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THE TRANSITION TO AIR BREATHING IN FISHES

V. COMPARATIVE ASPECTS OF CARDIORESPIRATORY REGULATION IN *SYNBRANCHUS MARMORATUS* AND *MONOPTERUS ALBUS* (SYNBRANCHIDAE)

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Summary

Extreme heart-rate lability accompanies the air-breathing cycles of *Synbranchus marmoratus* and *Monopterus albus*. When air is taken into the buccopharyngeal air-breathing organ of these fishes, heart rate increases sharply above pre-inspiration rates of 3–25 beats min^{-1} to as high as 40–45 beats min^{-1}. With time, and as O_2 is depleted from the air-breathing organ, heart rate gradually declines and drops to its lowest level with, or following, exhalation. Relationships between air breathing and sinus arrhythmia in *M. albus* were investigated by injecting variable gas volumes and O_2 mixtures into the cannulated air-breathing organ. Tests were also carried out on undisturbed fish breathing volitionally in atmospheres containing different O_2 levels. Both the volume and O_2 content of the inspired gas affect the level and duration of inspiration tachycardia. Additional factors affecting tachycardia are the heart rate prior to inspiration and the time since air was last held. *S. marmoratus* is a non-obligatory air breather and uses rhythmic branchial aquatic respiration to a greater extent than *M. albus*, an obligate air breather. While the heart rates of both species are increased during aquatic ventilation, the higher heart rate to ventilation ratio in *S. marmoratus* (2–3 *versus* approximately 1 in *M. albus*) seems attributable to its more proficient aquatic respiratory system. The available cardiorespiratory data for air-breathing fishes indicate that the scope of air-inspiration tachycardia is smaller in lungfishes and other primitive species than in most teleosts. This difference is mainly attributable to the greater chronotropic effect of sympathetic cardiac innervation in teleosts.

Key words: *Synbranchus marmoratus*, *Monopterus albus*, Synbranchidae, air-breathing fish, heart rate, sinus arrhythmia, tachycardia, bradycardia.

Introduction

Previous papers in this series have examined diverse aspects of the physiology of facultative and continuously air-breathing fishes. These works quantified the influence of aquatic O_2 tension ($P_{W}O_2$) on the thresholds for air breathing (Graham and Baird, 1982), established that hypoxia acclimation favourably affects aerial respiratory efficiency by modulating air-breathing frequency, haemoglobin O_2-affinity and air-breathing organ (ABO) volume (Graham, 1983), and demonstrated that both $P_{W}O_2$ and body size influence respiratory partitioning and the efficacy of branchial, cutaneous and ABO gas-exchanging surfaces (Graham and Baird, 1984; Graham *et al.* 1987). The objectives of the present study are to define the relationships between air-breathing and heart activity in two species of the family Synbranchidae, *Synbranchus marmoratus* Bloch and *Monopterus albus* (Zuiew), and to compare cardiorespiratory interactions among the air-breathing fishes.

Synbranchids occur circumtropically in freshwaters. Commonly referred to as swamp, or rice, eels (however, they are not real eels which are in the family Anguillidae), these fishes make extensive mud burrows, are protogynous hermaphrodites and use their modified buccopharyngeal chamber as an ABO (Taylor, 1831; Johansen, 1966; Liem, 1963; Rosen and Greenwood, 1976; Bicudo and Johansen, 1979). The ABO surface is covered by a vascular epithelium, and in *Monopterus* (an Asian genus) the gills are reduced to such an extent that this fish is an obligatory air breather (Lomholt and Johansen, 1974, 1976). Some species (e.g. *M. cuchia*) even have auxiliary respiratory sacs within the buccopharynx (Taylor, 1831; Munshi *et al.* 1989).

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Synbranchus (a neotropical genus), in contrast, has a complete set of gills and is not an obligatory air breather (Johansen, 1966; Graham *et al.* 1987). Other synbranchid specialisations for air breathing include a reduced, ventrally positioned, single opercular slit and several longitudinal buccal–branchial tissue folds that permit marked ABO expansion during air breathing (see Fig. 1A).

In most vertebrates, regulation of cardiac activity occurs through balanced inputs from the cholinergic (parasympathetic) and adrenergic (sympathetic) autonomic nervous system. Increased vagal (cholinergic) tone usually reduces both heart rate and contractility, whereas reduced vagal tone or an increase in adrenergic activity often increases both variables. Pronounced cardiac arrhythmias during air breathing have been documented in a number of fish species (Johansen *et al.* 1968*a*; Singh and Hughes, 1973; Lomholt and Johansen, 1976; Farrell, 1978; Smatresk, 1988, 1990). The most common pattern of arrhythmia is an inspiration-induced tachycardia followed by the gradual onset of bradycardia as the O_2 content of the breath falls, leading to full bradycardia with exhalation. This pattern appears to optimize ventilation and perfusion relationships by elevating blood flow to the ABO just after a breath is taken and then gradually attenuating flow as O_2 is depleted (Johansen, 1966, 1970).

Repetitive cycles of air-breathing tachycardia and ABO-deflation bradycardia have been demonstrated in both *Synbranchus* and *Monopterus* (Johansen, 1966; Lomholt and Johansen, 1974, 1976; Roberts and Graham, 1985). Several classes of receptor input are thought to contribute to the integration of aerial respiration and sinus arrhythmias in fishes, and recent studies have implicated mechanoreceptors within the ABO or along the airway (Milsom, 1990; Smatresk, 1988, 1990). The response levels of the heart-regulating mechanoreceptors have not, however, been defined for most air-breathing fishes. Also, few studies have considered whether the quality of the inspired gas affects the time course of sinus arrhythmia during the breath-hold. In this paper, we demonstrate ABO mechanoreceptor effects on the cardiorespiratory regulation of *S. marmoratus* and *M. albus* and present evidence that an ABO O_2 receptor influences instantaneous air-breath tachycardia and other cardiorespiratory variables.

Materials and methods

Specimens of *S. marmoratus* (150–960 g) were collected from streams in the Republic of Panama. Specimens of *M. albus* (40–700 g) were purchased in a Singapore fish market and from commercial dealers in San Diego and San Francisco, California. All fish were maintained in aerated laboratory aquaria (at 24–27 °C) and fed about every 4 days with pieces of cut mackerel and squid. Experiments with *S. marmoratus* were conducted on six fish between 1984 and 1987. With access to relatively few specimens, the work with *S. marmoratus* was more qualitative than for *M. albus* for which 18 specimens were studied between 1988 and 1993.

Specimen preparation

Fig. 1A illustrates the placement sites of electrocardiogram (ECG) and branchial electrodes and the branchial cannulae. To make these attachments, fish that had been starved for at least 48 h were anaesthetised in cool (8–10 °C) water for 15–30 min and then placed on a wet surgical table and covered with cool, wet towels. Pairs of stainless-steel or copper (30 and 36 AWG) electrode wires were implanted *via* small (22–25 gauge) hypodermic needles onto the ventral wall of the branchial chamber and adjacent to the heart (Fig. 1A), secured by self-tying skin loops and stitches, and directed dorso-anteriorly from the body. Branchial tubes (PE 50 or PE 90), with heat-flared and smoothed tips and side ports, were passed through the ventral branchial chamber wall *via* a hypodermic puncture, secured with two stitches, and directed anteriorly. Two cannulae were used so that simultaneous withdrawal and injection tests could be carried out and also to reduce the possibility that the thick tissue layers in the branchial folds might occlude the cannula tips and thus prevent experimental ABO-volume changes.

About 30 min was required to instrument a fish. After surgery, fish were placed in one of two different experimental chambers (Fig. 1B,C) and allowed 48 h for recovery and chamber acclimation. Experimental data were obtained over 3–6 days following recovery, during which time the fish was not fed. ECG and ventilation signals were amplified on either d.c. or impedance circuits and sent to a Gould 2400 chart recorder. In later studies, long-term records were stored on reel-to-reel magnetic tape. After the experiments had been completed, each fish was anaesthetised by chilling, all wires, catheters and sutures removed, and wet body mass determined.

