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RESPIRATORY PHYSIOLOGY OF INTESTINAL AIR BREATHING IN THE TELEOST FISH *MISGURNUS ANGUILLICAUDATUS*

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SUMMARY

The Japanese weatherloach (*Misgurnus anguillicaudatus* Cantor) can exchange gases both with water, *via* gills and skin, and with air, *via* the posterior region of the alimentary canal (intestine). Air breathing occurs by unidirectional ventilation of the alimentary canal with air taken in at the mouth and simultaneous expulsion of intestinal gas from the vent. Although the weatherloach is not an obligate air-breather, aerial gas exchange normally occurs even at 10°C in air-saturated water.

The alimentary canal was examined histologically to assess differences in capillary density and distribution and the diffusion distance for gases across those regions modified for aerial respiration. A respirometer system specifically designed for 2- to 3-g fish allowed continuous measurement of O_2 and CO_2 exchange *via* both aquatic and aerial routes at rest and at various ambient temperatures, and respiratory gas partial pressures. Air ventilation volumes, O_2 and CO_2 partial pressures of exhaled gas, O_2 extraction, and O_2 and CO_2 exchange *via* the intestine were also determined, allowing the role of the intestine in total gas exchange in the weatherloach to be determined and compared with aerial gas exchange organs in other fishes.

The alimentary canal is divided into three zones, an anterior glandular portion separated by a spiral section from the posterior, respiratory zone which has the greatest capillary densities and shortest gas diffusion distances. At rest (20°C), the intestine takes up about 20% of total O_2 but accounts for less than 3% of total CO_2 elimination (gas exchange ratio = 0·08 for intestine). O_2 extraction averages 50%. Increasing temperature causes only slight increases in total metabolic rate (Q_{10} for $\dot{M}_{O_2}$ = 1·5–1·8), but highly significant increases in intestinal gas exchange relative to total gas exchange develop as temperature rises. Intestinal gas exchange also rises with decreasing O_2 availability. A strong hypoxic drive and weak hypercapnic drive exist for aerial ventilation of the intestine, but are reduced or absent for aquatic ventilation of the gills. In spite of having to function in respiration, absorption, secretion and buoyancy regulation, the potential effectiveness of intestinal gas exchange is shown to be similar to that of other structures used for aerial gas exchange in facultative air-breathing fish.

Key words: air breathing, intestine, teleost, physiology.

INTRODUCTION

The breathing of air to supplement aquatic gas exchange by gills and skin has arisen independently in several different groups of fishes (see Johansen, 1970; Munshi, 1976; Graham, Rosenblatt & Gans, 1978*b*; Randall, Burggren, Farrell & Haswell, 1981*a*; Magnuson, Keller, Beckel & Gallep, 1983). Because of this diverse ancestry, modern air-breathing fishes show remarkable diversity in air-breathing organs, with different species using various combinations of gills, skin, buccal and pharyngeal cavities, as well as the swimbladder and digestive tract (Johansen, 1970; Munshi, 1976; Singh, 1976; Randall, Cameron, Daxboeck & Smatresk, 1981*b*).

Perhaps one of the more unusual (and certainly the least well understood) air-breathing organs in fishes is the alimentary canal. Several members of the families Loricaridae and Callichthyidae, for example, periodically draw air into the stomach or intestine for gas exchange. Gas bubbles are subsequently exhaled either through the mouth or through the vent (Gee & Graham, 1978; Kramer & McLure, 1981; Graham & Baird, 1982). In some species, certain regions of the alimentary canal are specialized for gas exchange, while in others the dual functions of digestion/absorption and gas exchange apparently occur simultaneously along much of the alimentary canal. Clearly, intestinal gas exchange and digestion/absorption are not mutually exclusive, since feeding and breathing behaviour can alternate within seconds in several species of intestinal breathing fishes (Jeuken, 1957; W. W. Burggren & B. R. McMahon, unpublished observations).

Relatively little is known of the respiratory efficiency of intestinal gas exchange systems. Many authors from Erman (1808) to Calagareanu (1907) and Lupu (1914) and most recently Jeuken (1957) have attempted analyses of intestinal respiration in one or more fishes of the genus Cobitidae. Although these authors clearly demonstrated intestinal breathing in these fish, most of their experiments were carried out under distinctly non-physiological conditions. Consequently, these early studies did not allow quantitative assessment of either the role played by the intestine in respiration under normal (quiescent) conditions, or its efficiency relative to skin, gills or other structures potentially used for aerial gas exchange.

More recently, changes in frequency of air ventilation of the alimentary canal with variation in ambient O_2 and its role in overall O_2 consumption ($\dot{M}_{O_2}$) under more physiological conditions have been reported for several other fishes using alimentary gas exchange (Liem, 1967; Gee & Graham, 1978; Kramer & McLure, 1981; Graham & Baird, 1982; Graham, 1983). However, data on tidal volume, minute ventilation, O_2 extraction, gas partial pressures and accurate measurement of any aspect of CO_2 elimination for intestinal breathers are lacking. Thus, the effectiveness of intestinal gas exchange has yet to be assessed. This paucity of information may result largely from that fact that intestinal breathers are often relatively small fish and consequently quite difficult to study physiologically.

The aim of the present study was to investigate intestinal breathing in the Japanese weatherloach *Misgurnus anguillicaudatus* to assess the effectiveness and flexibility of the alimentary canal for gas exchange, and to allow comparison with different aerial

respiratory structures in other fishes. *M. anguillicaudatus*, like *M. fossilis*, is known to be an air-breather which periodically swallows air, passing it through the alimentary canal and out through the vent (Wu & Chang, 1945). Although *M. anguillicaudatus* is small, several factors made this species appropriate for our study, including its anguilliform shape with cloaca positioned far back, its obviously well-developed tendency for aerial respiration, and its burrowing habit. An additional reason for choosing this experimental animal was that *M. anguillicaudatus* is very eurythermal, thriving at temperatures from 2 to 30°C (Axelrod & Schultze, 1955). While respiration in some larger eurythermal air-breathing fish has been studied – for example, the gar, *Lepisosteus* (Rahn *et al.* 1971), and the bowfin, *Amia* (Johansen, Hansen & Lenfant, 1970) – most air-breathing fishes live in the more thermally stable habitats. The varied effects of temperature change on the partitioning of gas exchange between air and water are not well understood (see Burggren, Feder & Pinder, 1983, for discussion of this problem) and therefore variation in gas exchange in response to change in temperature in *M. anguillicaudatus* was studied.

MATERIALS AND METHODS

Experiments and dissections were performed on 24 *M. anguillicaudatus* (mean mass $\pm$ 1 S.D. = $2{\cdot}09 \pm 0{\cdot}35$ g) obtained from commercial suppliers. Four different groups of animals were purchased in January, February, March and August 1983. Each group, which were of similar mean body mass, was acclimated at 20°C and 12 h:12 h light:dark photoperiod for at least 3 weeks prior to experimental use. Nonetheless, under control conditions small but significant differences in air-breathing frequency, air-breath volume and total CO_2 elimination occurred between groups. Other morphological and physiological variables measured in the four groups were not statistically different under identical control conditions. Since each type of environmental perturbation (e.g. temperature change, hypoxia etc.) was compared directly with control values for each group rather than among groups, we assumed that these slight differences were inconsequential to our major findings.

Morphological studies

Dissections and photography, as well as injections and casts of the alimentary canal and circulatory system, were performed either on terminally anaesthetized (MS 222, 1:5000, buffered to pH 7) or freshly killed specimens. Histology of alimentary canal and gill tissue was performed after initial fixation in buffered formalin and later transfer to Carnoy's fixative. Tissues were cleared with Histoclear and embedded in paraffin wax. Sections were cut (5–8 μm) using an A. O. Spencer 820 Microtome and stained with Heidenheim's haematoxylin and eosin to reveal general cellular structure or with periodic acid Schiff (PAS) reagent and fast green to show mucus cells. In two fish, India ink was injected into the circulation *via* the ventricle prior to fixation to help visualize small blood vessels and sinuses. This procedure did not show details of intestinal capillaries, but did highlight gill lamellar sinuses.

