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BIOMODAL GAS EXCHANGE DURING VARIATION IN ENVIRONMENTAL OXYGEN AND CARBON DIOXIDE IN THE AIR BREATHING FISH *TRICHOGASTER TRICHOPTERUS*

BY WARREN W. BURGGREN*

The University of British Columbia, 2075 Wesbrook Mall, Vancouver, B. C., Canada V6T 1W5

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SUMMARY

Gas exchange in the gourami, *Trichogaster trichopterus*, an obligate air breather, is achieved both by branchial exchange with water and aerial exchange via labyrinth organs lying within the suprabranchial chamber.

Ventilation of the suprabranchial chamber, $\dot{M}_{O_2}$, $\dot{M}_{CO_2}$, gas exchange ratios of both gills and labyrinth organs, and air convection requirements have been measured under conditions of hypoxia, hyperoxia or hypercapnia in either water or air.

In undisturbed fish in control conditions (27 °C), air breathing frequency was 12 breaths/h, gas tidal volume 30 μl/g, total oxygen uptake 5·2 μM/g/h and total carbon dioxide excretion 4·1 μM/g/h, indicating a total gas exchange ratio of approximately 0·8. The aerial labyrinth organs accounted for 40% of oxygen uptake but only 15% of carbon dioxide elimination.

Hypoxia, in either inspired water or air, stimulated air breathing. Total $\dot{M}_{O_2}$ was continuously maintained at or above control levels by an augmentation of oxygen uptake by the labyrinth during aquatic hypoxia or by the gills during aerial hypoxia. Hypoxia had no effect on $\dot{M}_{CO_2}$ partitioning between air and water. Hypercapnia in water greatly stimulated air breathing. About 60% of total $\dot{M}_{CO_2}$ then occurred via aerial excretion, a situation unusual among air breathing fish, enabling the overall gas exchange to remain at control levels. Aerial hypercapnia had no effect on air breathing or O_2 partitioning, but resulted in a net aerial CO_2 uptake and a decrease in overall gas exchange ratio.

Trichogaster is thus an air breathing fish which is able to maintain a respiratory homeostasis under varying environmental conditions by exploiting whichever respiratory medium at a particular time is the most effective for O_2 uptake and CO_2 elimination.

INTRODUCTION

Most interest in the physiology of the air breathing fishes has centred upon the relative gas exchanging performances of the gills and of the aerial exchange organ,

*Present address: Zoology Department, University of Massachusetts, Amherst, Ma. 01003, U.S.A.

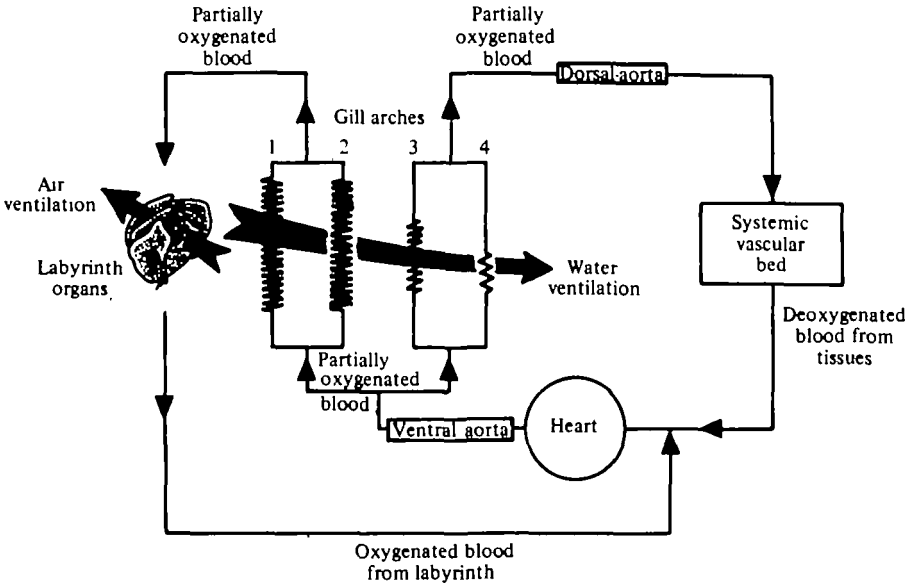

Fig. 1. Diagrammatic representation of the circulation of the air breathing fish *Trichogaster trichopterus*.

which may be a buccopharyngeal structure, opercular cavity and gill elaborations, a modified swimbladder or combinations of these and other organs (see Johansen, 1970; Munshi, 1976 for reviews). Partitioning of O_2 and CO_2 transfer between air and water is highly variable between species, and is a function of gill and aerial organ surface area, blood–water and blood–air diffusion barriers, the ventilation–perfusion ratios of the individual organs and subunits, and the ability of the respective respiratory media to serve as an O_2 source and CO_2 sink.

The variability and extremes on both a seasonal and daily basis of respiratory gas pressures in stagnant water habitats of air breathing fishes are well documented (Dehadrai & Tripathi, 1976), and are often advanced as major factors in the adaptational significance of bimodal breathing in fishes. Yet, it is not clear for many air breathing fish whether marked changes in the performance of the various respiratory organs can occur to fully or partially compensate for gas exchange in a hypoxic or hypercapnic medium. That is, can air breathing fish maintain a respiratory homeostasis in a dynamic environment by turning to whichever respiratory medium is at that time most effective for O_2 uptake and CO_2 excretion, or is the respiratory performance of their gas exchange organs fixed within fairly broad physiological and morphological limits? Ventilation of both the gills and air breathing organs of some air breathing fish have been shown to change, often disproportionately, after experimental hypoxic or hypercapnic exposure in one of the two respiratory media (see Hughes & Singh, 1970*a*; Wood & Lenfant, 1976; Farrell & Randall, 1978). However, a comprehensive investigation of actual gas exchange partitioning between respiratory organs and its quantitive relationship to tidal air ventilation and its control has not been made for a single species under regulated and varying environmental conditions.