Effects of changes in ABO volume and the O_2 content of the ABO gas

Controlled ventilation chamber

Fish fitted with both electrode sets and the branchial chamber tubes were slipped gently and tail first into a slightly inclined horizontal tube (Fig. 1B). The front of the tube was then sealed with a rubber stopper into which were mounted two stainless-steel hypodermic needles that were connected to the branchial PE tubes on the inside of the chamber and to syringes on the outside. The electrode wires were fed out between the chamber wall and the stopper. The tube was filled with water that was recirculated by an oscillating pump (Fisher 13-874-32) operated at low voltage. An O_2 electrode (Yellow Springs Instrument Co., no. 5750) was placed in the loop to monitor $P_{W_{O_2}}$, which was kept at, or below, 4 kPa to ensure that fish would remain heavily reliant upon air-breathing. For most tests, this system was maintained between 25 and 27 °C by thermostattically controlled water heaters.

Inclining the tube slightly (Fig. 1B) enabled positioning of an air pocket in the anterior top section of the chamber, just over the head of the fish. This was convenient in preventing gas bubble entrainment in the circulating water loop during experiments and it additionally provided air access for the fish,

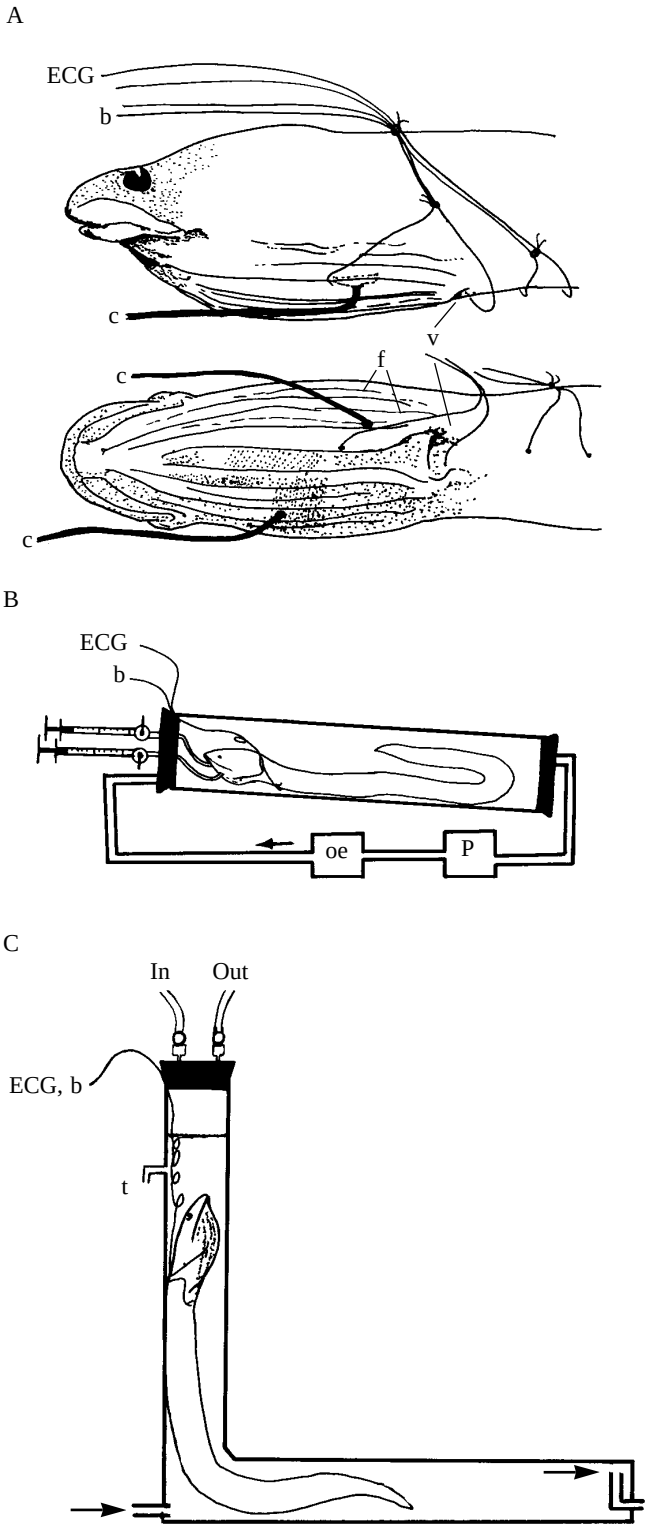

Fig. 1. (A) Lateral and ventral views of the head of *Synbranchus marmoratus* showing the positions of the branchial chamber cannulae (c) and the branchial (b) and ECG electrodes. Also shown are the small, ventral opercular valve (v) and longitudinal tissue folds (f) in the floor of the branchial chamber. (B) The horizontal tube system showing the connection between the branchial cannulae and syringes used for the 'push–pull' and 'volume overflow' tests. The branchial (b) and ECG electrodes were passed between the chamber wall and stopper. The arrow shows the direction of the water flow through the pump (p) and the oxygen electrode (oe). (C) L-shaped tube design. Gases of known P_{O_2} were circulated through the open space at the top of the chamber *via* the 'in' and 'out' ports in the rubber stopper. The pressure transducer port (t), a few centimetres below the water level, was connected to a Statham gauge which monitored water-head pressure to indicate when air breathing occurred (see text and Fig. 2). The electrodes attached to the fish were passed through the stopper. Water circulation indicated by arrows.

thus reducing stress levels as well as the chance of accidental suffocation (*M. albus* is an obligatory air breather). Inclination also aided breath exhalation, which is carried out most effectively when the fish can raise its snout to about 45 ° with respect to the horizontal plane (Graham and Baird, 1984). Inclination also lessened the tendency of the fish to roll up on and kink its wires and tubes, although this occasionally happened.

Experiments

The objective of these tests was to determine how heart rate was affected by ABO inflation and deflation and whether the O_2 content of the ABO gas affected heart rate. For *S. marmoratus*, volume effects were tested by incremental injections of small (1–3 ml) volumes of air or gas of known O_2 content at regular intervals. These began at 'zero' ABO volume (i.e. the fish was 'between' air breaths, had no gas in its ABO and had a low heart rate) and continued until the organ was full, as indicated by a distended branchial chamber and expanded longitudinal tissue folds, and by the 'overflow' of gas from the opercular slit during injection. This series was followed by a stepwise volume withdrawal and the sequence was then repeated.

Several techniques were employed to ensure that the injections began at zero ABO volume. First, previous experiments showed that, provided there is sufficient room for *S. marmoratus* to raise its head to an angle of 45 ° or more, this fish will void all gas from its ABO during a normal expiration (Graham and Baird, 1984). Also, no gas is present in the ABO of *S. marmoratus* during long periods of aquatic ventilation (Graham *et al.* 1987). Preliminary tests with *M. albus* also indicated complete ABO emptying and the absence of residual ABO gas. Thus, by beginning experiments after expirations, the assumption of a 'zero volume' ABO was reasonable. Also, with careful placement of the catheter in the ventral wall of the branchial chamber, we could extract most of the injected gas, and this could be verified with a volume measurement. Nevertheless, it is likely that a fraction of the injected gas was not retrieved. In cases where the unrecovered volume was suspected of being excessive (20 %) or where we were not certain that the ABO was gas-free, the zero volume state could be established by purging the ABO with a large bolus of water and postponing the experiments for 1 h, during which breathing behaviour was continually monitored.

For *M. albus*, two types of experiments were carried out to

determine the effects of gas quality on air-breath tachycardia. First, 'volume-overflow' studies tested the effects of O_2 levels in a fully inflated organ. This procedure incorporated a series of large volume injections (see below) of gas of known O_2 content, over a relatively short period (2 min). An earlier study on *S. marmoratus* (Graham and Baird, 1984) had established the quantitative relationship between ABO volume (v in ml) and body mass (m in g) as:

$$\log v = -0.825 + 0.737 \log m , \quad (1)$$

and values determined from this equation are similar to ABO volume data for *M. albus* (Lomholt and Johansen, 1974). From this regression, it was possible to calculate the injection volume required to overflow the ABO and thus to keep it at, or near, maximal inflation (as indicated by equation 1, this volume varied with fish body size). The injections began when the fish was post-expiration (ABO empty, low heart rate), four injections were given at 2 min intervals, and heart rate was measured 1 min after injection.