Casts of the alimentary canal of intact, freshly killed fish were made by injecting freshly prepared methacrylate (Batson's) compound into the intestine *via* a cannula tied into the vent. Injection was maintained at low rate and low pressure (i.e with no apparent deformation of the body wall) until the casting compound began to appear in the buccal cavity. Once the compound had polymerized, all tissues were dissolved by immersion in 30% KOH for 24 h. Knowing the density of the casting compound, the volumes of the various regions of the intestine lumen could be determined by weighing the relevant portions of the cast. Intestines of two fish were fixed, embedded and stained as above. Representative sections from glandular stomach and from anterior, middle and posterior regions of the intestine were examined morphometrically to test whether significant differences in capillary diameter and density, or in diffusion distance, could be ascertained among various regions of the alimentary canal. Measurements were made using a micrometer eyepiece calibrated with a ruled slide.

Physiological studies

Respirometry

Measurements of total O_2 uptake ($\dot{M}_{O_2,tot}$), and CO_2 elimination ($\dot{M}_{CO_2,tot}$), and their partitioning between aquatic sites ($\dot{M}_{O_2,aq}$, $\dot{M}_{CO_2,aq}$) and aerial (intestinal) sites ($\dot{M}_{O_2,int}$, $\dot{M}_{CO_2,int}$) were made in the apparatus shown in Fig. 1. The respirometer chamber consisted of a sealed 200 ml polystyrene reservoir and a connected glass tube (15 cm long and 11 mm in diameter) which held the fish. The holding tube was of a size which allowed the fish to swim forwards and backwards in the tube to reach the air surface but not to turn around. A screen restrained the fish from swimming into the main reservoir. Weatherloaches in their natural habitat voluntarily seek small confined spaces under rocks or burrow into the substratum, and they appeared to tolerate this confinement well.

The respirometer was filled with water to within 5 mm of the top. The remaining space contained ports for air flow into the respirometer and out to the gas analyser system (Fig. 1). The bend in the tube ensured that gas bubbles exhaled from the vent during each breathing sequence were diverted away from the air in the reservoir and up the tube to be collected as a single bubble in a V-shaped notch. This eliminated gas was immediately taken into an air-tight Hamilton syringe for volume measurement (air ventilation volume, V_{air}) and subsequent analysis. The rate of air ventilation ($\dot{V}_{air}$ in $\mu l\,g^{-1}\,h^{-1}$) was calculated as the product of V_{air} and the air-breathing frequency (f_{air}).

The fish ventilated its gills from the fixed volume of water in the respirometer. In one group of fish, gill ventilation frequency (f_{gill}) was recorded by visual observation. To prevent stagnation in the holding tube, a peristaltic pump was used to circulate water slowly (25 ml min^{-1}) through the tube and back to the reservoir. This ensured that the water passing the gills and skin of the fish had gas partial pressures similar to those of the water in the reservoir. A rapidly spinning stir bar prevented the water stratifying in the reservoir and helped to maintain an equilibrium between the water

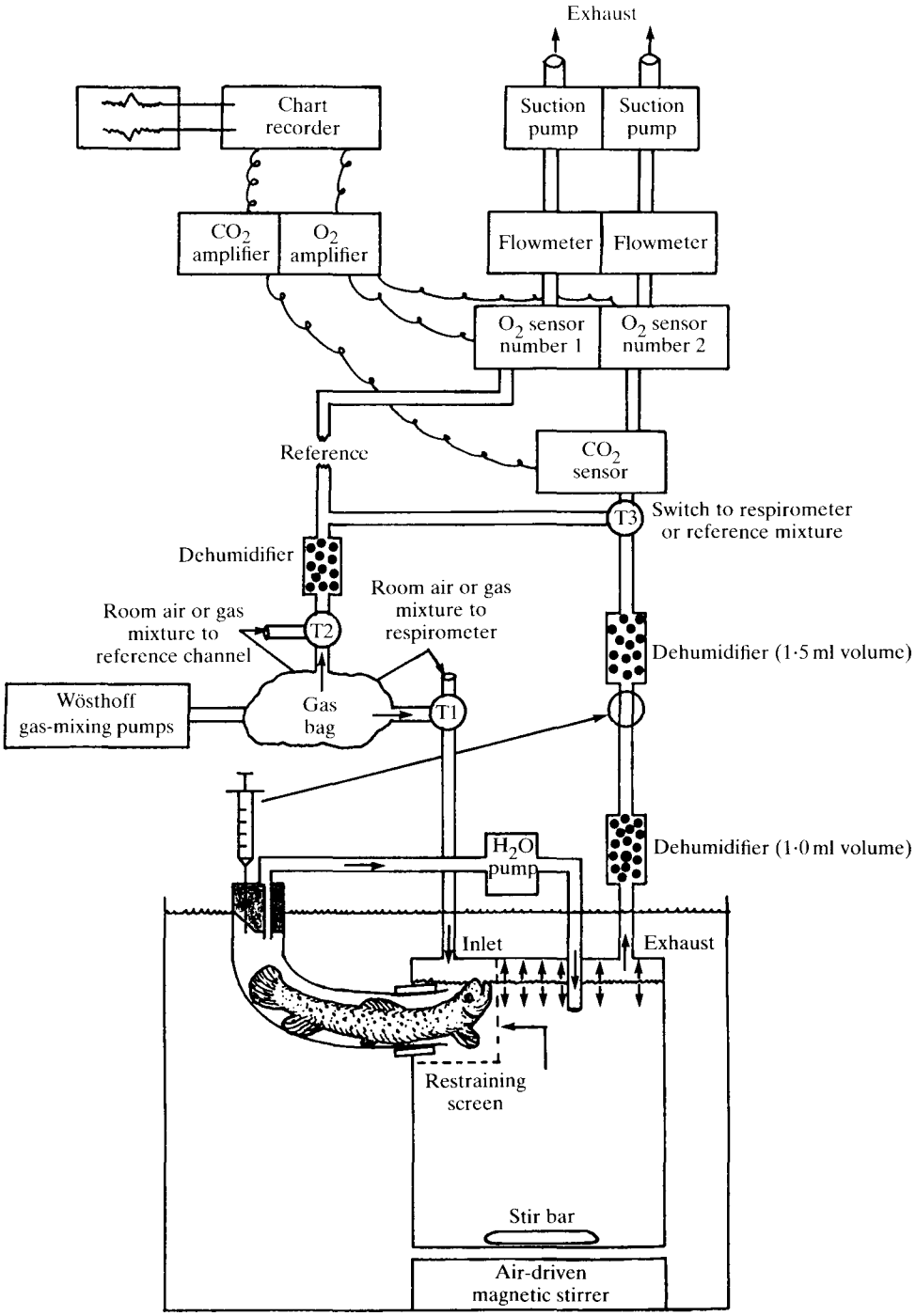


Fig. 1. Schematic diagram of the experimental apparatus used for measuring aerial and aquatic gas exchange in *Misgurnus anguillicaudatus*. See Materials and Methods for further details.

and flowing air. The entire apparatus was almost completely submerged in a water bath, which maintained experimental temperature constant and also partially screened the fish from visual disturbance.

