E.) P_{w,O_2} and P_{w,CO_2} values (mmHg) are given for two ambient conditions.)

	Hypoxic water (P_{w,O_2} 22.3 \pm 3.2; P_{w,CO_2} \leq 2)	Hypoxic and hypercapnic water (P_{w,O_2} 23.1 \pm 2.7; P_{w,CO_2} 12.0 \pm 1.2)
<i>Ancistrus chagresi</i>		
\bar{X}	8.0 \pm 1.7	5.7 \pm 1.7
Range	1.8–13.6	0–11.1
N	20	17
<i>Hypostomus plecostomus</i>		
\bar{X}	5.6 \pm 1.1	2.7 \pm 0.8
Range	2.4–9.2	0–5.2
N	20	17

activity within 2–6 h of first exposure. Neither the initial air-breathing frequency nor the time required for these species to cease air breathing were significantly affected by repeated or prolonged exposure to hypercapnia.

Following hypercapnia tests, P_{w,O_2} was allowed to decline gradually (6–10 h) to air-breathing threshold levels, and both species resumed air breathing (Fig. 3 B). When this response to hypoxia occurred, *Ancistrus* again had a higher air-breathing frequency than *Hypostomus*. The air-breathing frequency of *Hypostomus* in hypoxic and hypercapnic water was in fact significantly lower than that measured in hypoxia alone (Table 2, cf. Figs. 1, 3 B). The air-breathing frequency of *Ancistrus*, although also slightly depressed in hypoxic and hypercapnic water, was not significantly different from its rate in hypoxic water (Table 2).

The added effects of hypercapnia and hypoxia on air-breathing frequency were apparent during abrupt exposure to these conditions (Fig. 3 C). Fish that had been in aerated water for 24 h or longer responded to the reduction of P_{w,O_2} and rise in P_{w,CO_2} with an air-breathing frequency that was greater than that observed during the gradual onset of these conditions (cf. Fig. 3 B, C). The air-breathing frequency found for *Ancistrus* was similar to its rate in hypercapnia alone (Fig. 3 A), but the response of *Hypostomus* was more pronounced. Both these sensitivity differences are consistent with previously described differences in air-breathing threshold (Table 1).

Temperature effects

The air-breathing threshold P_{w,O_2} of *Ancistrus* is temperature-independent from 25 to 35 °C (Fig. 4). At 15 and 20 °C, however, P_{w,O_2} is significantly increased ($P < 0.05$, t test). It is also elevated at 35 °C but not significantly different from the 25–32 °C values. *Ancistrus* did not survive very long in hypoxic water above 32 °C and frequency measurements were confined to the 20–30 °C range (Fig. 4). The air-breathing frequency increase is small from 20 to 25 °C ($Q_{10} = 1.5$), but is much larger from 25 to 30 °C ($Q_{10} = 7.2$).

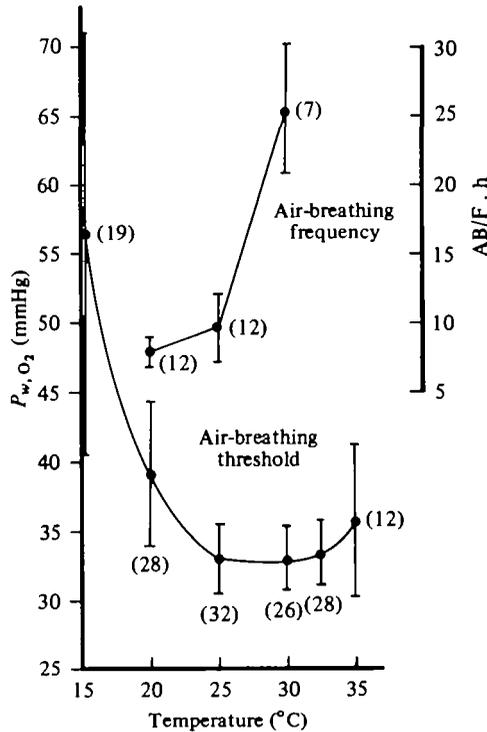


Fig. 4. Effects of temperature on the mean P_{w, O_2} air-breathing threshold and the mean air-breathing frequency of *Ancistrus chagresi*. Numbers with threshold data indicate total number of fish observed, Numbers with the frequency data are the total observation periods conducted on 5 fish. Lines are 95% confidence intervals of the mean. P_{w, O_2} ranged from 8 to 12 mmHg during all air-breathing frequency determinations.