The present investigation reports on bimodal gas exchange in the blue or opa

gourami, *Trichogaster trichopterus*. *Trichogaster*, like *Anabas*, *Macropodus*, and *Betta*, is an Anabantidae, whose air breathing modifications typically take the form of so-called labyrinth organs. These organs derive from the epibranchial regions of the first and second branchial arches, and extend dorsally as shelly, plate-like organs to fill the upper region of the opercular cavity, or suprabranchial chamber (Munshi, 1968; Peters, 1978). Fig 1 depicts the organization of the circulation in the gourami. All efferent blood from gill arches 1 and 2 enters the labyrinth complex, and all oxygenated blood draining the labyrinth joins into the jugular veins and flows on to the heart (Henninger, 1907; Munshi, 1968). Hence, blood in the ventral aorta is partially oxygenated, a condition typical of many air breathing fish (Satchell, 1976). Only the efferent vessels of gill arches 3 and 4 merge to form the dorsal aorta. Gill arches 1 and 2 in most Anabantidae are large and are fully developed, whereas gill arches 3 and particularly 4 are much reduced in size and filament numbers (Munshi, 1968; Burggren, unpublished).

The gourami continuously ventilates its gills with water, and never ventures onto land. However, it is an obligate air breather at temperatures above 20–25 °C (Das, 1927; Burggren, unpublished) and will quickly show signs of distress if denied access to air.

METHODS

Experiments were performed on 15 adult blue gouramis, *Trichogaster trichopterus* (mean mass 7·97 ± 1·87 g), which either were hatched and reared in the laboratory or obtained from local suppliers. The fish were maintained at 27 °C in de-ionized Vancouver tap water for at least 1 month before experimentation. All experiments were carried out at 27° ± 1/2 °C on fish which had been fasting for at least 24 h.

Air breathing frequency and expired and inspired gas volumes were determined in a miniaturized, adapted version of an apparatus described by Lomholt & Johansen (1974). Individual fish were placed in a glass vessel (vol 931 ml), the top of which consisted of an inverted funnel open to the atmosphere. When the vessel was filled with water, surface access for air breathing was limited to an area 10–12 mm in diameter in the stem of the funnel. Upon surfacing, the inspiration of gas caused an increase or decrease, respectively, in the volume of water displaced by the fish. Since the funnel lumen was the sole opening of the water chamber to the atmosphere, breathing movements caused a displacement of water up or down the funnel stem (and hence a change in the hydrostatic pressure head) which was proportional to tidal volume. A Grass PT4 A volumetric pressure transducer writing out on a Bausch and Lomb VOM 7 chart recorder was connected via a cannula to the water chamber. The internal membrane in this transducer is extremely compliant compared to conventional pressure transducers, and produces a very large output signal in response to small changes in volume. Thus, while changes in the pressure head of the system produced by emptying and filling of the fish's suprabranchial chamber were too small to be accurately measured by conventional means, these small pressure heads caused comparatively large volume changes in the volumetric transducer and hence in its output signal. The apparatus was calibrated for volume changes by using a syringe to simply add or withdraw a known volume of water from the chamber. Calibration lines were constructed by producing volume changes from 0 to 300 μl in 20 μl increments. Volume changes as

small as 5 μl could be detected with this transducer, so the 75–500 μl tidal volumes of the gourami could be readily measured. An overestimation of tidal volume by this technique would result if any portion of the fish's head or body was raised above the surface during the ventilatory act. In undisturbed gouramis, however, only the mouth breaks the water meniscus (Peters, 1978) so errors in tidal volume from this source were negligible. The water chamber contained a magnetic stirring bar, and was immersed in a thermostatted water bath screened from the investigator. Fish were allowed to acclimate to the apparatus for at least 18 h before experiments were begun.

Air or gas mixtures delivered from a Wösthoff gas mixing pump continuously ventilated the funnel mouth. Aquatic gas partial pressures were controlled by bubbling appropriate gas mixtures through the water. Because of the very small diameter of the surface access hole and the extreme sensitivity of the transducer to changes in water level, gas mixtures could not be bubbled through the water during actual periods of ventilation monitoring. However, the water volume was sufficiently large and the individual 1 h monitoring periods short enough so that large changes in water gas tensions due to aquatic respiration did not occur during the course of the experiments (see below). The air–water interface had a very small surface area and control measurements in the apparatus without the presence of a fish revealed that significant diffusion of respiratory gases across this interface did not develop during the measurement periods.

In a second series of experiments oxygen uptake ($\dot{M}_{O_2}$) and carbon dioxide excretion ($\dot{M}_{CO_2}$) in μM/g/h by both the gills (plus any skin contribution) and the labyrinth organs of individual fish were simultaneously determined. An air-tight glass vessel (vol. 255 ml) was fitted over the inverted funnel at the top of the water chamber. By measuring the changes in gas partial pressures of both the liquid and gas phase occurring in this closed system during bimodal gas exchange by a gourami, total $\dot{M}_{O_2}$ and $\dot{M}_{CO_2}$ as well as their partitioning between gas and water were calculated. Water and air from the respirometer were sampled with gas-tight glass syringes, and gas partial pressures in both respiratory media were determined with Radiometer O_2 and CO_2 electrodes connected to a modified Radiometer 27 or Beckman 160 gas analyser.

Respirometry of this type can be problematic for several reasons. If respiration by the fish is allowed to continue for a long period of time in a closed system such that large and easily measurable changes in gas partial pressures occur, then the changing quality of the respiratory media may begin to affect directly respiratory performance. Thus, partial pressure changes in the respiratory media should be kept small. The capacitances of water and air for CO_2 and air for O_2 are large, while the O_2 capacitance for water is small. Hence, disproportionate changes in O_2 and CO_2 partial pressures will occur, especially with large imbalances in gas exchange partitioning by the fish. With the above considerations in mind, 1 h periods of bimodal respiration were chosen for the experimental measurement period, while in between periods the respirometer was left open and the water equilibrated with air. A 1 h period of closed respirometery during bimodal respiration usually resulted in an average decrease in P_{O_2} of 15–25 mmHg in water and 1–2 mmHg in gas, and an average increase in P_{CO_2} of 0·6–1·2 mmHg in water and 0·2–0·4 mmHg in gas.

To measure accurately these very small changes, the scales on the gas analysers were considerably expanded. The outputs from Wösthoff gas mixing pumps were

cascaded to provide humidified calibration gases in 0·5 mmHg partial pressure increments, and the electrodes were calibrated between each hour measurement period. The accurate measurement of small differences in P_{CO_2} is particularly difficult, and can be complicated by the properties of the gas electrodes themselves (Boutilier *et al.* 1978). Several P_{CO_2} electrodes and electrode solutions were considered before one of exceptional stability and responsiveness was located. This electrode was fitted with a Teflon rather than silicon rubber membrane for additional stability. Further sources of error in $\dot{M}_{CO_2}$ measurement can arise from conversion of P_{CO_2} to CO_2 content using a solubility factor for distilled water if the fresh water contains carbonates (Dejours, Armand & Verriest, 1968). However, total alkalinity as $CaCO_3$ in the tap water used in all experiments was less than 2·5 ppm, so the solubility values given for carbonate-free water were used in all calculations.