A second set of experiments, the 'push–pull' tests, was designed to measure the effects of different O_2 levels in a relatively constant ABO volume. This was done by causing an abrupt change in ABO O_2 content in the absence of a change in ABO volume. First, the ABO was partially filled by injecting enough gas (about 75 % of maximum v, see equation 1) to accelerate the heart while not fully distending the ABO. An additional volume (20 % of maximum v) of experimental gas was then injected through one branchial cannula and the same volume was withdrawn (either simultaneously or immediately following injection) from the second cannula and heart rate was determined. After the test, the initial gas volume was withdrawn (i.e. leaving the ABO completely, or nearly, empty) and, after waiting for 1 min, another gas injection and push–pull test were carried out.

Cardiac responses following volitional inspiration of mixed gases

Test chamber

Experiments were also carried out using an L-shaped tube (Fig. 1C) that was placed in a waterbath. The water in this tube was slowly circulated and oxygen levels monitored. As above, $P_{W_{O_2}}$ was kept below 4 kPa to ensure dependence on the aerial oxygen supply. Tests were carried out at water temperatures between 23 and 27 °C. Electrode leads, with sufficient slack to permit free vertical movement by the fish, were passed up through the vertical arm. From about 2 cm below the water level, the tube was completely wrapped in black plastic and the fish often retreated to this area. Tests with *S. marmoratus* carried out in this tube included the placement of a peritoneal catheter for determination of the effect of atropine (1 mg kg^{-1}) on cardiorespiratory interactions.

Experiments

For tests of cardiac responses to different gas mixtures, the vertical tube was sealed with a rubber stopper containing in- and out-flow ports so that a controlled, mixed-gas respiratory atmosphere could be maintained above the water surface (Fig. 1C). These tests were carried out on *M. albus* fitted with ECG and ventilation electrodes but without branchial cannulae. Connection of a Statham pressure transducer to the pressure port (Fig. 1C) enabled continuous monitoring of the pressure change caused by air breathing (i.e. inspiration inflated the ABO and thus displaced water and raised hydrostatic pressure; Fig. 2). Data obtained with this system allowed investigation

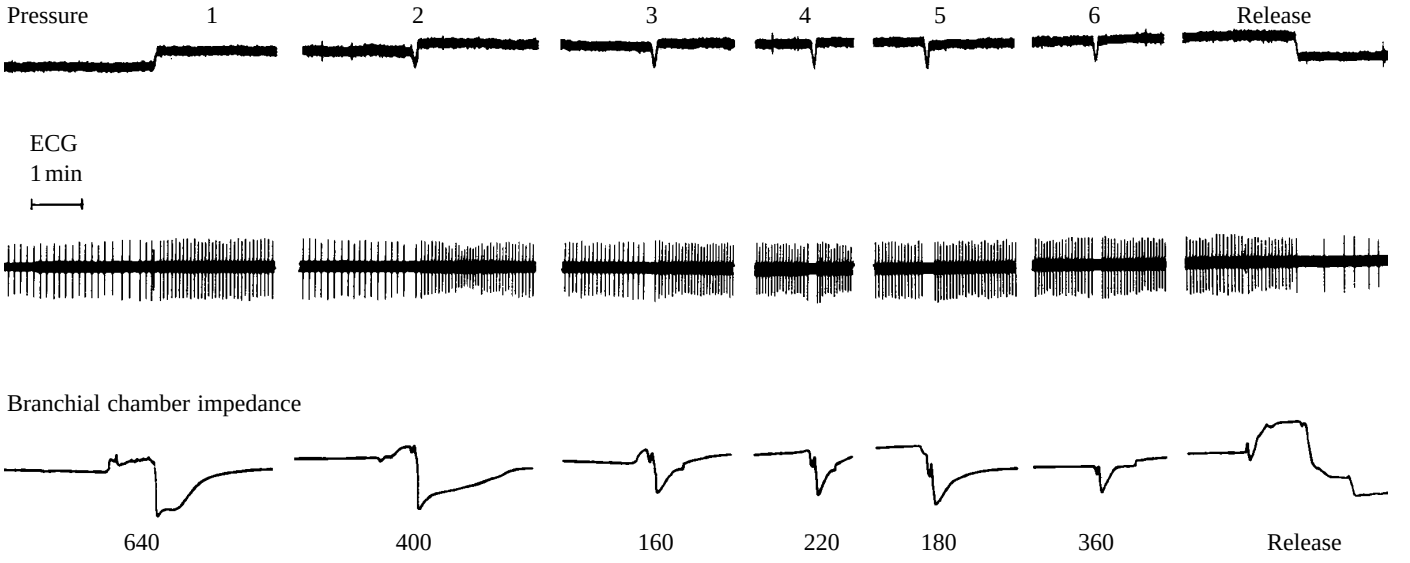

Fig. 2. Sections of a 33 min record showing six air breaths by a 560 g *Monopterus albus* in the L-shaped tube (Fig. 1C). Numbers below each panel indicate breath duration (s). The relative pressure trace (top) shows the step increase in water depth associated with inspiration followed by a series of five 'V-shaped' exhalation–inhalation breathing cycles and then a final decrease with air-release. The ECG trace (middle) shows the arrhythmia coincident with inflation and deflation. The impedance record (lower trace) registers displacement of the branchial chamber wall with air-breathing events (T=25 °C).

of both instantaneous and long-term features of mixed-gas inspiration, without the disturbance associated with the close proximity of an investigator, manual gas injections or a variable injection rate or volume. A Wösthoff pump (Bochum, Germany) was used to mix air with either N_2 or O_2. Experimental atmospheres in these tests ranged from 100 to 1.5 % O_2, and even 100 % N_2 was used for short periods.

Results

Synbranchus marmoratus

Fig. 3 shows simultaneous branchial and ECG signals recorded for *S. marmoratus* while air breathing in normoxic (air = 21 % O_2), hypoxic (10.5 % O_2/89.5 % N_2), and hypercapnic (10 % CO_2/90 % air) atmospheres. Sequential spikes in the branchial records indicate exhalation of a breath followed by inhalation. For these data, the inter-breath interval (IBI), the time between the release of old and the taking of new air, is relatively short (5–40 s). Depending upon conditions, the IBI for *S. marmoratus* can vary considerably (Johansen, 1966). Graham and Baird (1984) measured a mean IBI of 15 min for this fish; however, the observational range extended from 1 to 42 min. Air exhalation can also lead directly to the onset of aquatic ventilation (see below).

The ECG records for *S. marmoratus* (Fig. 3) show a slowing of the heart just prior to breath release, a brief interval of heart stoppage, and tachycardia following inspiration; the pattern first described by Johansen (1966). Fig. 3 also shows that inspired gas quality affects both the extent and duration of the inspiration tachycardia. Under normoxic conditions (Fig. 3A), breaths were held for longer times and supported a greater range of cardiac activity than breaths taken in hypoxia (Fig. 3B). Exposure to hypercapnia for 2 h or longer (Fig. 3C) resulted in a more variable, but usually shorter-duration, air-breathing pattern and a greater degree of arrhythmia.