Synchronous air breathing

Analysis of 4.5 h of air-breathing records for *Ancistrus* and *Hypostomus* reveals a significant ($P < 0.05$) temporal clumping of air breathing, which indicates that both species breathe air synchronously. No interspecific differences in synchrony level were indicated in these records. The demonstration of synchronous air breathing in *Hypostomus* contrasts with the results of Kramer & Graham (1976) and probably reflects sampling error in their study as well as the variability of the behaviour of *Hypostomus*.

DISCUSSION

Air breathing in Hypostomus and Ancistrus

This study demonstrates differences in the air-breathing responses of *Ancistrus* and *Hypostomus* that may influence microhabitat selection by these species in their stream environment. In progressive hypoxia, *Hypostomus* switches to air breathing at a higher P_{w, O_2} than *Ancistrus*, but below 33 mmHg P_{w, O_2} , the point where *Ancistrus* commences air breathing, *Hypostomus* breathes less frequently than *Ancistrus* (Fig. 1). Although 'air breathing' seems to be primarily a respiratory adaptation, *Hypostomus* does

occasionally gulp air in 'aerated' laboratory aquaria while *Ancistrus* does not. Gee (1976) suggested *Hypostomus* may utilize increased buoyancy provided by air in its stomach to facilitate movement and feeding, and in normoxic streams, this species has been observed to gulp air prior to grazing on non-supportive substrates (M. Power, pers. comm.). By contrast air breathing in *Ancistrus* appears to have an exclusively respiratory function.

The different air-breathing rates of these two species and the larger inverse correlation between air breathing and hypoxia suggest that *Ancistrus* is a less efficient air-breather (i.e. less of the inhaled aerial O_2 is actually delivered to respiring tissue) than *Hypostomus*. Gee (1976) determined that equal-sized individuals of these two species have similarly sized stomachs that, during air breathing, are totally emptied by exhalation and then filled to capacity upon inhalation. Since these species have similar routine O_2 consumption rates (J. B. Graham, in prep.) and because equivalent air volumes are swallowed, it could be expected that two equal-sized fish of each species would hold an air breath for the same time. *Ancistrus*, however, breathes air more often than *Hypostomus* and frequency differences change with P_{w,O_2} (Fig. 1).

Measurements of expired gas O_2 content are needed to compare air-breathing efficiencies. An additional aspect of the efficiency question, however, concerns the extent to which aerial O_2 that is absorbed by blood haemoglobin (Hb) in the stomach may be lost through the gills to hypoxic water before it is distributed to tissues. By virtue of 'in-series' circulation, O_2 -rich blood leaving the stomach mixes with other venous blood and circulates through heart and gills before reaching capillaries (Johansen, 1970), making a reversed O_2 diffusion gradient in gill circulation likely. Both these species continue to ventilate their gills while air breathing in hypoxic water and, because *Ancistrus* normally remains dependent upon exclusively aquatic ventilation down to a lower P_{w,O_2} , it may be less able to control branchial O_2 loss (see below), which would cause a more rapid increase in air breathing with declining P_{w,O_2} (Fig. 1).

Comparative measurements of respiration in hypoxic water have not been made, but the lower P_{w,O_2} threshold of *Hypostomus* is consistent with the hypothesis that it is a more efficient air breather than *Ancistrus*. Air-breathing species often have a diminished capacity for aquatic respiration when compared to a closely-related but less proficient air-breathing species or a related species that has no air-breathing capacity (Munshi, 1976; Singh, 1976; Graham, Kramer & Pineda, 1978; Hulbert *et al.* 1978; Johansen, Mangum & Weber, 1978). This is because the evolution of air-breathing is, in most species, tied to specializations (e.g. reduced gill area, gill by-passing shunts, reduced ventilatory musculature and changes in Hb concentration and Hb- O_2 affinity) that, while minimizing the potential for O_2 loss from gills, also reduce the scope of aquatic respiration (Johansen, 1970; Hughes, 1972, 1976; Stevens & Holeyton, 1978; Farrell & Randall, 1978, and references above). Therefore, the initiation of air breathing by *Hypostomus* at a higher P_{w,O_2} than *Ancistrus* may reflect limitations imposed on branchial respiration by the presence of specialisations favouring efficient aerial respiration once in hypoxia.