Labyrinth ventilation frequency and tidal volume were determined over a range of (1) either water or gas hypoxia and hyperoxia (P_{O_2} 37, 75, 150 and > 600 mmHg) and (2) either water or gas hypercapnia (P_{CO_2} 0, 15, 30 and 45 mmHg). $\dot{M}_{O_2}$ and $\dot{M}_{CO_2}$ were determined at a set level of gas or water hypoxia (P_{O_2} 54 mmHg) or hypercapnia (P_{CO_2} 21 mmHg). In each experiment, unless specified, at least air was available for labyrinth ventilation or the water ventilating the gills was air-equilibrated; ie. fish were not simultaneously exposed to hypoxia and/or hypercapnia in both respiratory media.

Significance levels of all data were assessed with Student's *t* test for independent means, and fudicial level of $P < 0{\cdot}05$ was chosen for differences of means.

RESULTS

Undisturbed *Trichogaster trichopterus* under control conditions surfaced to breathe approximately once every 4–6 min (Fig. 2), with a mean apnoea length of $4{\cdot}7 \pm 2{\cdot}2$ min. The ventilatory act consisted first of breaking the water surface with the mouth, followed immediately by the expiration of gas from the suprabranchial chambers. After rapidly inspiring the fish left the surface, the entire process of labyrinth ventilation requiring less than $\frac{1}{2}$ sec and often taking only $\frac{1}{8}$ sec. In normoxic conditions *Trichogaster* never took more than a single breath before leaving the water surface. The reader is referred to Peters (1978) for details of ventilation mechanics in the gourami.

Inspired volumes during control conditions were approximately 27–32 μl/g (Figs. 3 and 4), and were 11–15% greater than expired volumes. Considerable differences in suprabranchial chamber gas volume between sucessive interbreath intervals occurred, as evidenced in the variation in the base-line volume level at the start of different expirations (Fig. 2). The progressive change toward a smaller suprabranchial chamber volume during the interbreath interval resulted from a proportionately greater labyrinth oxygen removal than carbon dioxide addition (see below). All values of suprabranchial chamber tidal volume (V_s in μl gas/g) reported below have been calculated on the basis of inspired gas volumes, measured as the volume change from the point of maximum expiration to the point at which inspiration was terminated (Fig. 2).

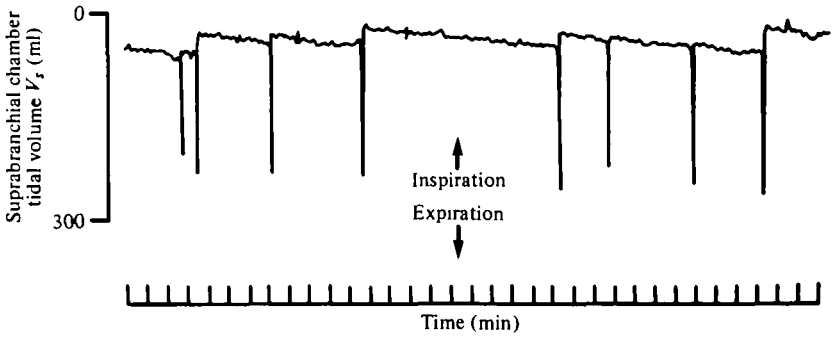

Fig. 2. Representative records of suprabranchial chamber air ventilation during control conditions in an 8·3 g *Trichogaster trichopterus*. Time marker in minute intervals.

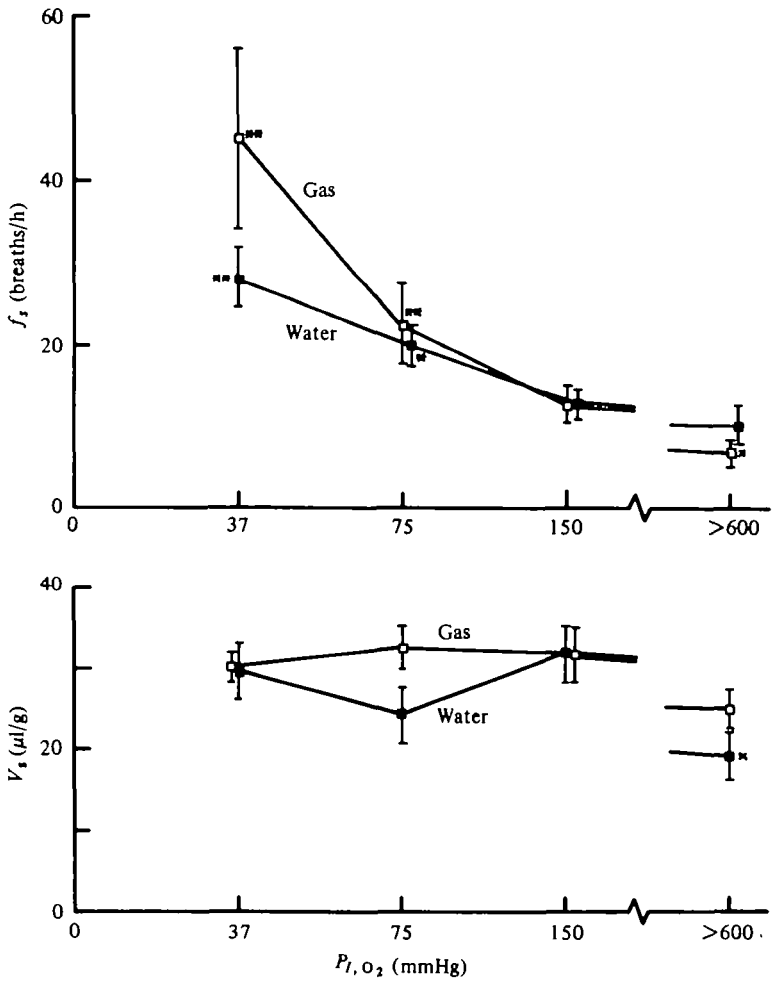

Fig. 3. Effect of changes in inspired water or air oxygen partial pressure on air breathing frequency and suprabranchial chamber tidal volume. Mean values ± 1 S.E. determined in seven fish are given. Where mean values are significantly different from control levels a single asterisk ($0{\cdot}025 \leq P < 0{\cdot}05$) or double asterisk ($P < 0{\cdot}025$) indicates the level of significance.