S. marmoratus frequently utilises aquatic ventilation (Graham *et al.* 1987) and Fig. 4 shows the transition from air breathing to aquatic ventilation. At the onset of aquatic ventilation, the heart rate was relatively fast and the ratio of heart rate to ventilation was 3.6. As ventilation rate increased, this ratio fell to 2.4 (Fig. 4). A 1 mg kg^{-1} dose of atropine, a cholinergic antagonist, accelerated the heart rate of *S. marmoratus* and obliterated the arrhythmic pattern associated with normal air breathing (Fig. 4B). Administered *via* the peritoneum, atropine began to have an effect within 1 h and its influence on the heart remained strong for nearly 20 h. Air-injection experiments (Fig. 4C) revealed that heart rate is dependent upon ABO volume. Withdrawal of 3 ml of gas from the ABO caused bradycardia; however, before additional withdrawals could be made, this fish spontaneously ejected all the ABO gas, which slowed the heart to its pre-inflation bradycardic level.

Monopterus albus

M. albus utilises aquatic ventilation to a lesser extent than *S. marmoratus* and has a lower heart rate to ventilation ratio. Fig. 5 compares heart rate during aquatic ventilation and air breathing. These data were obtained using the system described in Fig. 1C and, during the aquatic respiration phase, the fish positioned its head just below the water surface and thus ventilated in more oxygenated water. The ventilation rate of this fish was similar to that of *S. marmoratus* (Fig. 4A); however, the ratio of heart rate to ventilation was smaller (above 2.0 in *S. marmoratus* and approximately 1.0 in *M. albus*).

Features of the air-breathing sinus arrhythmia in *M. albus* are described in Table 1 and Figs 2 and 6. Table 1 shows mean data for 10 sequential and spontaneous air breaths recorded for five fish under an air atmosphere (25–27 °C). This table expresses the relative increase in post-air-breath heart rate in terms of the sinus arrhythmia index (SAI) which ranged from 48 to 117 % for these five fish. Table 1 also shows that, even though there was not much variability among the five fish in terms of average breath duration (5–9 min), the individual variation was quite large, as in *S. marmoratus*.

Fig. 2 shows the ECG record for a 560 g *M. albus* immediately before, and after, a series of six volitional air breaths (in normoxia) spanning 33 min. Also shown in the record are step changes in water pressure and the branchial activity associated with ABO ventilation. This figure demonstrates the cumulative effect of sequential air breaths on heart rate. The first breath was taken after a 35 min IBI, during

Table 1. *Sinus arrhythmia data for five* Monopterus albus *measured over 10 sequential air breaths in normoxic water*

Individual	Mass (g)	Pre-air-breath heart rate (beats min^{-1})	Inspiration heart rate (beats min^{-1})	SAI (%)	Exhalation heart rate (beats min^{-1})	Mean breath duration (s)
1	450	18 (6)	39 (6)	117	22 (9)	287 (115)
2	690	20 (7)	36 (5)	80	33 (4)	423 (51)
3	150	22 (9)	41 (6)	86	31 (9)	498 (149)
4	190	25 (8)	37 (6)	48	27 (6)	368 (74)
5	220	21 (9)	42 (8)	100	26 (7)	551 (138)

SAI is the sinus arrhythmia index and is expressed as a percentage increase in heart rate with inspiration. For fish 1, the calculation is 100(39–18)/18=117 %.

Values are means (S.E.M.) for five fish over 10 sequential breaths; T=25–27 °C.

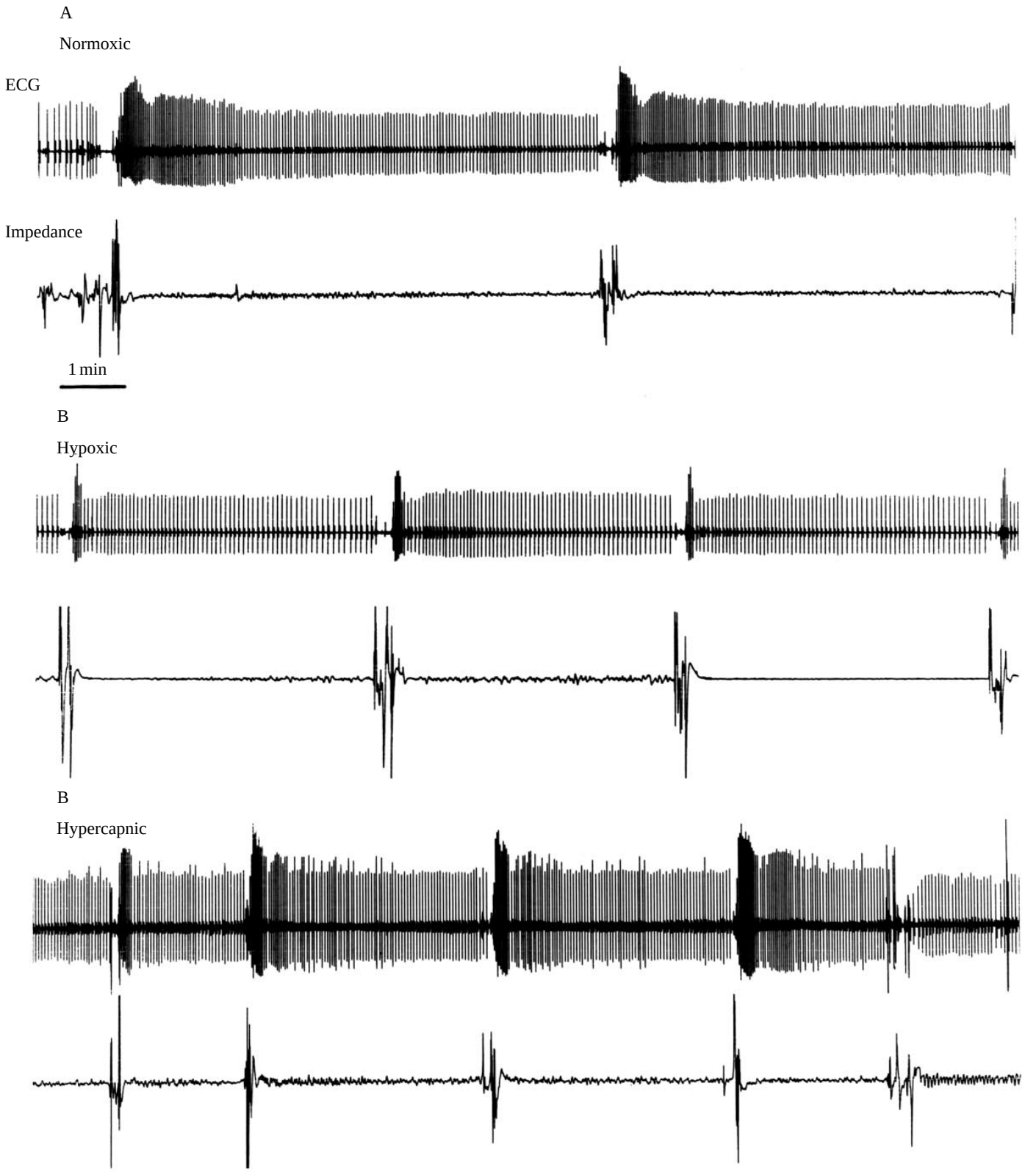

Fig. 3. ECG and branchial impedance activity records for a 260 g *Synbranchus marmoratus* air breathing in (A) normoxic (21 % O_2), (B) hypoxic (10.5 % O_2) and (C) hypercapnic (10 % CO_2, 90 % air) atmospheres in the L-shaped tube shown in Fig. 1C. At the time records were taken, the fish had been air breathing in the specified atmosphere for several hours. The time scale (1 min) shown between A and B applies to all three records (T=27 ˚C).