Hypoxia, hypercapnia and the control of aerial respiration

Although hypoxia acclimation enabled *Ancistrus* and, to a lesser extent *Hypostomus* to reduce air-breathing frequency in hypoxia (Fig. 2), it is surprising that this treatment did not affect the O_2 air-breathing threshold of either species. The latter finding is similar to that of Gee (1980) for the mud minnow (*Umbra*) but contrasts with results of Bicudo & Johansen (1979), who found that hypoxia-acclimated swamp eels (*Synbranchus*) 'switched on' air-breathing at a higher P_{w,O_2} than did fish from normoxia.

Since both *Ancistrus* and *Hypostomus* ventilate their gills in hypoxia it could be expected that hypoxia acclimation would result in physiological adjustments that augment both aerial and aquatic respiration (J. B. Graham, in prep). It is not known at the present time if both respiratory modes are enhanced equally, but such a balance might result in a constant O_2 -air-breathing threshold. However, another possibility is that the 'switching on' of air breathing is controlled by external O_2 receptors located in the aquatic ventilation pathway. If this were the case, then the extent to which an air-breathing species could, through adaptive processes, gain independence from aquatic hypoxia and increase its reliance on aerial respiration would be limited by the degree to which its gill function in water could be reduced (Johnsen, 1970; Hughes & Singh, 1970a, b). Hypoxia acclimated *Synbranchus*, for example, may have shifted to a greater dependence on aerial respiration, which would explain a lower O_2 threshold (Bicudo & Johansen, 1979). Due to the amphibious capabilities of this species and because its gills and buccopharyngeal epithelium are utilized for aerial respiration even while in water, *Synbranchus* may initially possess efficient alternative means of handling ancillary gill functions (Graham, 1976), which may be further refined in the course of hypoxia acclimation (Bicudo & Johansen, 1979).

Both CO_2 and pH are important regulators of air-breathing frequency in some species (Hughes & Singh, 1970a, b; Johansen, 1970; Singh, 1976). Thus, it is noteworthy that the CO_2 air-breathing thresholds observed for *Ancistrus* and *Hypostomus* (Table 1) are high compared to those recorded for other species (from 1–5 mmHg in *Amia*, *Neoceratodus*, and *Synbranchus*; Johansen, 1970). Also hypercapnia evoked only a short period of air breathing in *Ancistrus* and *Hypostomus* followed by a rapid compensation for CO_2 and the resumption of exclusively aquatic respiration. A decline in response to CO_2 was also observed in the characin *Piabucina* (Graham, Kramer & Pineda, 1977). Few other studies, however, have documented the long-term effects of hypercapnia on fish air-breathing activity. We conclude that the high CO_2 thresholds found for *Ancistrus* and *Hypostomus*, along with their ability to compensate for the effect of this gas, indicate that CO_2 is not important in the regulation of facultative air-breathing in these species.

The mechanisms of both CO_2 -initiation of air breathing and CO_2 compensation are unknown but probably involve short-term changes in Hb- O_2 affinity (Weber, Wood & Davis, 1979) and shifts in acid-base status and in the ratios of excreted bicarbonate and CO_2 (Burggren, 1979; Cameron, 1979).