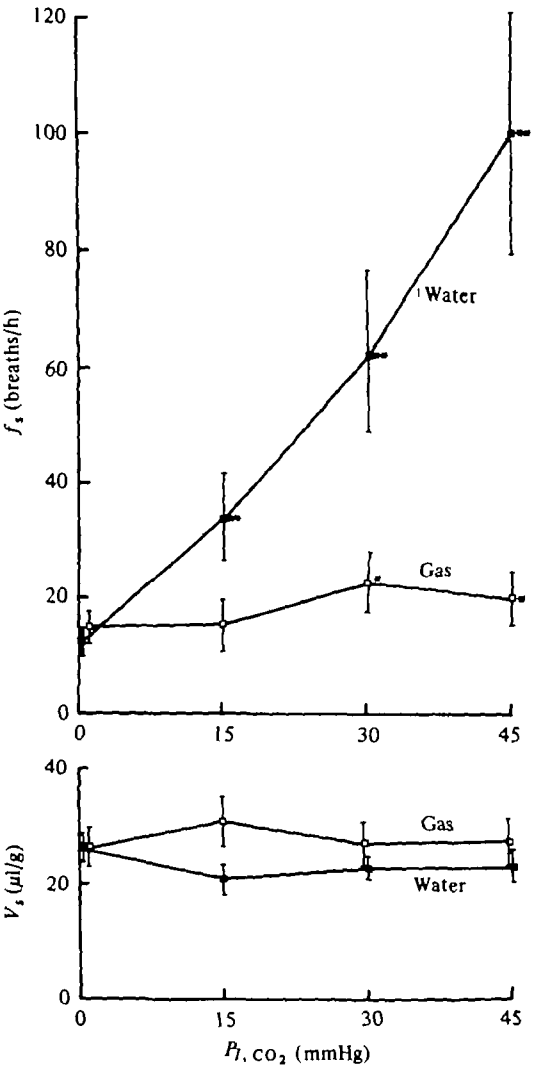

Fig. 4. Effect of changes in inspired water or air carbon dioxide partial pressure on air breathing frequency and suprabranchial tidal volume. Mean values ± 1 S.E. determined in seven fish are given. Significance coding as described in legend to Fig. 3.

Aerial ventilation responses

(1) *Hypoxia and hyperoxia*

A reduction in the inspired oxygen partial pressure (P_{I,O_2}) of either gas or water caused a stimulation of air breathing, particularly at the lowest oxygen levels (Figs. 3, 5). At a water P_{I,O_2} of 37 mmHg breathing rate was more than double control levels, and was almost trebled when gas at this same partial pressure was inspired. Although severe hypoxia proved a stimulus to aerial breathing frequency, gas tidal volume of the suprabranchial chamber showed no significant change even with the most profound hypoxic conditions in either water or air. Total ventilation of the suprabranchial

chambers ($\dot{V}_g$ in μl gas/g/h) increased slightly as the P_{I,O_2} of water or air fell to approximately 75 mmHg, and then at progressively lower levels of O_2 began to increase at a greater rate, particularly when the suprabranchial chamber was being ventilated with hypoxic gas.

The inspiration of hyperoxic gas produced a significant reduction in aerial breathing frequency from normoxic levels, whereas a non-significant change accompanied the inspiration of hyperoxic water (Fig. 3). V_g fell slightly during water hyperoxia, but did not change significantly during gas hyperoxia. The net effect was that $\dot{V}_g$ decreased by one-half during the inspiration of hyperoxic gas, but showed a non-significant reduction during irrigation of the gills with hyperoxic water.

(2) *Hypercapnia*

The inspiration of hypercapnic gas had no effect on ventilation of the labyrinth organs in *Trichogaster* until a P_{CO_2} of approximately 30 mmHg had been reached (Figs. 4, 5). At and above this level of hypercapnia significant but small increases in breathing frequency occurred. V_g showed no significant changes during progressive hypercapnia, and consequently total suprabranchial chamber ventilation volume remained unchanged from control levels even during the inspiration of gas with a P_{CO_2} as high as 45 mmHg (Fig. 4). In contrast, ventilation of the gills with only mildly hypercapnic water caused a profound increase in air breathing frequency, and at a water P_{CO_2} of 45 mmHg breathing frequency had increased over control values by nearly 700% (Fig. 4). V_g was unchanged from control levels even at high levels of CO_2 in the water, but as a consequence of the enormous increase in breathing frequency, $\dot{V}_g$ more than doubled with every 15 mmHg increase in water P_{CO_2} (Fig. 5).

(3) *Hypoxic and hyperoxic hypercapnia*

The inspiration of water with both a P_{O_2} of 60 mmHg and P_{CO_2} of 15 mmHg greatly stimulated air breathing in *Trichogaster*. $\dot{V}_g$ under these conditions was 1672 ± 360 μl/g/h, compared to 550 and 650 μl/g/h during comparative levels of solely water hypoxia or hypercapnia, respectively. This increase in $\dot{V}_g$ during hypoxic hypercapnia was largely the product of an increase in breathing rate to over 60 breaths/h.

Experiments were designed to test the effect on ventilation of the suprabranchial chamber produced by an increase in water P_{CO_2} to 29–33 mmHg, both when fish had free access to air, and then during the inspiration of pure oxygen gas and water with a P_{O_2} of greater than 600 mmHg. Whereas aquatic hypercapnia alone produced a very large increase in air ventilation from 387 ± 88 to 1979 ± 400 μ/g/h (n = 4, $\pm$S.E.), $\dot{V}_g$ when very high O_2 levels in air and water attended aquatic hypercapnia, 394 ± 37 μl/g/h/, was not significantly changed from control values.