Gas flow through the respirometer and analyser system was generated by a suction pump and flowmeter system (R/2a Applied Electrochemistry) at a rate of 4·0 ml min^{-1}. Because of the slow air flow through the respirometer, the rapid spinning of the water in the reservoir, plus the large surface area (22 cm^2) and low volume (< 40 ml) of the air–water interface, gas drawn *via* the intake port into the air space at the top of the respirometer came into a new partial pressure equilibrium with the underlying water before leaving the respirometer. Since gas bubbles expelled from the vent never contacted the gas in the respirometer but were immediately withdrawn from the system, any change in the O_2 and CO_2 concentrations of gas passing through the top of the respirometer chamber directly reflected a change in aquatic gas exchange *via* the gills and skin. After leaving the respirometer the equilibrated gas passed through two dehumidification columns (total volume less than 10 ml) before entering first a CO_2 analyser and then one channel of a differential O_2 analyser (Applied Electrochemistry 9–3A O_2, CD-3A CO_2). Either room air or a designated gas mixture could be drawn through the respirometer as dictated by the position of tap 1 (Fig. 1). A second suction pump/flowmeter system also drew a designated dehumidified gas (either room air or gas mixture) through the (reference) channel (channel 1) of the O_2 analyser. Flow through each channel was adjusted to 4·0 ml min^{-1}. The O_2 analyser was set to display the differential between the two channels, i.e. the difference between respirometer input and output gas. Analogue signals from both analysers were displayed on a two-channel recorder (Fisher Recordall) and analysed as described below. By varying the position of tap 3, the CO_2 analyser and channel 2 of the O_2 analyser could monitor either dehumidified gas directly from the respirometer (for actual measurement of metabolic rate) or air or other gas mixture which was continually passing through the reference channel (channel 1) of the O_2 analyser. For experiments involving exposure to variation in O_2 or CO_2 levels, both the gas phase above the fish and the reference channel of the analyser system were flushed with gas at the same partial pressures as that to which the water had initially been equilibrated. Calibration gas and experimental gas mixtures were made with Wösthoff gas-mixing pumps and pumped directly into gas-tight Teflon storage bags. These gases were used for calibration of the two analyser systems. Calibration was performed frequently throughout the experiments.

Calculation of O_2 and CO_2 exchange

Aquatic exchange

Aquatic gas exchange, i.e. the combined exchange of gills and skin, was calculated from the change in O_2 and CO_2 concentration as gas passed through the respirometer and became equilibrated with the water containing the fish, together with the rate of gas flow and body mass.

Aerial exchange

Each gas bubble released from the vent was collected immediately (usually <5 s) after release. Due to the short distance (4 cm) between vent and collection site we have assumed that no significant gas exchange could have occurred between the expelled bubble and the surrounding water. After its volume (approx. 80 μl) had been recorded, each bubble was injected as a discrete bolus into the gas inflow to the analysers at the injection port (Fig. 1). This injected gas was dehumidified in a small column (length 10 cm, volume 1 ml) before passage through the analysers. Since all tubing was of small bore the gas passed through the analysers as a bolus and appeared on the records as peaks of higher (CO_2) and lower (O_2) gas concentration. The CO_2 analyser system was first calibrated using bolus injections of 20, 40, 60, 80 and 100 μl of 0·1 % CO_2 in N_2. In practice the amount of CO_2 the fish added to the gas bubble ($\dot{M}_{CO_2,inj}$) could be determined relatively simply. Knowing the concentration of CO_2 in the inhaled gas (= baseline), the CO_2 content of the experimental bolus could be determined by integration of the area under the peak and comparison with the calibration values.

Calculation of the O_2 that had been consumed from each exhaled bubble ($\dot{M}_{O_2,inj}$) was more complex. The area defined under the (negative) peak on the O_2 differential record represented the O_2 'deficit' of the injected bubble as compared to the gas flowing through the reference channel. The extent of this deficit was assessed by comparison of the area under the curve with values obtained from a calibration curve produced by similar bolus injections (20, 40, 60, 80 and 100 μl) of N_2. Some of the O_2 deficit of the bubble is actually accounted for by CO_2 addition to the bubble. Subtraction of this amount from the deficit above yields the amount of O_2 removed. Knowing the O_2 content of the gas when inhaled (= baseline), the amount of O_2 removed by the fish could thus also be calculated.

Partial pressures of O_2 and CO_2 in the exhaled bubble were calculated using the gas content and volume of the bubble and barometric pressure, water vapour pressure and temperature. V_{air} is defined as the exhalant volume. This is approximately 10 % lower than the inhaled volume, because during gas exchange O_2 removal exceeds CO_2 addition.

Experimental protocol

Each fish was allowed to acclimate to the experimental apparatus for 12 h at 20 °C. In those experiments where temperature was the major experimental variable, the fish breathed air and air-saturated water. Data sets were routinely collected for 2- to 4-h periods, initially at 20 °C. The temperature of the respirometer was then lowered to 10 °C over the course of 2 h, and a second data set measured at this temperature. The experimental temperature was then raised to 30 °C over the course of 4 h and a third data set taken. The respirometer temperature was then lowered back to the original starting temperature, 20 °C, and a final data set was measured.

In another series of experiments, fish were maintained at 20 °C while the partial pressures of both inspired gas and water were adjusted to a P_{CO_2} of 3·1, 7·2 or

14·4 mmHg (P_{O_2} = 150 mmHg; 1 mmHg = 133·3 Pa) or a P_{O_2} of >400, 150, 105 or 45 mmHg (P_{CO_2} < 1 mmHg). Each fish was exposed to each gas partial pressure for 1 h in sequence, with a 1-h period of acclimation at air saturation between data sets.

Statistical analysis

All data were analysed to provide mean values ± standard error. Treatment effects (temperature, inspired gases) were assessed with a one-way analysis of variance (ANOVA), using a significance level of $P < 0{\cdot}05$. Where ANOVA revealed significant treatment effects, differences between specific means were compared using Student's *t*-test.

RESULTS

Morphology of the alimentary canal

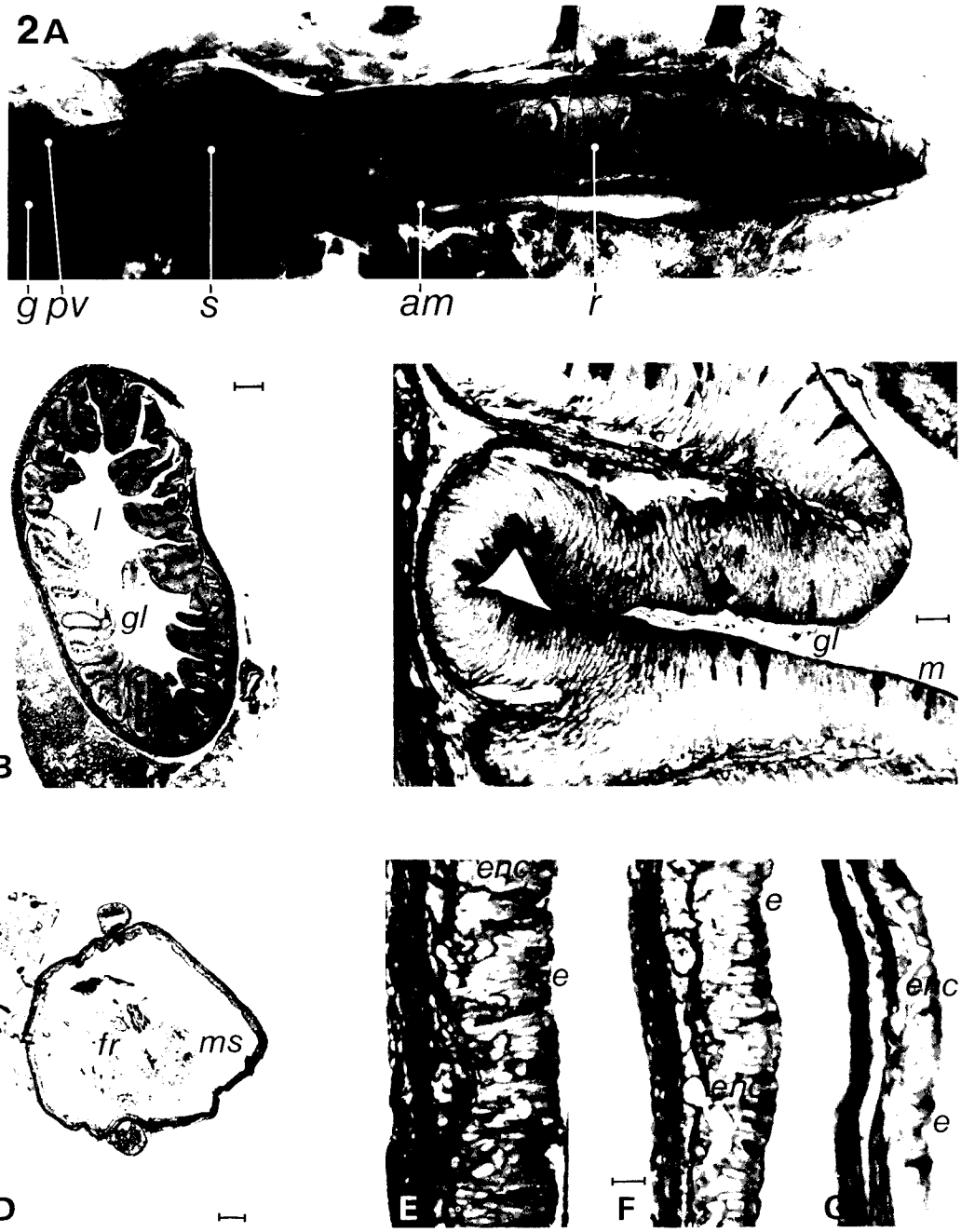
The alimentary canal of *Misgurnus anguillicaudatus*, like that of many other Cobitidae, is divided into three distinct zones (Fig. 2). The anterior zone, which is enveloped on its ventral and lateral margins by the large, bilobed liver, appears to constitute the major digestive region of the gut. The walls of this zone appear thick and well muscled (Fig. 2B,C). Its inner surface shows many longitudinal fissures. Both pancreatic and gall-bladder secretions enter this zone. Partially digested food was almost always found in this anterior region upon dissection.