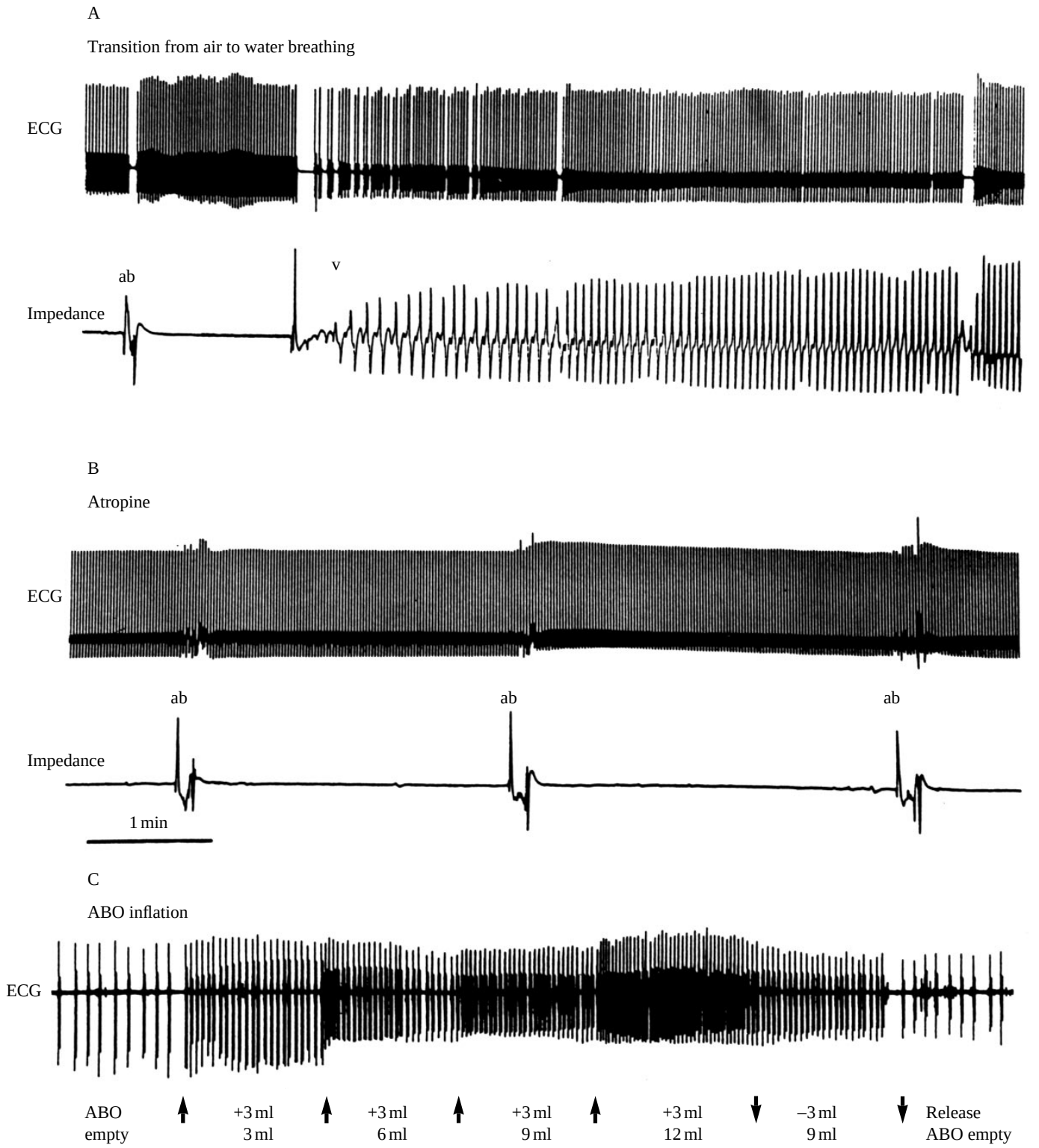

Fig. 4. ECG and branchial chamber impedance records for a 390 g *Synbranchus marmoratus*. The 1 min time scale shown under trace B applies to both A and B. (A) The effect on heart rate of a spontaneous transition from air breathing to gill ventilation. The impedance trace shows a typical air-breath signal and the associated cardiac acceleration (ab). Cyclic aquatic gill ventilation (v) begins about 80 s after the air breath and results in a lower level of tachycardia than followed the air breath. After 5 min of aquatic ventilation, the maximum ventilation rate is about 18 ventilations min^{-1} and the ratio of heart rate to gill ventilation is 2.4. (B) ECG and branchial impedance records showing air breathing (ab) following atropinisation (1 mg kg^{-1}). (C) Effect on heart rate of four manual air injections (3 ml each) into the air-breathing organ (ABO), followed by the withdrawal of 3 ml, and then the spontaneous release of all ABO gas by the fish. Numbers below the trace indicate injected and cumulative volumes at each step (T=27 °C). On the basis of equation 1, the estimated ABO volume of this fish was 12 ml.

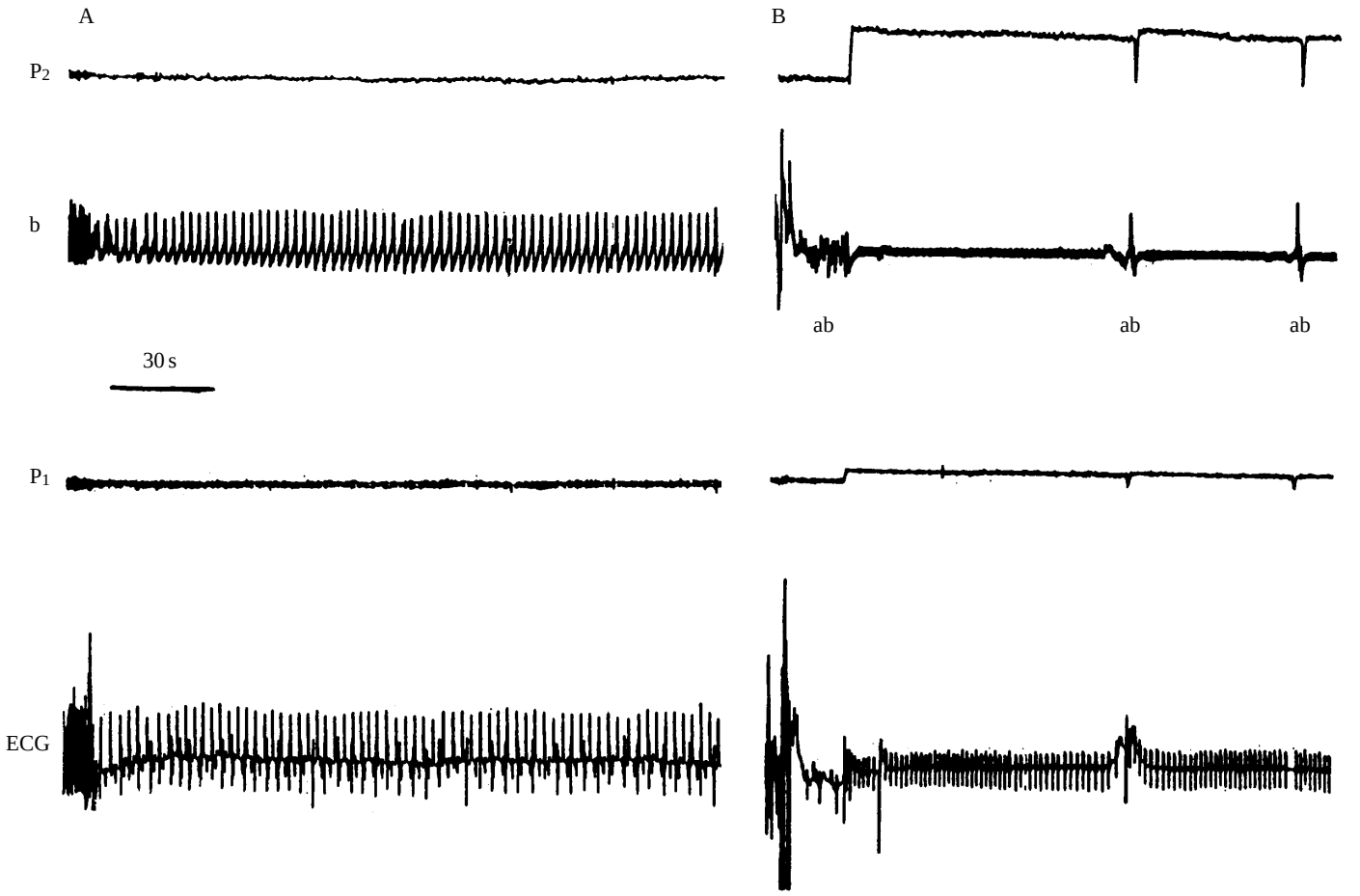

Fig. 5. (A) Records of aquatic branchial ventilation (b), the ECG and relative water-head pressure (P_1) in the L-shaped tube (Fig. 1C) for a 40 g *Monopterus albus*. Also shown is a simultaneous amplified pressure signal (P_2). Note that the pressure traces are stable because the fish was not air breathing. At maximal ventilation rate (23 ventilations min^{-1}), the heart rate to ventilation ratio is 1.0. (B) Record during air breathing showing episodic branchial movements and corresponding pressure oscillations with the initial, second and third air breaths (ab) in a series (see Fig. 2) (T=27 °C). Scales for time, relative pressure and ECG are the same in both A and B.