Air-water partitioning of gas transfer

(1) *Oxygen*

Undisturbed *Trichogaster* under normoxic conditions consumed oxygen at a rate of approximately 5·3 μM-O_2/g/h at 27°C (Table 1). Of this total $\dot{M}_{O_2}$, approximately 42% was accounted for by the labyrinth organs, the remaining 58% of oxygen uptake arising almost entirely from gas exchange by the gills (Fig. 5). (The skin of the gourami is covered in relatively coarse, thick scales, and accounts for only about 10% of to

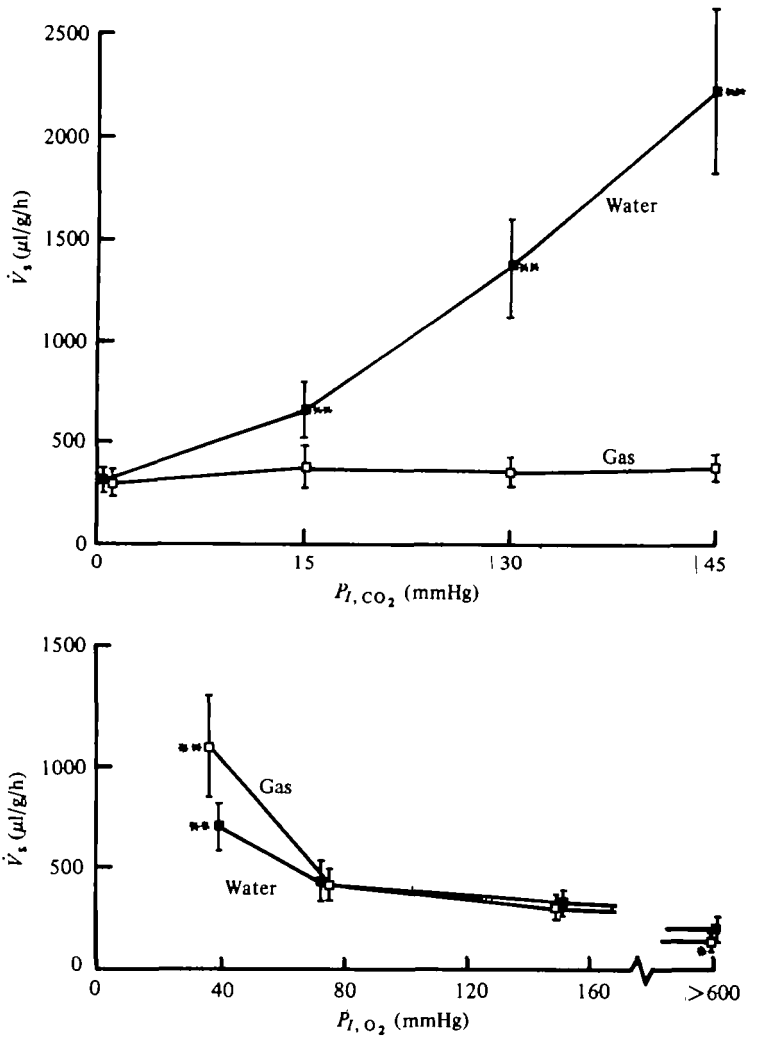

Fig. 5. Effect on suprabranchial chamber ventilation of changes in inspired water or gas oxygen and carbon dioxide partial pressures. Mean values ± 1 S.E. determined in seven fish are given. Significance coding as described in legend to Fig. 3.

aquatic exchange even in air exposed fish (Burggren & Haswell, 1979). Therefore it is reasonable to assume that most aquatic gas transfer is occurring across the branchial membranes.) When water P_{I,O_2} was reduced to 56 ± 6 mmHg, while air was still available for ventilation of the labyrinth organs, no significant reduction in total $\dot{M}_{O_2}$ of *Trichogaster* occurred (Table 1). Under these conditions, however, the gills accounted for only 30% of the oxygen uptake, with the labyrinth thus assuming the role of the major oxygen exchanging organ. When instead, the P_{O_2} of the inspired gas rather than inspired water was reduced to 54 ± 3 mmHg, there again was no significant change in total $\dot{M}_{O_2}$, but the labyrinth organs of *Trichogaster* now accounted for less than 15% of total oxygen uptake (Fig. 6).

TABLE 1. *Oxygen uptake and carbon dioxide elimination (μM gas/g body w/h) and the gas exchange ratio of the gills and the labyrinth organs of the gourami,* Trichogaster trichopterus *at 27°C*

(In all experimental conditions either air of air-equilibrated water was available to the fish, as was appropriate (see text for details). Values given are mean values ± 1 S.D. from seven fish. Asterisks indicate significant differences from control values. *$0.025 \leq P < 0.05$. ** $P < 0.025$.)

	Control (air-equilibrated water plus air)	Hypoxic water (P_{O_2} 56 ± 6 mmHg)	Hypoxic gas (P_{O_2} 54 ± 3 mmHg)	Hypercapnic water (P_{CO_2} 21 ± 2 mmHg)	Hypercapnic gas (P_{CO_2} 21 ± 1 mmHg),
$\dot{M}_{O_2}$ gills	3·1 ± 1·3	1·3 ± 0·4*	5·0 ± 2·0*	3·8 ± 1·4	3·4 ± 0·7
$\dot{M}_{O_2}$ lab.	2·1 ± 0·2	3·5 ± 1·2**	1·0 ± 1·3**	3·4 ± 0·7**	2·5 ± 1·1*
$\dot{M}_{O_2}$ total	5·2 ± 1·3	4·8 ± 1·3	6·0 ± 2·8	7·1 ± 1·7	5·9 ± 1·4
$\dot{M}_{CO_2}$ gills	3·4 ± 0·8	3·4 ± 1·0*	4·7 ± 1·3*	2·3 ± 1·5*	5·4 ± 1·7**
$\dot{M}_{CO_2}$ lab.	0·5 ± 0·3	0·6 ± 0·2	0·7 ± 0·4	3·6 ± 2·1**	1·5 ± 1·4 (net uptak
$\dot{M}_{CO_2}$ total	4·1 ± 0·8	4·0 ± 1·0	5·0 ± 2·1	5·9 ± 3·0**	3·9 ± 1·1
R gills	1·24 ± 0·45	2·74 ± 2·01**	0·93 ± 0·29*	0·62 ± 0·42*	1·73 ± 0·44*
R labyrinth	0·25 ± 0·14	0·17 ± 0·07	4·96 ± 5·37**	1·07 ± 0·56**	0**
R total	0·79 ± 0·19	0·86 ± 0·20	0·93 ± 0·47*	0·81 ± 0·29	0·69 ± 0·27

