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Cardiorespiratory responses to hypercarbia in tambaqui *Colossoma macropomum*: chemoreceptor orientation and specificity

K. M. Gilmour[1,2,*], W. K. Milsom[1,3], F. T. Rantin[1], S. G. Reid[1,4] and S. F. Perry[1,2]

[1]*Department of Physiological Sciences, Federal University of São Carlos, Via Washington Luiz km 235, São Carlos, SP, 13565-905, Brazil*, [2]*Department of Biology, University of Ottawa, 150 Louis Pasteur, Ottawa, ON, K1N 6N5 Canada*, [3]*Department of Zoology, University of British Columbia, Vancouver, BC, V6T 1Z4 Canada* and [4]*Department of Life Sciences, University of Toronto at Scarborough, Toronto, ON, M1C 1A4 Canada*

*Author for correspondence (e-mail: Katie.Gilmour@science.uottawa.ca)

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Summary

Experiments were carried out to test the hypothesis that ventilatory and cardiovascular responses to hypercarbia (elevated water P_{CO_2}) in the tambaqui *Colossoma macropomum* are stimulated by externally oriented receptors that are sensitive to water CO_2 tension as opposed to water pH. Cardiorespiratory responses to acute hypercarbia were evaluated in both the absence and presence of internal hypercarbia (elevated blood P_{CO_2}), achieved by treating fish with the carbonic anhydrase inhibitor acetazolamide. Exposure to acute hypercarbia (15 min at each level, final water CO_2 tensions of 7.2, 15.5 and 26.3 mmHg) elicited significant increases in ventilation frequency (at 26.3 mmHg, a 42% increase over the normocarbic value) and amplitude (128%), together with a fall in heart rate (35%) and an increase in cardiac stroke volume (62%). Rapid washout of CO_2 from the water reversed these effects, and the timing of the changes in cardiorespiratory variables corresponded more closely to the fall in water P_{CO_2} (Pw_{CO_2}) than to that in blood P_{CO_2} (Pa_{CO_2}). Similar responses to acute hypercarbia (15 min, final Pw_{CO_2} of 13.6 mmHg) were observed in acetazolamide-treated (30 mg kg^{-1}) tambaqui. Acetazolamide treatment itself, however, increased Pa_{CO_2} (from 4.81±0.58 to 13.83±0.91 mmHg, mean ± S.E.M.; N=8) in the absence of significant change in ventilation, heart rate or cardiac stroke volume. The lack of response to changes in blood P_{CO_2} and/or pH were confirmed by comparing responses to the bolus injection of hypercarbic saline (5% or 10% CO_2; 2 ml kg^{-1}) into the caudal vein with those to the injection of CO_2-enriched water (1%, 3%, 5% or 10% CO_2; 50 ml kg^{-1}) into the buccal cavity. Whereas injections of hypercarbic saline were ineffective in eliciting cardiorespiratory responses, changes in ventilation and cardiovascular parameters accompanied injection of CO_2-laden water into the mouth. Similar injections of CO_2-free water acidified to the corresponding pH of the hypercarbic water (pH 6.3, 5.6, 5.3 or 4.9, respectively) generally did not stimulate cardiorespiratory responses. These results are in agreement with the hypothesis that in tambaqui, externally oriented chemoreceptors that are predominantly activated by increases in water P_{CO_2}, rather than by accompanying decreases in water pH, are linked to the initiation of cardiorespiratory responses to hypercarbia.

Key words: tambaqui, *Colossoma macropomum*, hypercarbia, blood pressure, ventilation, blood flow, acetazolamide, CO_2, pH.

Introduction

Renewed interest in CO_2/H^+ chemoreception in fish over the last few years has resulted in considerable advances. It is now clear that exposure to environmental hypercarbia (elevated water P_{CO_2}) initiates a host of cardiorespiratory adjustments in fish that do not simply reflect impairment of blood O_2 transport, as earlier work suggested (Randall, 1982; Smith and Jones, 1982). Rather, changes in CO_2 and/or pH can elicit cardiorespiratory responses directly, through interaction with specific CO_2/H^+ chemoreceptors. Interspecific variation in the particulars of such cardiorespiratory responses is high, and cardiovascular responses to hypercarbia, in particular, have been assessed in relatively few species. However, most fish examined to date exhibit a striking increase in ventilation (e.g. Dejours, 1973; Janssen and Randall, 1975; Smith and Jones, 1982; reviewed by Gilmour, 2001), together with a fall in heart rate (Perry et al., 1999; Reid et al., 2000; Sundin et al., 2000; McKendry et al., 2001; Perry and McKendry, 2001). Depending on the species, the hypercarbia-induced hyperventilation and bradycardia may also be accompanied by changes in blood pressure, cardiac output and systemic resistance (Perry et al., 1999; Reid et al., 2000; Sundin et al., 2000; McKendry et al., 2001; Perry and McKendry, 2001).