which there was no aquatic ventilation. This breath was held for 640 s and was followed immediately by breath 2 (400 s). Heart rate prior to the first breath was about 7 beats min^{-1}, abruptly increasing to 14 beats min^{-1} (SAI=100 %) when breath 1 was taken. Tachycardia following breath 2 peaked at about 18 beats min^{-1} and by breath 6 tachycardia just after inspiration was 21 beats min^{-1}. Thus, there was a steady rise in the mean heart rate over the six-breath sequence. The pressure record indicates that breaths 2, 3 and 4 were of a larger volume than the others. Although our techniques did not allow quantification of this difference, the possibility that this larger volume affected heart rate is unlikely as the relatively smaller-volume breaths (5 and 6) were accompanied by higher heart rates. Upon the expiration of breath 6, heart rate fell abruptly to 6 beats min^{-1}.

Additional aspects of the sinus arrhythmia of *M. albus* are shown in Fig. 6, which covers almost 3 h of air breathing and three complete air-breath and IBI cycles. Although both air-breath and IBI durations vary, the effects of inspiration, time and exhalation on heart rate are clearly evident.

Overflow injections of gases with different O_2 levels were given over a short period (8 min) in order to assess the effect of the oxygen content of ABO gas on heart rate. Fig. 7 gives the results for a 690 g *M. albus* (individual 2, Table 1) given four 20 ml injections at 2 min intervals. The injection series began with the ABO empty and with initial heart rates that varied between 5 and 12 beats min^{-1}. In all cases, heart rate increased with the first injection; however, except for 10 % CO_2, the relative increase was directly proportional to the amount of O_2 contained in the injected gas. Similarly, with the exception of the 10 % CO_2 injection, and allowing for differences in the pre-injection rates, heart rate following the final overflow injection in each series was directly proportional to the O_2 content in the injected gas. That O_2 content was, in fact, determining maximum heart rate in these tests is demonstrated by the pronounced tachycardia resulting from the overflow injection of 20 ml of pure O_2 into the previously N_2-inflated ABO (Fig. 7). Similar mixed-gas effects on heart rate were verified in the eight other fish tested.

Table 2 shows results of a push–pull test carried out on a *M.*

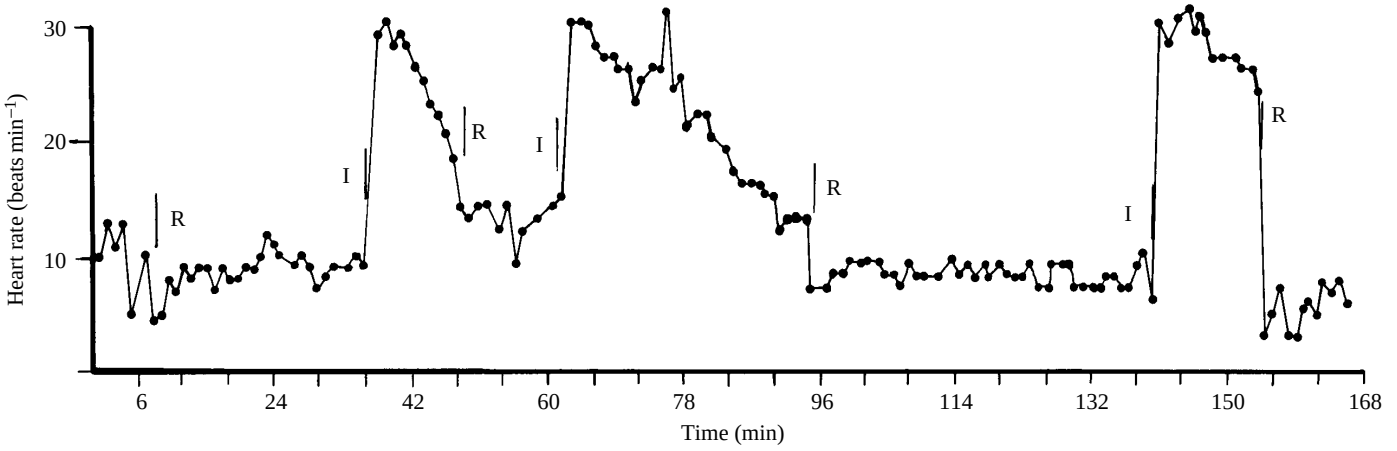

Fig. 6. A 3 h record of heart rate and air breathing in a 170 g *Monopterus albus*. Vertical lines indicate times of air-breath inspiration (I) and release (R) (*T*=23 °C).

albus (tests of this type were completed on four fish). In this series, 3 ml of experimental gas was injected and the same volume was then immediately withdrawn (i.e. organ volume expanded for 10 s) and the ensuing heart responses were monitored over six 10 s intervals. The ABO was then deflated and, after 1 min, another gas was injected. Table 2 indicates that the small, brief inflation pulse initiated tachycardia, but that the extent to which tachycardia developed depended on both the pre-inflation heart rate and the relative O_2 content of the injected gas. In five of the seven injections shown in Table 2, tachycardia developed within 10 s of gas injection. In these cases, pre-injection rate was 18–24 beats min^{-1}, whereas in the two cases where tachycardia did not develop, pre-inflation heart rate was 30–36 beats min^{-1}. The effect of gas quality can be seen from the heart rates at 20 and 30 s. In three of the four cases where the injection contained less than 21 % O_2 (i.e. injection of N_2 or re-injection of 'used' air), heart rate began to decrease by 20 s after injection. This contrasts with the tachycardia induced by injection of air and pure O_2, which required about 30 s to develop maximally.

The rapidity of the onset of a gas-quality component of air-breath tachycardia in *M. albus* was investigated with the apparatus shown in Fig. 1C. Fig. 8, based on experiments carried out over a period of several days with three specimens, shows that inspired-breath O_2 content affects both breath duration and heart rate. A reduction in inspired O_2 content

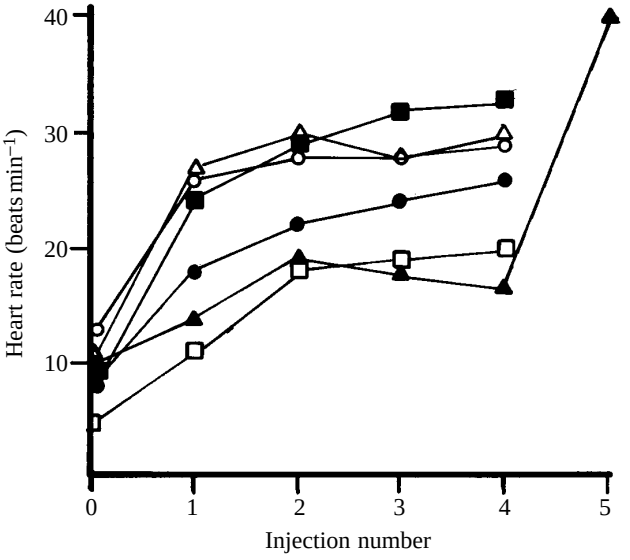

Fig. 7. Effects on heart rate of a series of four 20 ml overflow injections of gases containing different proportions of O_2 into the ABO of *Monopterus albus* (690 g, estimated ABO volume 19 ml). Experimental gases are: air (filled squares) and pure N_2 (filled triangles). Mixtures are 18.9 % O_2 (open triangles), 15.7 % O_2 (open circles), 10.5 % O_2 (filled circles) and 90 % air/10 % CO_2 (open squares). Injection 5 shows the effect of replacing N_2 with O_2 (*T*=25 °C).