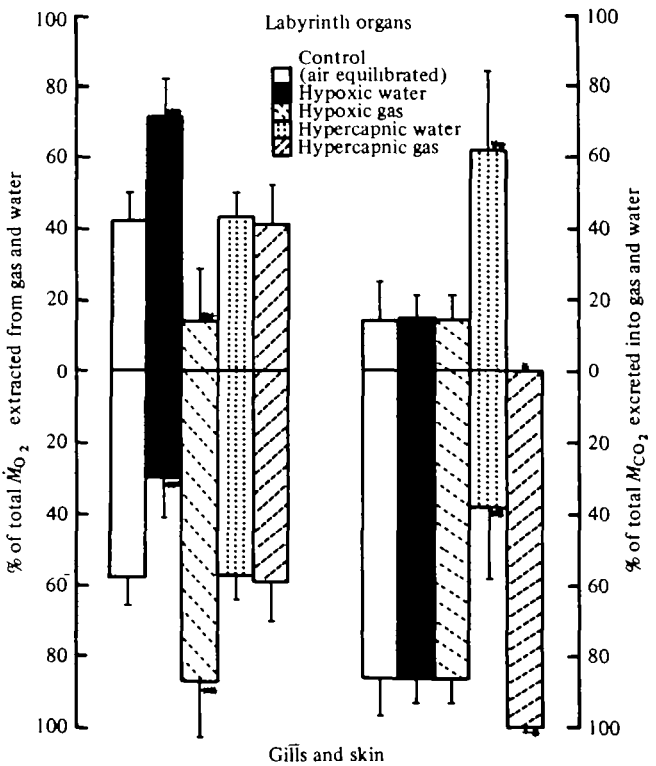

Fig. 6. Partitioning of $\dot{M}_{O_2}$ and $\dot{M}_{CO_2}$ between gills and labyrinth in *Trichogaster trichopterus* during different levels of P_{O_2} and P_{CO_2} in air and water. See text for details of levels of experimental hypercapnia and hypoxia. Mean values ± 1 S.E. were determined in seven fish. Significance coding as described in legend to Fig. 3.

The distribution of oxygen uptake between the gills and labyrinth evident during control (normoxic) conditions was independent of increases in the P_{CO_2} of inspired water or air (Fig. 6). Total oxygen uptake by *Trichogaster* was significantly elevated during the inspiration of hypercapnic water, presumably reflecting the energetic costs of a greatly increased air breathing frequency (Fig. 4) and necessary movement through the water column which occurs under these conditions.

(2) *Carbon dioxide*

Total $\dot{M}_{CO_2}$ of *Trichogaster* under control conditions was approximately 4·1 μM-CO_2/g/h (Table 1). Of this total $\dot{M}_{CO_2}$, approximately 85% was excreted into the water via the gills. Under control conditions then, the gas exchange ratio for the gills (R_g) was 1·20 compared to a gas exchange ratio for the labyrinth (Rl) of only 0·25. The labyrinth thus serves primarily as an organ of oxygen uptake during normoxia. The overall gas exchange ratio (R_{total}) for undisturbed *Trichogaster* under control conditions was 0·79 (Table 1).

The inspiration of hypercapnic water (P_{CO_2} 21 ± mmHg) was accompanied by a considerable redistribution $\dot{M}_{CO_2}$ partitioning between air and water. Aquatic CO_2 excretion fell to less than 40% of total $\dot{M}_{CO_2}$ (Fig. 5). Consequently, during aquatic hypercapnia R_{lab} increased to approximately 1·1 as the labyrinth became the major organ of CO_2 excretion (Table 1). The inspiration of hypercapnic water was accompanied by a significant increase in $\dot{M}_{CO_2}$. Rather than indicating an uptake of CO_2 from the water, this increase was the result of a rise in metabolic rate. This is evident from the facts that $\dot{M}_{O_2}$ was also elevated, probably reflecting the increased labyrinth ventilation effort (Fig. 5), and that there was no significant change in the overall gas exchange ratio during 1 h of aquatic hypercapnia (Table 1). Ventilation of the suprabranchial chamber with hypercapnic gas (P_{CO_2} 21 ± 1 mmHg), although eliciting no changes in $\dot{V}_g$, produced a reversal in the direction of CO_2 movement across labyrinth membranes. A net uptake of 1·5 μM-CO_2/g/h from the labyrinth gas into the blood occurred under these conditions, but branchial $\dot{M}_{CO_2}$ and R_g rose significantly above control levels, and the overall gas exchange ratio of *Trichogaster* showed only a non-significant decrease (Table 1).

The partitioning of $\dot{M}_{CO_2}$ in the gourami was not influenced during the inspiration of hypoxic water or gas (Fig. 6). While either hypoxia or hypercapnia may indirectly influence overall gas transfer through changes in metabolic rate, O_2 and CO_2 partitioning between gills and the labyrinth organs appear to change independently of each other.

Labyrinth convection requirement

The relationship between the air convection requirement of the labyrinth organs and suprabranchial chamber ventilation under different experimental conditions is shown for five *Trichogaster trichopterus* in Fig. 7. The air convection requirement under control conditions was approximately 100–220 μl air/μM-O_2 consumed. The air convection requirement of the labyrinth during the inspiration of hypercapnic gas (P_{CO_2} 21mmHg), which produced no change in $\dot{V}_g$, remained unchanged from control levels. Ventilation of the gills with hypoxic water (P_{O_2} 60 mmHg), which stimulated large increases in $\dot{V}_g$, was accompanied by either no change or a small increase in the

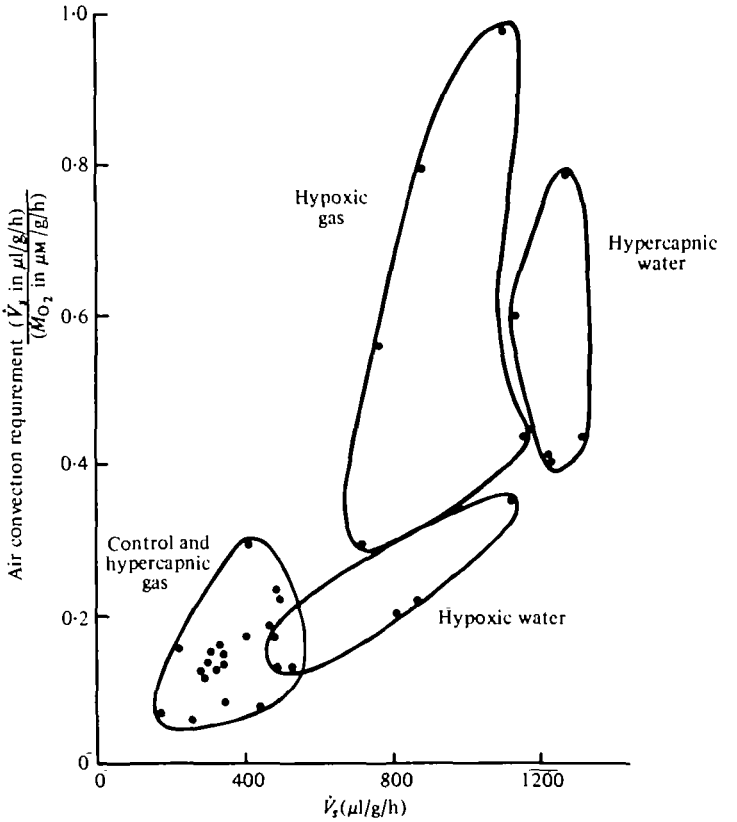

Fig. 7. Relationship between air convection requirement and ventilation of the suprabranchial chamber during different environmental conditions in five *Trichogaster trichopterus*. See text for details of experimental conditions.