The microscopic anatomy of this anterior zone of the alimentary canal (Fig. 2B,C) is typical of intestinal tissue from aquatic teleost fish. There are distinct bands of both circular and longitudinal muscle and the internal surface is complexly folded. Striated muscle is clearly visible within at least the circular muscle bands of several sections. The luminal epithelium is composed largely of columnar epithelial cells together with large numbers of mucus cells (Fig. 2C). The vasculature penetrates each mucosal fold or villus and capillaries can be seen at the bases of, but not penetrating, the epithelial cell layer.

The spiral region of the alimentary canal represents a transitional zone from the anterior 'digestive' zone to the posterior 'respiratory' zone. This transition is characterized by the presence of a highly variable degree of spiral rotation (Fig. 2A). Observations on nine fish showed a mean rotation of 490 ± 130° (±1 S.E.). In

Fig. 2. General and microanatomy of the alimentary canal of *Misgurnus anguillicaudatus*. (A) Dissection showing anterior glandular (stomach) region, spiral and respiratory regions of the canal and their blood supply. *g*, anterior (stomach) region; *s*, spiral valve; *r*, respiratory region of intestine; *pv*, hepatic portal vein entering liver; *am*, anterior mesenteric artery. (B) Low-power view of section across anterior region (stomach). Scale bar, 100 μm. (C) Higher magnification of B to show cellular structure. Scale bar, 10 μm. (D) Low-magnification view of section across posterior (respiratory) region. Scale bar, 100 μm. (E,F,G) Higher magnifications of epithelium in the respiratory region showing thinning of epithelial walls increasing posteriorly (E to G) and details of intra-epithelial capillary networks. *e*, intestinal epithelium; *enc*, intestinal capillary network; *l*, lumen; *ms*, mucous sleeve; *fr*, food residue; *m*, mucous cells (very intensely stained); *gl*, glandular region of stomach, *c*, sub-epithelial capillary. Scale bar, 10 μm.

anaesthetized specimens, peristalsis was commonly observed in this zone. This area is strongly muscular as is the anterior region, but the height of the epithelial cell layer is progressively reduced, as are the number and depth of villi.



The posterior 60% of the alimentary canal, the intestine, is always very thin-walled and routinely contained gas in *M. anguillicaudatus*. Faecal material was not commonly present and, where present, it seemed to be contained in a mucus envelope (Fig. 2D). The internal walls of this zone are comparatively smooth and its diameter relatively constant. This zone still contains all of the muscular and epithelial elements of the preceding sections but they are substantially reduced in depth giving this zone a very thin wall (Fig. 2D–G). The internal surface is relatively smooth and the epithelium flattened to such an extent that it resembles pavement, rather than columnar, epithelium. The many capillaries invading this epithelial layer often come within micrometres of the luminal boundary (Fig. 2D–G). Both of the above factors clearly reduce the diffusion distance across the luminal boundary. Mucus cells are still present in the epithelium but there appear to be fewer than in the other zones.

Arterial blood is supplied to the alimentary canal by the anterior mesenteric artery, which gives off 12–17 lateral branches as it runs along the dorsal surface (Fig. 2A). These branches eventually break up into a fine capillary network and reconvene into venules and small veins which eventually coalesce to form a branch of the hepatic portal vein running along the ventral surface. From a gross anatomical view, the three zones of the gut appear to be equally well supplied with blood.

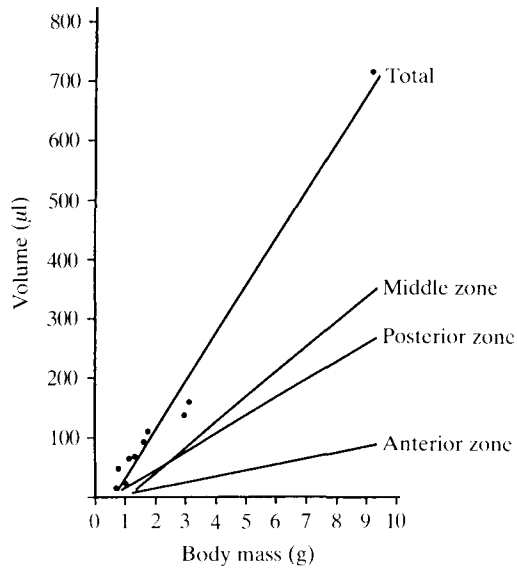


Fig. 3. Regional and total volumes of the gut as a function of body mass in *Misgurnus anguillicaudatus*. Lines represent linear regressions of data taken from methacrylate casts (see text). The equations and correlation coefficients for each line are: total volume = $-58m + 82$, $r = 0{\cdot}97$, $N = 10$; middle volume = $-48m + 42$, $r = 0{\cdot}95$, $N = 10$; posterior volume = $-5m + 29$, $r = 0{\cdot}98$, $N = 10$; anterior volume = $-6m + 11$, $r = 0{\cdot}99$, $N = 8$, where m is mass. All regressions are significant ($P < 0{\cdot}01$). Actual points are presented for total gut volumes, but are omitted for regional volumes for clarity of presentation.

Table 1. *Variation in capillary morphometrics and in lumen–capillary diffusion distance in several regions of the alimentary canal of* Misgurnus anguillicaudatus

		Respiratory		
	Stomach	Anterior	Mid	Posterior
Fish 1				
Capillary density/50 μm	1·9 ± 0·2	2·2 ± 0·4 (NS)	3·3 ± 0·03 (<0·01)	3·4 ± 0·2 (<0·01)
Capillary diameter (μm)	3·2 ± 0·2	3·4 ± 0·4 (NS)	3·7 ± 0·2 (NS)	3·2 ± 0·2 (NS)
Diffusion distance (μm)	42·8 ± 3·3	21·4 ± 1·5 (<0·01)	17·2 ± 0·8 (<0·01)	11·9 ± 0·4 (<0·01)
Fish 2				
Capillary density/50 μm	1·5 ± 0·3		4·2 ± 0·3 (<0·01)	
Capillary diameter (μm)	2·3 ± 0·3		3·6 ± 0·2 (<0·01)	
Diffusion distance (μm)	32·4 ± 1·5		11·2 ± 0·6 (<0·01)	

Data are mean ± 1 S.E. for 10 measurements, for each of the regions shown, for two fish.
Comparison for significant differences by Student's t-test with $P < 0{\cdot}01$ serving as the fiducial limit.
Capillary density was estimated as the number of capillaries in a cross-sectional segment.
Diffusion distance (μm) is from the capillary endothelium to the lumen edge.

Morphometrics of the alimentary canal

Total volume of the alimentary canal, as well as the relative volumes of each of the three structural zones, is clearly a linear function of body mass (Fig. 3). At 2 g, a mass typical of the fish used in the physiological experiments, the volume of the alimentary canal was approximately 115 μl, of which the anterior, spiral and posterior (air-breathing) zones had volumes of 20, 43 and 52 μl, respectively. Thus the anterior (glandular) zone occupied less than 20 % of the alimentary canal, compared to the more than 45 % occupied by the respiratory zone.