Environmental and metabolic factors influencing air breathing

Although the air-breathing frequency of *Ancistrus* varied with temperature (Fig. 4), its P_{w,O_2} air-breathing threshold was temperature independent from 25 to 35 °C. For *Umbra*, a more eurythermal species, Gee (1980) found a direct relationship between temperature and P_{w,O_2} air-breathing threshold (12 mmHg at 5 °C; 42 mmHg at 30 °C). In the case of *Ancistrus* the rise in threshold P_{w,O_2} below 25 °C and the slight increase at 35 °C are probably a result of the separate effects of atypical thermal extremes on metabolic O_2 demand (higher in warm temperature) and on O_2 delivery capacity (cool temperature depresses heart rate and ventilation, and lowers diffusion rate). Air-breathing frequency typically increases with temperature (Johansen, 1970). *Ancistrus* in these tests were acclimated to 25–27 °C and the Q_{10} of the frequency change from 20 to 25 °C was very small compared to that from 25 to 30 °C (Fig. 4). In addition to the direct effect of increased temperature on metabolism, the large Q_{10} may also reflect the secondary effects of temperature on tissues and organ systems (e.g. pH changes, reduced Hb- O_2 affinity, increased heart and ventilatory activity and a lowered O_2 extraction efficiency).

Our studies with *Ancistrus* and *Hypostomus* clearly demonstrate the distinction between species that 'switch on' air breathing in response to factors that directly limit aquatic respiration (i.e. aquatic hypoxia and, to a lesser extent, hypercapnia) and species such as *Amia* (Johansen *et al.* 1970) and *Lepisosteus* (Rahn *et al.* 1971) that, independent of ambient conditions, either initiate air breathing or 'turn up' air-breathing frequency in response to a heightened metabolic O_2 demand; this is most usually caused by increased ambient temperature or activity. In his review, Johansen (1970) pointed out this distinction, but, with few exceptions (see Gee, 1980), subsequent air-breathing fish literature has been insensitive to it. In *Ancistrus* and, for the most part, *Hypostomus*, air breathing is primarily a facultative response to aquatic hypoxia. Even though temperature, P_{w,O_2} , and activity all affect air-breathing frequency, these species exclusively use aquatic respiration in normoxic water. Neither abrupt temperature changes nor typical activity levels, including feeding and aggressive interactions, will cause either species to breathe air. This is different from *Amia* and *Lepisosteus*, both of which breathe air in normoxia and elevate their air-breathing rates in hypoxia and with increased temperature or activity.

Facultative air-breathing responses in different species

Facultative air-breathing doubtlessly increases the survival probability of fishes forced to endure periodic exposure to water unsuitable for aquatic respiration. During the tropical dry season, for example, fish that typically occur in normoxic flowing streams may become trapped for weeks or months in stagnant ponds that are hypoxic and hypercapnic (Lowe-McConnell, 1975; Kramer *et al.* 1978). Moreover, the inhabitants of some tropical ponds may have to endure hypoxia every night irrespective of season (Kramer *et al.* 1978).

In recent years the category 'facultatively air-breathing fish' has been expanded by discovery of and experimentation with various species (e.g. *Piabucina*, Graham *et al.* 1977; *Hoplosternum*, Gee & Graham, 1978; *Hoplerythrinus*, Stevens & Holeton, 1978;

Umbra, Gee, 1980; *Corydoras*, Kramer & McClure, 1980) that are not obligate air-breathers, but breathe air in normoxic water and merely 'increase' air-breathing frequency in order to endure hypoxia. Our studies illustrate the important need to contrast this class of facultative air-breathing with that of species like *Ancistrus* and *Hypostomus* in terms of the scope and magnitude of the behavioural, anatomical, physiological, and biochemical adjustments that may occur during the transition to air breathing. For a normoxic air-breather, hypoxia simply requires the fish to increase the frequency of an established behaviour pattern. In the long term, adjustments that increase air-breathing capacity and efficiency (see above) probably occur. But, greater changes must occur in fishes that 'switch on' facultative air-breathing. The need to periodically swim to the surface for air is a fundamental departure in behaviour from the usual benthic habits of *Ancistrus* and *Hypostomus*, and it probably also requires the diversion of energy resources normally used for other activities (Kramer & McClure, 1981). In addition, changes in the stomach may be required to facilitate aerial respiration and these may affect feeding efficiency. Our studies demonstrate that the transition to air-breathing is followed by refinements that favour increased air-breathing efficiency. This together with a predator-avoidance capability afforded *Ancistrus* and *Hypostomus* by synchronous air-breathing (Kramer & Graham, 1976; Gee, 1980) suggest that once initiated, air breathing becomes fully integrated into the natural activities of these species.

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