air convection requirement. Inspiration of either hypoxic gas (P_{O_2} 60 mmHg) or hypercapnic water (P_{CO_2} 21 mmHg) similarly generated large increases in ventilation of the suprabranchaial chamber, but under both of these conditions the air convection requirement of the labyrinth increased to 4–10 times over that evident during control ventilation.

DISCUSSION

Gas flow in and out of the suprabranchial chamber of *Trichogaster* is somewhat different from that of the air breathing organs of many other fishes, in that during each expiration practically all of the gas in the suprabranchial chamber is displaced out of the mouth with water from the opercular cavity (Peters, 1978). Thus, the initial gas P_{O_2} immediately after inspiration at the start of the breath hold must therefore be close to ambient (155 mmHg P_{O_2}). Given the oxygen uptake, breathing frequency, and tidal volume of *Trichogaster*, it can be calculated that on average 70% of the oxygen must be removed from the suprabranchial chamber gas during the mean 5 min interbreath period, which is comparable to rates of oxygen depletion from the lungs of *Protopterus* and *Lepidosiren*, and the buccal cavity of *Electrophorus* (see Johansen, 1970).

The first and second gill arches of *Trichogaster* are well developed, however, and more than half of the oxygen uptake is obtained from water under normoxic conditions (Table 1). During aquatic hypoxia, the reduction in oxygen uptake from water is compensated for by an increase in labyrinth oxygen uptake, achieved by an augmented ventilation of the suprabranchial chamber (Fig. 5). As the water ventilating the gills becomes progressively more hypoxic, the gradient driving O_2 diffusion from water into blood will rapidly deteriorate. Unless blood in the ventral aorta derived from the aerial exchange organ can be preferentially shunted through gill arches with a reduced diffusion capacity before delivery to the tissues, the loss of O_2 from blood in the gills into water will become a factor limiting branchial gas exchange during severe aquatic hypoxia in these fish. The large increase in labyrinth air ventilation in *Trichogaster* which finally developed below a water P_{O_2} of 60 mmHg probably reflects the deterioration of aquatic oxygen uptake due to a diminished or even reversed O_2 diffusion gradient at the gills. The labyrinth was sufficiently effective under these conditions to maintain total $\dot{M}_{O_2}$ at control (normoxic) levels. Another Anabantid, *Anabas testudineus*, also is able to maintain aerial $\dot{M}_{O_2}$ in almost totally deoxygenated water (Hughes & Singh, 1970*a*). Munshi (1968) has shown that the third and fourth gill arches of this fish are very much reduced in surface area, and serve largely as non-exchanging shunt vessels conveying partially oxygenated blood derived jointly from systemic and labyrinth veins into the dorsal aorta. There is a paucity of branchial morphometric data for *Trichogaster*, but gill arches 3 and particularly 4 are also clearly reduced in length and width compared to arches 1 and 2 (Burggren, unpublished; Fig. 1). A reduction of diffusion capacity of these 'shunt' gill arches in Anabantids could be an important factor in gas transport to the tissues.

The gas convection requirements for the labyrinth changed little during aquatic hypoxia (Fig. 7) and *Trichogaster* still extracted approximately 70% of the oxygen from each breath. Air and blood almost certainly reach equilibrium in the labyrinth because of extremely small diffusion distances, of the order of only 1200 Å (Schulz, 1960). The system thus must be perfusion limited and since oxygen uptake increases, so too must blood flow to the labyrinth. Both labyrinth blood flow and ventilation therefore will increase, maintaining a ventilation–perfusion relationship for the labyrinth similar to control levels during aquatic hypoxia. Hypoxia in the gas phase caused an increase in suprabranchial chamber ventilation, but labyrinth $\dot{M}_{O_2}$ was sharply curtailed and gill uptake increased to ensure the maintenance or slight elevation of oxygen uptake (Table 1). The gas convection requirement of the labyrinth increased 2–5 times during aerial hypoxia, but the gas medium in this instance also contained only one-third as much oxygen compared to the other experimental conditions.

The gourami clearly is able to maintain oxygen uptake in the face of environmental hypoxia by increasing uptake via either the labyrinth organs or the gills, depending on the suitability of the respiratory medium. Although not measured, *Trichogaster* probably increased gill ventilation to help maintain oxygen uptake in the face of aerial or aquatic hypoxia until, in the latter condition, the water-blood gradient for oxygen diffusion into the gills had deteriorated. Other amphibious fishes such as *Amia c[illegible]*, *Anabas testudineus* and *Neocerotodus forsteri*, although progressively relying upon aerial oxygen uptake as the aquatic environment becomes hypoxic, also increase gill

ventilation frequency or stroke volume in an initial attempt to maintain $\dot{M}_{O_2}$ (Johansen, Lenfant & Grigg, 1967; Hughes & Singh, 1970*b*; Johansen, Hanson & Lenfant, 1970; Singh & Hughes, 1973).

The partitioning of carbon dioxide excretion between the gills and the labyrinth organs of *Trichogaster* reveals that aerial $\dot{M}_{CO_2}$ is normally very small, as clearly reflected in the high gas exchange ratio for the gills and low exchange ratio for the labyrinth (Table 1). The preponderance of air breathing fishes normally show a similar distribution of CO_2 excretion between gills and aerial breathing organ (see Singh, 1976 for review). Rahn & Howell (1976) report that among bimodal breathers CO_2 elimination averages 76% from the gill-skin system and 24% for the aerial system.

There are, however, important aspects of CO_2 excretion in *Trichogaster* which become manifest only upon manipulation of CO_2 levels in inspired gas or water. Exposure to hypercapnic water caused a large increase in labyrinth ventilation. Total $\dot{M}_{O_2}$ and $\dot{M}_{CO_2}$ increased in *Trichogaster*, partially as a result of the increased energy expended on repeated surfacing and labyrinth hyperventilation (Table 1, Fig. 5). The air convection requirement for the labyrinth increased during exposure to hypercapnic water, indicating little increase from control levels in labyrinth blood flow and hence blood flow through gill arches 1 and 2. This is supported by the fact that there was little change of the partitioning of oxygen uptake between labyrinth and gills.