Histological sections through several gut regions of two fish were used to assess whether capillary diameter, capillary density (expressed as number of capillaries per sector, i.e. percentage of circumference) and diffusion distance (i.e. minimum distance from capillary wall to luminal boundary) differed among zones of the gut. There is a significant increase in capillary density and decrease in diffusion distance for the posterior compared with the anterior region (Table 1). Comparison of anterior and posterior sections within the respiratory region of the gut shows a similar result, indicating a progressive thinning of the respiratory epithelium caudally (Table 1).

Gas exchange: steady-state conditions

Ventilation of the intestine in unrestrained fish observed in aquaria occurred when the fish, which typically rested on or buried in the substrate, swam rapidly to the

surface, thrust its head into the air, and inhaled an air bubble. Inhalation lasted approximately 0·1 s. During, and for about 0·5 s after inhalation a stream of bubbles was released from the vent. Air breathing by weatherloaches in the respirometer system appeared similar, with the fish moving forwards and upwards in the tube, raising its head above water, then retiring back into the tube. In a typical 2-g weatherloach, V_{air} at 20°C was about 40 μl g^{-1} body mass (Fig. 4). Using these data and the alimentary canal volume estimated from casts for a 2-g fish (Fig. 3), we estimate that 55–85 % of the total gut volume contains gas that can be renewed at each air breath. The values are consistent with our dissections, in which both the middle and posterior intestinal regions were usually found to be gas-filled.

Air-breathing frequency varied considerably even in individual loaches, averaging about 10 breaths h^{-1} at 20 °C. Total ventilation rate ($\dot{V}_{air}$) thus ranged from 400 to 1200 μl air g^{-1} h^{-1} over the temperature range 10–30 °C (Fig. 4).

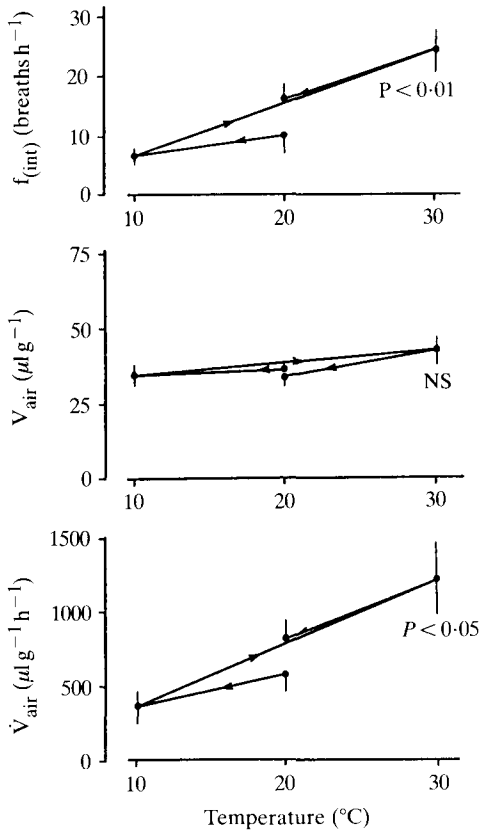


Fig. 4. Air-breathing variables as a function of body temperature in *Misgurnus anguillicaudatus*. Mean values ± 2 S.E. ($N = 5$) are presented. Significance level of ANOVA for treatment effect is indicated to the right of the data set for each variable. (NS, not significant, $P > 0·01$). Arrows on lines between mean values indicate the chronological order in which the data were acquired. See text for abbreviations.

The mean partial pressures of O_2 and CO_2 in the exhaled gas bubble were calculated to be 75 mmHg (P_{O_2}) and 4 mmHg (P_{CO_2}), respectively, at 20°C (Fig. 5). Partial pressures of exhaled gas bubbles varied not only among fish, but from breath to breath. Since there was also variation in exhaled volume, some of the fluctuation in partial pressure of exhaled gas could be due to a variable amount of gas turnover in the intestine with each breath. Occasionally, there were intervals between air breathing of 1 h or longer. P_{O_2} and P_{CO_2} of the gas subsequently expelled from the intestine after these long periods of aerial apnoea were not significantly different from those measured after the more typical intervals of 5–10 min, suggesting that the contribution of aerial gas exchange was greatest within the first minutes following air breathing. Given a P_{O_2} for inhaled gas of 150 mmHg and for exhaled gas of 70–75 mmHg (Fig. 5), intestinal O_2 extraction can be calculated to be approximately 50 %.

Intestinal respiration contributes about 20–25 % of $\dot{M}_{O_2,tot}$ at 20°C in resting weatherloaches in normoxic water acclimated to the apparatus (Fig. 6). However, only about 3 % of $\dot{M}_{CO_2,tot}$ occurs *via* this site, as reflected in the value of 0·075 for the gas exchange ratio for the intestine at 20°C (Fig. 6). CO_2 elimination thus occurs primarily across gills and skin ($R_{gas} = 0{\cdot}9$) (Fig. 7). The primary respiratory function of the intestine of *M. anguillicaudatus* is thus O_2 uptake. Interestingly, intestinal gas exchange varied comparatively little from fish to fish: most of the variation in total gas exchange occurred across the aquatic surfaces. Although under control conditions (20°C air saturation) most *M. anguillicaudatus* exhibited rhythmic buccal

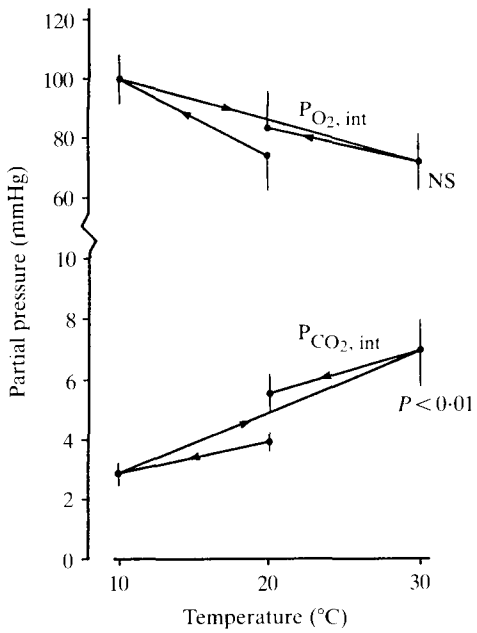


Fig. 5. Oxygen and carbon dioxide partial pressures in exhaled intestinal gas as a function of body temperature in *Misgurnus anguillicaudatus*. Statistical conventions as in Fig. 4 ($N = 5$).

ventilation of the gills, ventilation frequency (f_{gill}) was extremely variable both among fish and within individuals (Fig. 7). Periods of several minutes with no observable buccal or opercular movements were common but could not be clearly correlated with the frequency of aerial ventilation. Total respiratory rates ($\dot{M}_{O_2,tot}$, $\dot{M}_{CO_2,tot}$) at 20°C, as well as the gas exchange ratio for the entire fish (R_{tot}), were obtained by summation of aquatic and intestinal rates (Fig. 8). When $\dot{M}_{O_2,tot}$ and $\dot{M}_{CO_2,tot}$ are 7·4 and 6·0 μmol g^{-1} h^{-1}, respectively, then R_{tot} is 0·8 (Fig. 8).

Effects of temperature on gas exchange

Effects of acute temperature change on aerial, aquatic and total gas exchange in *M. anguillicaudatus* are indicated in Figs 4–8. Because of considerable variation in metabolic responses to acute temperature change between individuals, analysis of variance of the raw metabolic data indicated no significant effect of temperature on the absolute values of $\dot{M}_{CO_2,tot}$, $\dot{M}_{O_2,tot}$ or R_{tot} (Fig. 8). However, Q_{10} values calculated for both $\dot{M}_{O_2,tot}$ and $\dot{M}_{CO_2,tot}$ between 10 and 20°C and between 20 and 30°C were all significantly greater than one ($P < 0{\cdot}05$). There was, thus, a significant but comparatively slight effect of temperature on metabolism.