Table 2. *Cardiac responses of* Monopterus albus *following the nearly simultaneous injection/withdrawal (push–pull method) of ABO gases containing different amounts of* O_2

	Instantaneous heart rate (beats min^{-1})						
		Time post-injection (s)					
Gas sequence	Pre-breath	10	20	30	40	50	60
Air	18	36	36	42	42	42	36
'Used' air	36	36	30	30	24	24	24
Air	18	30	30	36	30	30	24
'Used' air	24	36	36	30	24	30	30
N_2	30	30	24	24	18	24	18
N_2	18	24	18	18	18	18	18
O_2	18	36	36	42	36	36	42

Gases were delivered in the order shown.

Instantaneous heart rates are indicated for the pre-injection state and over each 10 s interval of the first minute following gas manipulation.

Data are for fish 3 in Table 1; *T*=25–27 °C.

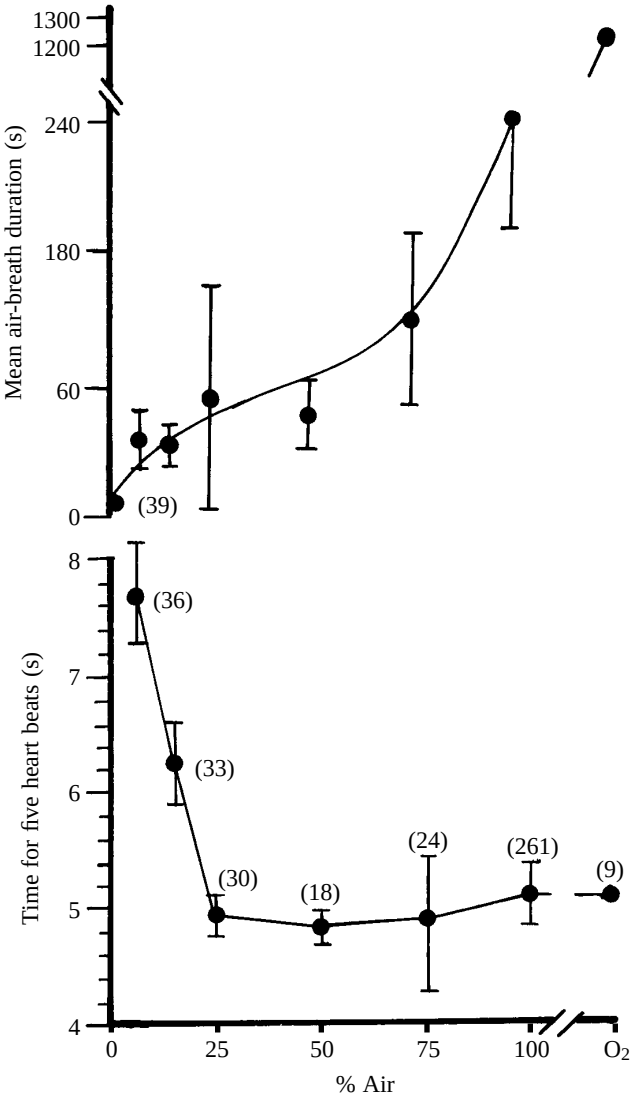

Fig. 8. Effects of different air-phase O_2 levels on the mean air-breath duration and instantaneous heart rates of three *Monopterus albus*. Sample sizes are given in parentheses. Values are means ± S.E.M. (T=24–26 °C).

causes a general decline in mean breath duration, but post-inspiration tachycardia (defined here as the time required for the first five heart beats) was unaffected by oxygen levels as low as 5.2 %. Inspiration of both 3.1 and 1.5 % O_2, however, caused the immediate (1–2 s) behavioural response of air ejection and affected air-breath tachycardia (Fig. 8). Many of the breaths taken in 1.5 % O_2 were released before 10 heart beats had occurred (11–27 s), and exposure to pure N_2 greatly slowed the heart and, in most cases, the gas was not held for long enough to record more than two or three heart beats.

Discussion

Cardiorespiratory integration in the Synbranchidae

In fishes, as in most vertebrates, heart rate depends upon the balance between inhibitory vagal (cholinergic) inputs and stimulatory (adrenergic) effects of either sympathetic nerve fibres or circulating catecholamines (Laurent *et al.* 1983; Taylor, 1987; Farrell and Jones, 1992). Vagal inhibition can affect both chronotropic and inotropic cardiac action and may be elicited by stress stimuli, by hypoxia or by input from both mechano- and chemoreceptors. Excitatory heart stimulation can occur as a result of reduced vagal tone, an increase in the central-vessel blood volume (i.e. by Starling's law), the action of blood-borne catecholamines or the direct effect of adrenergic nerve (sympathetic innervation) activity.

From experimental manipulation of ABO-gas volume and content, and by allowing fish to breathe air as required without disturbance in experimentally determined gas mixtures, this study has demonstrated the presence of volume- and O_2-mediated ventilatory sinus arrhythmia in both *S. marmoratus* and *M. albus.*

Volume effects

The presence of a volume-mediated cardiac arrhythmia in these two synbranchids is in agreement with previous studies on this group (Johansen, 1966; Lomholt and Johansen, 1974). In the air-breathing teleosts that have been studied to date (summarised in Table 3), ABO deflation elicits bradycardia and reduces ABO perfusion while spontaneous or experimental ABO inflation initiates tachycardia, increases ABO perfusion and reduces aquatic ventilation. In both *S. marmoratus* (Johansen, 1966) and *M. cuchia* (Lomholt and Johansen, 1974), heart rate directly affects ABO perfusion because blood ejected from the heart flows into the ventral aorta and to the branchial arches and buccopharyngeal epithelium (Rosen and Greenwood, 1976). In most species, the transduction of ABO-volume change occurs *via* mechanoreceptors sensitive to either wall stretch or displacement. Cardiorespiratory integration is mediated by the interconnection of the ABO and the cardiac and respiratory control systems *via* vagal loops consisting of sensory and motor tracks (Taylor, 1987; Smatresk, 1988, 1990).

Oxygen effects

Evidence for an O_2-dependent component of cardiac regulation in *M. albus* has been obtained in this study. The overflow experiments examined the effect of the O_2 content of ABO gas on heart rate at maximum ABO volume and demonstrated (Fig. 7) that a series of gas deliveries, given over a short period, have an additive effect on heart rate. However, both the relative increase in heart rate following the first injection and heart rate after the final delivery were strongly influenced by gas O_2 content. The effect of 18.9 % O_2 (i.e. 90 % air) on heart rate was diminished by the addition of 10 % CO_2. Also, continued exposure to N_2 over the four-injection sequence resulted in an absence of tachycardia, an effect that was rapidly reversed by O_2 injection (Fig. 7).