A stepwise increase in water P_{CO_2} will initially result in a reversal of the CO_2 diffusion gradient from blood to water, but this is probably a transient situation. During a 1 h exposure to water with a P_{CO_2} of 21 mmHg, approximately 2·4 μM CO_2/g/h were excreted into the water, indicating not only that afferent branchial blood P_{CO_2} had risen to at least slightly above 21 mmHg P_{CO_2}, but also that this situation must have occurred relatively early into the measurement period to account for a large net aquatic excretion of CO_2. Both the branchial fraction of total CO_2 excretion as well as the gas exchange ratio of the gills fell significantly from control values during aquatic hypercapnia (Table 1), suggesting that the absolute magnitude of the P_{CO_2} gradient from blood to water had been reduced from control levels once a steady state was achieved.

The gas exchange ratio for aerial exchange organs in air breathing fishes rarely exceeds 0·4–0·6 when aquatic CO_2 excretion is blocked by either air exposure or aquatic hypercapnia, and so blood P_{CO_2} progressively rises and pH falls (Hughes & Singh, 1971; Singh & Hughes, 1971; Singh, 1976; Randall, Farrell & Haswell, 1978; Wright & Raymond, 1978). *Trichogaster trichopterus*, however, unlike most other air breathing fishes which have been examined, was able through aerial hyperventilation to utilize its aerial exchange organ as a highly effective alternative route for CO_2 excretion when experiencing aquatic hypercapnia. The P_{CO_2} of the suprabranchial chamber is kept low by the pronounced hyperventilation with air (Fig. 5), and so a very large CO_2 gradient favourable for the elimination of CO_2 from the labyrinth organs will develop when *Trichogaster* is in hypercapnic water. In addition, the presence of high levels of carbonic anhydrase in the labyrinth has been shown to aid aerial CO_2 excretion in this fish (Burggren & Haswell, 1979).

Whereas aquatic hypercapnia proved to be a profound stimulus to air breathing, the inspiration of CO_2 enriched gas had little or no effect on air breathing. Moreo[illegible]

the gas convection requirement remained unchanged (Fig. 7), indicating that no gross changes in labyrinth perfusion developed. In short, the only factor affecting labyrinth CO_2 transfer which changed upon a sudden exposure to aerial hypercapnia was that the P_{CO_2} diffusion gradient from blood to labyrinth gas became reversed, since a net labyrinth uptake of 1·5 μM-CO_2/g/h now occurred (Table 1). However, an augmented aquatic CO_2 elimination developed, and net CO_2 excretion in *Trichogaster* during exposure to aerial hypercapnia remained almost unchanged from control levels, with only a slight, non-significant decrease in the overall gas exchange ratio. Thus, although aerial hypercapnia is rarely, if ever, encountered in nature, these data show the potential for the gills to assume the total role in CO_2 excretion, even when aquatic $\dot{M}_{CO_2}$ must be elevated well above normal levels.

There is a total lack of a ventilatory response in the gourami to aquatic hypercapnia in the presence of very high oxygen partial pressures. This experiment, plus the fact that some of the greatest ventilatory efforts observed in the present study occurred during mild but concomitant aquatic hypoxia and hypercapnia could be taken as evidence that labyrinth ventilatory responses in *Trichogaster* induced by changes in inspired CO_2 could largely be attempts to augment an O_2 uptake and delivery to the tissues being disrupted by a hypercapnic acidosis. Yet, there is a clear independence of $\dot{M}_{O_2}$ and $\dot{M}_{CO_2}$ partitioning between gills and labyrinth under a variety of environmental conditions (Fig. 6). There may be as yet unknown complex interactions between O_2 and CO_2 sensitive elements in the regulation of air breathing in *Trichogaster*.

Johansen *et al.* (1968) have suggested that changes in the oxygen partial pressures of the gas in the mouth of the air breathing eel *Electrophorus* are responsible for normal spontaneous breathing, and the reduction in ventilation of the suprabranchial chamber during hyperoxic breathing and increase during hypoxic breathing in the present study indicates that O_2 is also involved in regulation of breathing in *Trichogaster*. Oxygen chemoreceptors in *Electrophorus* may be located in the buccal mucosa (the air breathing organ) or in the blood pathways close to it, for ventilatory responses to changes in inspired gas were immediate in the electric eel. This is not the case in *Trichogaster*, where ventilation may take many seconds or minutes to respond to a stepwise change in O_2 partial pressure, indicating a more central location for purported chemoreceptors involved in oxygen regulation. Since aerial hypoxia is rarely, if ever, encountered in the natural environment, there may not have been strong selection pressures for the evolution of a chemo-sensitive control system able to differentiate between the reduced systemic blood oxygen resulting from gill ventilation with hypoxic water and that resulting from labyrinth ventilation with hypoxic gas. In the natural environment, an increase in ventilation of the suprabranchial chamber stimulated by low blood oxygen levels can only enhance aerial oxygen uptake, and is therefore always an appropriate response to tissue hypoxia. Under experimental conditions of breathing severely hypoxic gas, increased labyrinth ventilation may serve to produce the decrease in blood O_2 which in fact is reflexly stimulating aerial hyperventilation in the first place.

Chemoreceptors specifically responsive to carbon dioxide are not located on the labyrinth organs or directly in the efferent circulation in *Trichogaster* since aerial hypercapnia, unlike aquatic hypercapnia, had no influence on air breathing. However, the

specific locations of oxygen and carbon dioxide sensitive elements modulating ventilation in the gourami are not clear, nor are they for aquatic fishes generally (Johansen, 1970; Elancher & Dejours, 1974; Bamford, 1974).

The arrangement of the circulation of gills and aerial exchange organ as well as the relative dependence upon water and air for CO_2 and O_2 transfer probably are as important as patterns of environmental oxygen and carbon dioxide fluctuations, in terms of the selective pressures for a particular control system regulating a particular respiratory gas. Although much of the physiology of bimodal gas exchange remains to be described for *Trichogaster* and other air breathing fishes, it is clear that the simultaneous exploitation of aerial and aquatic respiration carries with it a great respiratory flexibility, particularly in forms such as the gourami where effective aerial CO_2 elimination can be achieved by the aerial organs.

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