Although the volume of air exhaled per breath was not affected by temperature, the frequency of ventilation (f_{int}) increased significantly as temperature rose. Thus

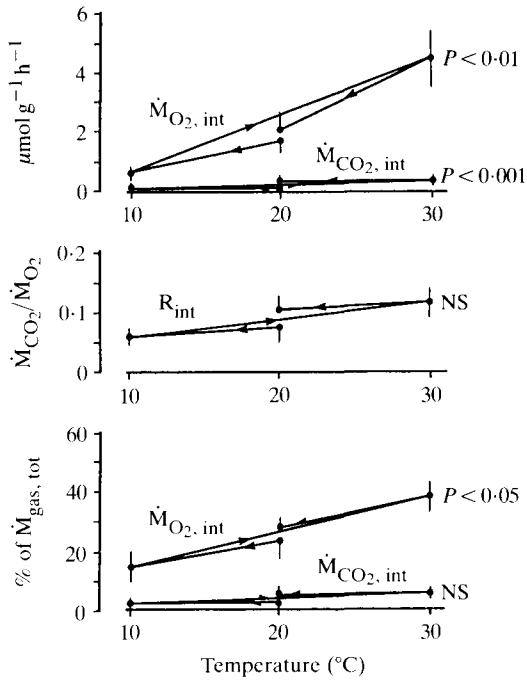


Fig. 6. Oxygen uptake and carbon dioxide elimination *via* the intestine (expressed both in absolute terms and as a percentage of total gas exchange rates) as a function of body temperature in *Misgurnus anguillicaudatus*. Also indicated is the gas exchange ratio, R, for the intestine. Statistical conventions as in Fig. 4 ($N = 5$).

the rate of air ventilation ($\dot{V}_{air}$) and both $\dot{M}_{O_2}$ and $\dot{M}_{CO_2}$ increased significantly between 10 and 30°C (Figs 4, 6). Although the increase appears greater for $\dot{M}_{O_2}$, R_{int} was not significantly affected (Fig. 6). The percentage contribution of aquatic to total $\dot{M}_{O_2}$ fell significantly with rise in temperature (Fig. 7), while the contribution of the intestine doubled (Fig. 6). Aquatic $\dot{M}_{O_2}$ nonetheless accounted for over 60% of total $\dot{M}_{O_2}$, even at 30°C. Intestinal P_{CO_2} increased significantly as temperature increased (Fig. 5). In contrast, P_{O_2} fell but the decrease was not significant. A final set of data was collected after a return to 20°C. Although slight hysteresis occurred for every variable measured, values at 20°C following acute exposure to 10 and 30°C were not significantly different ($P > 0{\cdot}1$) from those measured in the initial 20°C period.

Respiratory responses to hypoxia and hyperoxia

Respiratory responses to combined aerial and aquatic hypoxia ($P_{I_{O_2}}$ 105 and 45 mmHg) and hyperoxia ($P_{I_{O_2}} > 400$ mmHg) in *M. anguillicaudatus* are presented in Fig. 9. Acute hypoxic exposure caused a large and significant increase in the frequency of aerial ventilation. Thus, although V_{air} remained unchanged, $\dot{V}_{air}$ increased significantly to about four times normoxic levels at the lower $P_{I_{O_2}}$. The

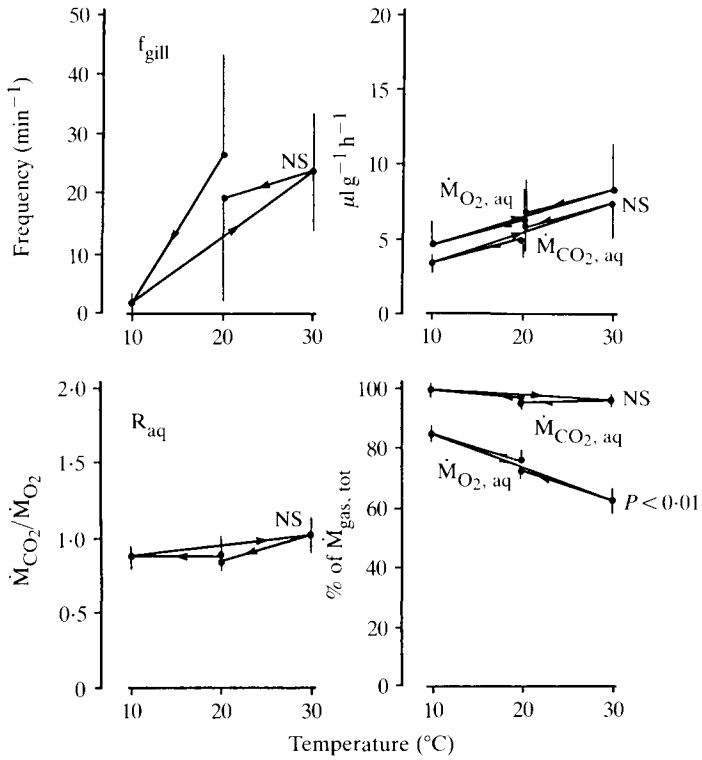


Fig. 7. Aquatic (aq) gas exchange and gill ventilation frequency as a function of body temperature in *Misgurnus anguillicaudatus*. Statistical conventions as in Fig. 4 ($N = 5$).

increase in intestinal ventilation was only moderate between $P_{I_{O_2}}$ of 150 and 105 mmHg, increasing primarily between 105 and 45 mmHg.

Not surprisingly, the P_{O_2} of exhaled intestinal gas decreased from about 80 mmHg in normoxia to below 30 mmHg when $P_{I_{O_2}}$ was 45 mmHg. Calculated O_2 extraction by the intestine fell only slightly from about 50 % in normoxia to about 45 % at a $P_{I_{O_2}}$ of 45 mmHg. $\dot{M}_{O_2,int}$ increased considerably with hypoxic exposure.

Exposure to hyperoxia ($P_{I_{O_2}} > 400$ mmHg) resulted in a slight but significant depression in $\dot{V}_{air}$ from normoxic levels, produced by a fall in f_{int} rather than V_{air} (Fig. 9). Frequency of aquatic ventilatory movements was highly variable in *M. anguillicaudatus* (Fig. 9) and showed no correlation with ambient O_2 over the entire range tested.

Respiratory responses to hypercapnia

Combined aerial and aquatic hypercapnia up to a $P_{I_{CO_2}}$ as high as 14 mmHg produced no significant effect on aquatic ventilation and only a muted effect upon intestinal breathing (Fig. 10). Although mean values of f_{int} increased nearly three times in the five loaches examined, two of these fish showed little if any aerial ventilatory response even at the highest $P_{I_{CO_2}}$. Thus, the small increase in mean $\dot{V}_{air}$ with increasing $P_{I_{CO_2}}$ failed to be significant ($P > 0{\cdot}10$). The P_{CO_2} of the exhaled gas increased significantly with rising P_{CO_2} as expected, but $\dot{M}_{CO_2,int}$ and consequently R_{int} increased significantly. Neither $P_{O_2,int}$ nor $\dot{M}_{O_2,int}$ showed any significant correlation with hypercapnic exposure.

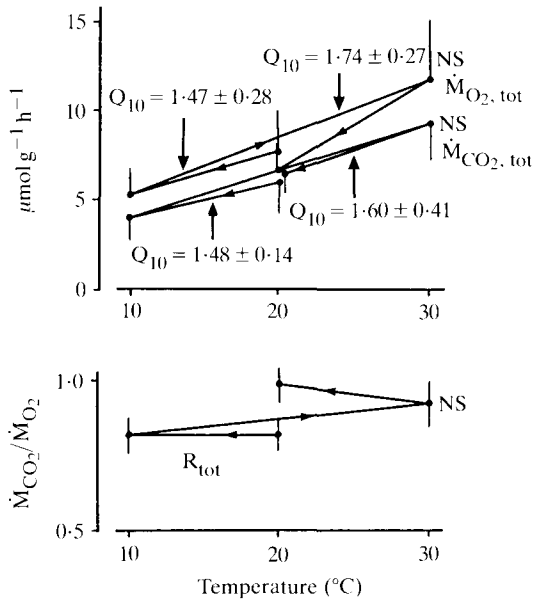


Fig. 8. Total oxygen uptake and carbon dioxide elimination and the gas exchange ratio, R, as a function of body temperature in *Misgurnus anguillicaudatus*. Also indicated is the Q_{10} for $\dot{M}_{O_2}$ and $\dot{M}_{CO_2}$ for two 10° ranges. Statistical conventions as in Fig. 4 ($N = 5$).