The 'push–pull' experiments had the objective of changing ABO-O_2 level while minimally affecting ABO volume. It can be seen in Table 2 that slight volume changes taking place during this technique would trigger a tachycardia, but that the

Table 3. *Heart-rate responses to air intake in aquatic air-breathing fishes*

Species	Temperature (°C)	Heart rate (beats min^{-1}) Pre-air-breath	Heart rate (beats min^{-1}) Post-air-breath	Reference
Neoceratodus forsteri	18	14	18[1]	Johansen *et al.* (1968*a*)
Protopterus aethiopicus	25	34 (2.1)	34 (2.0)[2]	Johansen and Lenfant (1968)
	25	33	36[1,3]	Johansen *et al.* (1968*a*)
	25	36	36	Szidon *et al.* (1969)
Lepidosiren paradoxa	27	29[4]	32	Axelsson *et al.* (1989)
Lepisosteus oculatus	18–21	35	37[5]	Smatresk and Cameron (1982)
	–	60	70	Smatresk (1988)
Amia calva	25–30	33	34[6]	Johansen *et al.* (1970*a*)
Arapaima gigas	26–30	34 (11)	34 (10)	Farrell (1978)
Electrophorus electricus	28	28	66	Johansen *et al.* (1968*b*)
	–	14	30	Johansen *et al.* (1970*b*)
Hoplerythrinus unitaeniatus	26–30	61 (12)	82 (23)	Farrell (1978)
Ancistrus chagresi	25	110	150	Graham (1983)
Clarias batrachus	25	30	39	Jordan (1976)
Monopterus cuchia	20	14–16	34–38[1]	Lomholt and Johansen (1976)
	28–30	12	70	
Monopterus albus	25	9 (2.1)	31 (2.5)	This study
	30	17 (1.6)	55 (1.2)	
Synbranchus marmoratus	20–22	6	27	Johansen (1966)
	–	21	35	Johansen *et al.* (1970*b*)
	25	5–10	40	Roberts and Graham (1985)
Anabas testudineus	25	30	57[7]	Singh and Hughes (1973)

Values in parentheses, where given, are S.E.M.

The species are arranged in phylogenetic order and, beginning with *Arapaima*, all listed species are teleosts.

[1]Data obtained by manual air inflation/deflation.

[2]Data compiled from reference given, Figs 2, 10 and 12.

[3]Lung deflation reduced heart rate from 18 to 12 beats min^{-1} (see Fig. 15 of reference).

[4]Resting heart rate. Rates following vasoactive drug administration were: atropine 32 beats min^{-1}, propranolol 25 beats min^{-1}.

[5]Pre- and post-air-breath differences not significant.

[6]Data compiled from reference, Fig. 11.

[7]Rates and amount of increase varied with activity and water O_2 content.

intensity and duration of this response were ultimately determined by gas quality and heart rate prior to gas injection. Observations of freely air-breathing *M. albus* also indicated an effect of inspired O_2 on breath duration and showed that fish would void severely hypoxic and anoxic breaths within a few seconds of inspiration (Fig. 8). The presence of an ABO chemosensor is suggested by the rapidity of this gas-voiding reflex, which was about 2–4 times faster than would be expected if stimulation had occurred *via* remote vascular receptors located downstream from the branchial chamber (Eclancher, 1975; Eclancher and Dejours, 1975).

The effect of branchial-volume displacement on heart rate could also be demonstrated by injecting a moderately large bolus of water into the branchial chamber of *M. albus* between air breaths. In tests on seven fish, water injection elicited a tachycardia and this tachycardia was greater and was sustained for longer when hyperoxic as opposed to hypoxic water was injected. These observations suggest that an aquatic-O_2 sensing system, analogous to the one operating in air, has the capacity to affect heart rate. It is entirely possible that the same O_2 chemosensors could function in both water and air; however, this cannot be verified without further information regarding sensor morphology, location and development.

Fishes that respire aquatically have the capacity to monitor inspired $P_{W_{O_2}}$ with externally facing sensors located in the anterior branchial arches (Taylor, 1987; Smatresk, 1988; Milsom, 1990). Thus, it is not surprising that a functional air-breath O_2 sensor would occur in synbranchids because of their use of the buccopharyngeal cavity as an ABO. Our results for *S. marmoratus* and *M. albus* suggest that the ABO-O_2 receptor transduces information about inspired gas quality (O_2 content), which influences the intensity of the tachycardic response to inflation as well as the rate of bradycardia during the breath-hold.

Both *S. marmoratus* and *M. albus* normally reside in mud burrows that can be hypoxic (J. B. Graham, personal observation). These fish may also gulp air from enclosed gas pockets under floating vegetation. In such circumstances, the ABO-O_2 receptor could monitor the O_2 content of each breath and elicit a breath-voiding response if required. Pronounced effects on the heart rate and breath-voiding were found at O_2 levels between 1.5 and 3.1 %, but it is unlikely that *S. marmoratus* and *M. albus* would consistently encounter air oxygen levels this low. Thus, the ABO-O_2 sensor may be important in the modulation of mechanoreceptor and other stimuli affecting air-breath tachycardia, in attenuating tachycardia as breath P_{O_2} declines and, ultimately, in terminating the breath when the P_{O_2} drops to a level unfavourable for O_2 transfer to blood (Graham and Baird, 1984).

Additional aspects of heart control

In addition to the effects of inspired (or injected) gas volume and quality, this study has shown that inspiration tachycardia can be influenced by the length of time that a breath is held in the ABO and the heart rate prior to gas introduction. Even

when not air breathing, *S. marmoratus* and *M. albus* engage in long periods of branchial apnoea (see also Lomholt and Johansen, 1974; Graham *et al.* 1987). Bradycardia accompanies branchial apnoea (Figs 4A, 5A), and aquatic ventilation triggers tachycardia. In the latter case, stimulation of the heart would appear to result from the cyclic deformation of the branchial apparatus during ventilation and this contrasts with air breathing, where a single ABO inflation (wall stretching) event triggers tachycardia (Fig. 4C). Our data indicate that these two different volume-change transduction events have a similar effect on heart rate although, in both cases, the normoxic stimulation of peripheral chemoreceptors would also be an important factor in sustaining tachycardia during air breathing and aquatic ventilation. Similarly, differences in the heart rate to ventilation ratio found here for *M. albus* and *S. marmoratus* may reflect differences in the exchange capacity of their aquatic respiratory surfaces as well as variables such as P_{WO_2} and metabolic demand.

Comparative aspects of cardiorespiratory interaction

In contrast to our findings for synbranchids, in the electric eel *Electrophorus electricus*, ABO gas quality does not affect heart activity. Johansen *et al.* (1968*b*) reported no difference in the extent of tachycardia elicited by the injection of either pure O_2 or N_2 into the ABO of *E. electricus*. As with the synbranchids, *E. electricus* uses its buccal chamber as an ABO and retains a reduced, although functional, branchial epithelium. While branchial tissue atrophy might explain the lack of a specific response to either N_2 or O_2 in *E. electricus*, Johansen *et al.* (1968*b*) based their conclusions on only a few records made on specimens recovering from anaesthesia, which may have desensitised possible peripheral chemosensory responses. The records reported were too brief to determine whether centrally mediated receptors in either the blood or myocardium were subsequently affected.

A marked difference in the cardiac response to air breathing can be seen for lungfishes (*Neoceratodus*, *Lepidosiren*, *Protopterus*) and to some extent for the garfish (*Lepisosteus*) relative to the teleosts (Table 3, note that *Arapaima* and all of the species listed below it in this table are teleosts). Although air-breath-initiated changes in pulmonary and systemic blood flow are well documented for lungfish (Johansen *et al.* 1968*a*; Szidon *et al.* 1969), the role played by air-breath tachycardia in these fishes appears to be minor. The difference between lungfishes and teleosts could be attributable to differences in the extent of cholinergic inhibition. However, it seems more likely that this difference is a phylogenetic consequence of the divergent patterns of cardiac regulation that exist among air-breathing fishes. Lungfishes, for example, lack sympathetic cardiac innervation entirely, and there is only limited sympathetic innervation to the heart of *Lepisosteus* (Laurent *et al.* 1983).

The need exists for additional comparative studies controlling for the many variables likely to influence pre- and post-air-breath heart rate. Table 3 does, nevertheless, suggest a phyletic difference, based on innervation pattern, in the cardiac responses to air breathing. It further suggests that these differences have resulted in radically different vasomotor responses to ABO inflation. At one extreme is the highly integrated vasomotor control system of *Lepidosiren* and *Protopterus*, which regulates cardiac output to the nearly separate and parallel pulmonary and systemic circulations, in phase with the air-breathing cycle (Johansen *et al.* 1968*a*; Szidon *et al.* 1969). In contrast to the teleosts, most of which have an in-series ABO to systemic blood flow, these lungfish modulate the balance between pulmonary and systemic circulation by changing peripheral resistance.

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