DISCUSSION

Anatomical and morphometric observations

The gross anatomy of the alimentary canal has been described for the cobitid fishes *Misgurnus anguillicaudatus*, *M. fossilis*, *Cobitis taenia* and *C. barbatula* (Wu & Chang, 1945; Jeuken, 1957). Our own observations of *M. anguillicaudatus*, while confirming many aspects of these studies, extend upon or differ from these studies in several important aspects.

The alimentary canal appears morphologically and physiologically separated into a small, glandular, anterior region, used for digestion, and a larger posterior respiratory region, specialized for gas exchange. A middle transition zone, characterized by considerable spiral rotation in the long axis of the gut, serves to demarcate these two regions. The extent of spiral rotation of the gut has been reported to be considerably greater in *Misgurnus* than in other cobitid genera (Wu & Chang, 1945; Jeuken, 1957; Fig. 2A). However, our dissections of freshly killed or

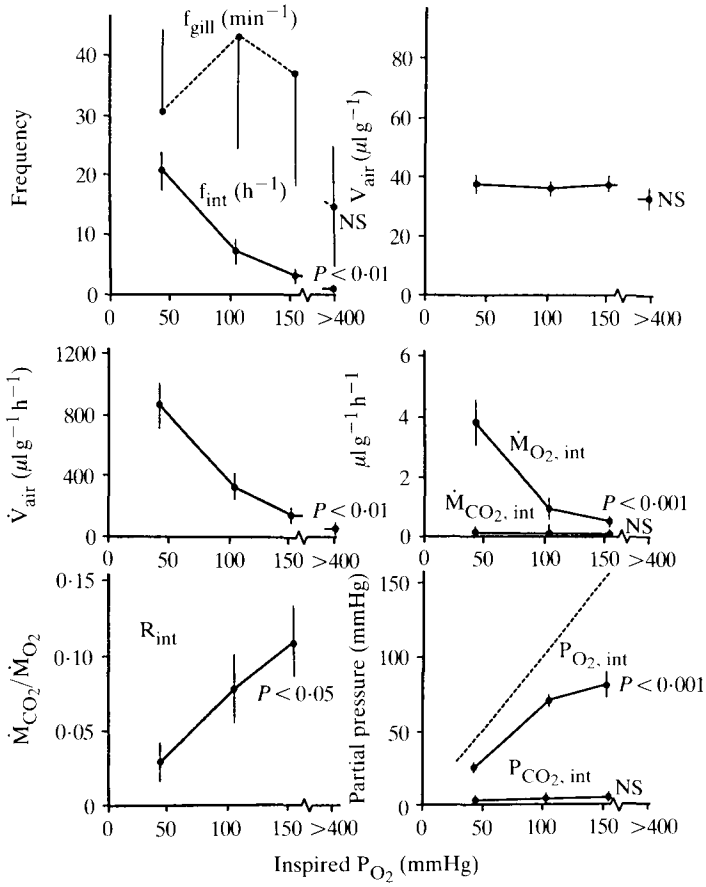


Fig. 9. Gas exchange variables for intestinal respiration as a function of P_{O_2} of inspired water and gas in *Misgurnus anguillicaudatus*. Statistical conventions as in Fig. 4 ($N = 5$).

anaesthetized preparations indicate that the degree of rotation of the gut can be altered by spontaneous contractions of the smooth muscle in the middle, transition zone. Consequently, at least in *M. anguillicaudatus*, the degree of spiral rotation can be highly variable even in an individual specimen.

Lupu (1914, 1925) described the function of the spiral region of the gut of cobitid fishes as the formation of the mucus sac surrounding the faecal material in the posterior intestine, and the passage of this sac and its contents back through the alimentary canal for its eventual expulsion from the animal. Our observations verify that this spiral region is highly contractile and also that it serves to compress the food in the mucus sac into a narrow string as it passes into the posterior region of the gut. The packaging of material in this fashion, while potentially reducing food assimilation in the posterior region of the gut, apparently maximizes the surface area of the intestinal epithelium that is in contact with inspired gas. Certainly, digestion and intestinal respiration are not mutually exclusive, as was suggested by Lupu (1925), since both *M. fossilis* (Jeuken, 1957) and *M. anguillicaudatus* (present study) frequently interrupt feeding to surface and breathe air.

This study is the first on cobitid fishes to quantify the volume of the various regions of the alimentary canal *in situ* in intact, freshly killed specimens. Clearly, the

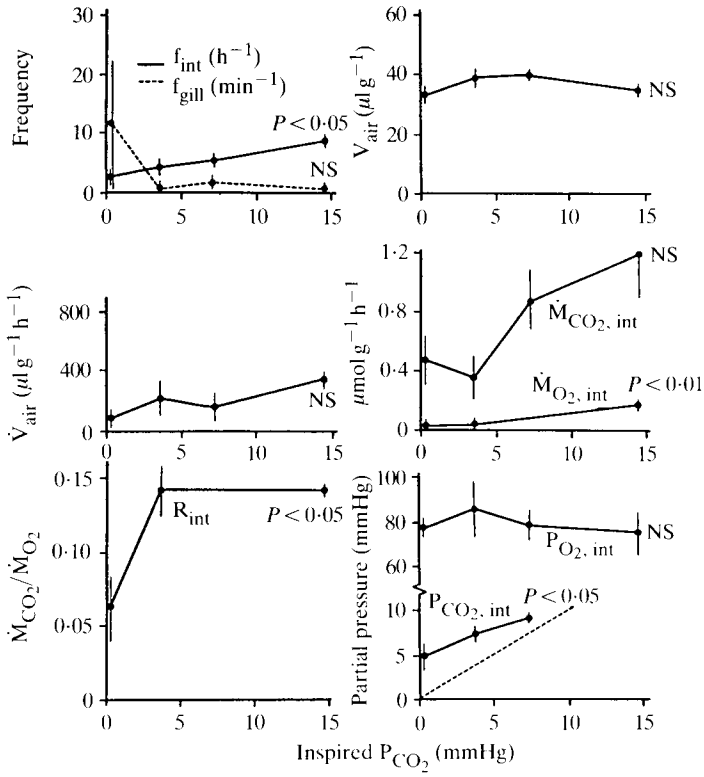


Fig. 10. Gas exchange variables for intestinal respiration as a function of P_{CO_2} of inspired water and gas in *Misgurnus anguillicaudatus*. Statistical conventions as in Fig. 4 ($N = 5$).

region of the gut specialized for digestion is proportionately small over the entire range of body mass examined (Fig. 3). Combining data on gut volumes with ventilation volumes measured *in vivo* (Fig. 4), we calculate that in *M. anguillicaudatus* a single air breath replaces at least 50–80 % of the total volume of the gut with fresh air. Since the digestive portion of the alimentary canal has a small but significant volume, and since some small volume of the respiratory portion may be taken up by food or faecal material, the volume of each air breath will probably approach the capacity of the respiratory portion of the intestine. Consequently, freshly inspired air will mix with only a small volume of residual gas in the gut. This will lead to a relatively high P_{O_2} and low P_{CO_2} of gas in the gut, thus maintaining large diffusion gradients between gas and blood.

The microvasculature of the stomach mucosa of *M. anguillicaudatus* (Fig. 2C) differs little from that of other vertebrates, but marked differences do occur in the vasculature of the posterior, respiratory portion. The capillary density in the respiratory portion of the gut is about twice that in the digestive region (Table 1). Capillaries penetrate between cells to create a distinct intra-epithelial capillary network in both *M. anguillicaudatus* (present study) and *M. fossilis* (Jeuken, 1957). This capillary arrangement, combined with a reduction in thickness of the epithelial cells themselves, results in a diffusion path for respiratory gas in the gut of *M. anguillicaudatus* which may be as little as 2 μm (Fig. 2E–G). These blood–gas diffusion distances are far less than the diffusion path across fish gills or skin, and approach the diffusion distances in mammalian lungs.

Gas exchange

General considerations

Total O_2 consumption ($\dot{M}_{O_2,tot}$) for *M. anguillicaudatus* reported in the present study is considerably lower than has been generally reported for *Misgurnus* sp. (see Jeuken, 1957, for a review), but is very similar to the values reported for other air-breathing fishes of similar size, such as *Trichogaster* (Burggren, 1979), *Calamoichthys* (Sacca & Burggren, 1979), *Hoplosternum* (Gee & Graham, 1978) and *Ancistrus* (Graham, 1983). The lower oxygen consumption for air-breathing fishes reported in studies more recent than those reviewed by Jeuken (1957) probably results from refinement of techniques resulting in less experimental stress to the fish. Intestinal gas exchange of *M. anguillicaudatus* accounts for about 25 % of $\dot{M}_{O_2,tot}$ at rest in normoxic water at 20°C. This reflects a degree of dependence upon aerial respiration *via* the gut similar to that measured under similar normoxic conditions in *Hoplosternum thoracatum*, another air-breathing fish using the gut as an aerial exchange organ (Gee & Graham, 1978). Intestinal oxygen extraction from each breath is highly variable in cobitid species (Jeuken, 1957). O_2 extraction from the gut of *M. anguillicaudatus* was also variable, averaging about 50 % of the O_2 inhaled per breath when fish were quiescent in aerated water at 20°C. This value is in the middle of the rather wide range of values reported for the air-breathing organs of other fishes (see Carter & Beadle, 1931; Burggren, 1979; Randall *et al.* 1981*a*). Higher aerial O_2

utilizations are routinely reported for air-breathing fish acutely exposed to hypoxic waters.

Increased O_2 utilization occurred in *M. anguillicaudatus* not only during hypoxia, but also with increasing temperature and following activity, all situations requiring increased O_2 uptake and transport.

Although data on CO_2 elimination are available for several air-breathing fishes (see Singh, 1976), the present study is one of the few on air-breathing (or aquatic) fish to have measured total $\dot{M}_{O_2}$ and $\dot{M}_{CO_2}$ simultaneously. Our experimental system allows calculation of respiratory exchange ratios (R) for both aquatic and for aerial respiratory systems, both in steady state and in response to changes in environmental parameters. The R_{tot} values measured for *M. anguillicaudatus* are similar to those for other fishes (Burggren, 1979; Burggren & Cameron, 1980; Randall *et al.* 1981*b*) and for non-fasting vertebrates generally. As in most other air-breathing fishes, as well as many amphibians, the air-breathing organ of *M. anguillicaudatus* is responsible for a greater O_2 uptake than elimination of CO_2, as reflected in R values of only about 0·1 at 20°C. There are several morphological and physiological factors that contribute to this disproportionate exchange of O_2 and CO_2 in the air-breathing organ of fishes, including the physicochemical nature of O_2 and CO_2, the anatomical position in the circulatory systems of the air-breathing organ relative to the gills and skin, the presence or absence of carbonic anhydrase in the air-breathing organ, the surface area of the gills, central and peripheral cardiovascular shunts, etc. (for extensive discussions see Randall *et al.* 1981*a*; Johansen & Burggren, 1985; Feder & Burggren, 1985). Which combination of these many factors relegates CO_2 elimination primarily to aquatic rather than aerial sites for gas exchange in *M. anguillicaudatus* requires further experimentation.

Influence of temperature on gas exchange

Although metabolic rate tends to increase with temperature in *M. anguillicaudatus* (Fig. 8), no significant change in $\dot{M}_{O_2,tot}$, $\dot{M}_{CO_2,tot}$, R_{tot} or $\dot{V}_{air}$ occurred over a full 20°C increase. These findings contrast with those for most fish species – aquatic or amphibious – which typically show relatively large increases in total metabolism in response to temperature increase. The weatherloach often encounters sudden and large changes in ambient temperature in its natural habitat, and a relatively temperature-insensitive metabolism is characteristic of eurythermal animals, both vertebrate and invertebrate (Hochachka & Somero, 1973; Burggren & McMahon, 1981).

Increasing environmental temperature causes some amphibious vertebrates to become more reliant on aerial exchange, while other species show little or no change in gas exchange partitioning between air and water (see Burggren *et al.* 1983). *M. anguillicaudatus* exhibits the latter pattern, with intestinal (aerial) O_2 uptake remaining below 40 % even at 30°C. Decreasing dissolved oxygen in the environment and increasing oxygen demand are often touted as major pressures for increased air-breathing at high temperature. The issue is considerably more complex, however, involving several ecophysiological considerations such as the metabolic cost of

transport to the water surface, the probability of a predatory encounter, the alteration in buoyancy caused by air breathing, etc. (for a discussion see Kramer, 1983; Feder, 1984; Burggren *et al.* 1983). Further experiments focusing on the eurythermal habitat of the weatherloach would be valuable in assessing the temperature-dependent respiratory responses of this fish.

Although most aspects of intestinal gas exchange did not change significantly with temperature, the P_{CO_2} of intestinal gas (and thus presumably blood P_{CO_2}) doubled between 10 and 30°C (Fig. 5). Similar findings have been reported for another air-breathing fish, *Synbranchus* (Heisler, 1980). Interpretation of these observations is beyond the scope of this study, since changes in blood P_{CO_2} during variation in environmental temperature may relate as much to temperature-dependent changes in acid–base status as to changes in metabolic production of molecular CO_2.

Respiratory effects of oxygen and carbon dioxide

The important supplemental role of intestinal respiration in *M. anguillicaudatus* is clear from the effects of environmental hypoxia, which results in a large increase in intestinal breathing but no significant increase in gill ventilation (Fig. 9). Similar increases in air-breathing frequency upon hypoxic exposure have been observed in other intestinal air-breathing fishes (Jeuken, 1957; Gee & Graham, 1978; Graham & Baird, 1982; Graham, 1983). In *M. anguillicaudatus*, the factorial increase in intestinal $\dot{M}_{O_2}$ is greater (8·5-fold) than the increase in ventilation frequency (6-fold), suggesting that the efficiency of O_2 transport across the intestine may improve during hypoxia. The increase in intestinal $\dot{M}_{O_2}$ might have been higher if the experimental apparatus could have limited hypoxia only to the water phase of the respirometer. In either case, an increased O_2 utilization under the present conditions could probably have been achieved by any number of factors, including increases in bulk flow of blood to the gut, recruitment of intestinal capillaries, or the blood–gas gradient across the intestinal wall.

Respiratory responses to a variety of ambient oxygen and carbon dioxide levels indicate that *M. anguillicaudatus* has a pronounced O_2 drive for intestinal but not branchial ventilation. A similar lack of aquatic ventilatory responses to hypoxia compared with strictly aquatic teleosts is clearly shown in a direct comparison of two closely related species of *Piabucina*, one bimodal and one aquatic, by Graham, Kramer & Pineda (1978*a*). A similar trend is also apparent for three cobitid species showing differential reliance upon air breathing (Jeuken, 1957). *M. anguillicaudatus* showed little (f_{int}) or no (f_{gill}) significant response to hypercapnic exposure (Fig. 10), indicating that CO_2 is a minor contributor to respiratory drive. Hypoxic ventilatory drive decreases as the hypercapnic drive increases in a series of vertebrates progressively more dependent on aerial respiration (see Johansen, 1970; Randall *et al.* 1981*a*; Glass & Wood, 1983). *M. anguillicaudatus* appears to bear features of respiratory control that represent a mid-point in this physiological continuum.

In summary, the intestinal gas exchanger of the Japanese weatherloach, *M. anguillicaudatus*, is revealed as a relatively efficient O_2 uptake system functioning as an accessory O_2 source when the animal is quiescent in aerated water. However,

the intestine assumes an increasingly important role under conditions of increased O_2 demand or reduced ambient O_2. In this respect, the alimentary canal emerges as a gas exchange organ of quantitatively similar performance to that of other structures used by fish for aerial gas exchange.

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