These cardiorespiratory responses to hypercarbia appear to be triggered by peripheral chemoreceptors that are located primarily, although probably not exclusively in at least some species (Reid et al., 2000; Milsom et al., 2002), on the gills. Bilateral denervation of the gills was sufficient to abolish most or all of the hypercarbia-induced changes in ventilation and/or cardiovascular variables in spiny dogfish *Squalus acanthias* (McKendry et al., 2001), channel catfish *Ictalurus punctatus* (Burleson and Smatresk, 2000), traira *Hoplias malabaricus* (Reid et al., 2000) and tambaqui *Colossoma macropomum* (Sundin et al., 2000; Milsom et al., 2002; Florindo et al., 2004). Similarly, bilateral extirpation of the first gill arch prevented or greatly attenuated ventilatory and cardiovascular responses to hypercarbia in rainbow trout *Oncorhynchus mykiss*, pointing to the first gill arch as the chief location of chemoreceptors involved in initiating cardiorespiratory responses to hypercarbia in this species (Perry and Reid, 2002).

The branchial chemoreceptors in rainbow trout, Atlantic salmon *Salmo salar* and dogfish appear to respond primarily to changes in water CO_2 tension specifically; neither alteration of water pH nor manipulation of blood P_{CO_2} were effective in triggering cardiorespiratory responses (Perry and McKendry, 2001; Perry and Reid, 2002). Although denervation studies have identified the gills as the principal location of the chemoreceptors that initiate cardiorespiratory responses to hypercarbia in tambaqui (Sundin et al., 2000; Milsom et al., 2002; Florindo et al., 2004), the orientation of these receptors (whether they preferentially detect water or blood) as well as their sensitivity to CO_2 *vs* H^+, remain uncertain. The tambaqui is a hypoxia-tolerant (P_{50}=2.4 mmHg; Brauner et al., 2001) and hypercarbia-tolerant neotropical fish species that is found throughout the Amazon basin, often in floodplain lakes that are subject to large variations in O_2, CO_2 and pH. Owing to the frequent occurrence of hypercarbic conditions in its natural environment (water total dissolved CO_2 may range from 0.82 to 1.79 mmol l^{-1} depending on season and depth; Reid et al., 2000), and an anatomy that renders this species amenable to surgical sectioning of nerves innervating selected chemosensory areas, the tambaqui has been the focus of a concerted research effort to identify the locations and roles of chemoreceptors involved in respiratory reflexes to both hypoxia and hypercarbia (Sundin et al., 2000; Milsom et al., 2002; Reid et al., 2003; Florindo et al., 2004). Previous studies have focused primarily on reflex changes in ventilation and heart rate during hypoxic or hypercarbic exposures without monitoring blood gas or acid–base status. Blood gas and acid–base data were reported by Wood et al. (1998), but in the context of acid–base regulation in response to an acid challenge, and so without cardiorespiratory data. Thus, the objective of the present study was to characterize more fully blood gas and acid–base status, as well as the cardiovascular responses to hypercarbia, while testing the hypothesis that cardiorespiratory responses to hypercarbia in tambaqui are triggered by externally oriented branchial chemoreceptors that react specifically to changes in water CO_2 tension.

Materials and methods

Experimental animals

Juvenile tambaqui *Colossoma macropomum* Cuvier (1271±53 g, mean ± S.E.M.; *N*=17) were obtained from CAUNESP (Aquaculture Centre of the São Paulo State University – UNESP), Jaboticabal, SP, Brazil, and transported to the Federal University of São Carlos. These fish were third- or fourth-generation descendents of native tambaqui taken from the Amazon in 1993 and introduced into the south-eastern region of Brazil for aquaculture. Tambaqui were maintained in large fibreglass aquaria supplied with aerated water from an artesian well. The tanks were located outdoors and so exposed to a natural photoperiod. Temperature was maintained at 25°C, and fish were fed to satiation every second day.

Surgery was carried out on fish anaesthetized by immersion in an aerated solution of benzocaine (ethyl-*p*-aminobenzoate; 100 mg l^{-1}) and then transferred to an operating table where the gills were irrigated continuously with a more dilute anaesthetic solution (50 mg l^{-1}). For monitoring of blood gas and acid–base status using an extracorporeal circulation (Thomas, 1994), the caudal artery and caudal vein were cannulated (Axelsson and Fritsche, 1994); in addition, the caudal artery cannula was used for measurements of blood pressure (P_{DA}) while the caudal vein cannula also served for saline or drug administration. Following exposure of the haemal arch by means of a lateral incision at the level of the caudal peduncle, flexible polyethylene tubing (Clay-Adams PE50, Becton-Dickenson and Co., Sparks, MD, USA) was inserted into the vessels in the anterior direction. Cannulae were filled with heparinized (100 i.u. ml^{-1} ammonium heparin) modified (4.5 mmol l^{-1} $NaHCO_3$) Cortland saline (Wolf, 1963) and flushed daily. To measure cardiac output, a 3S ultrasonic flow probe (Transonic Systems, Ithaca, NY, USA) was placed around the ventral aorta, which was accessed *via* an incision through the overlying epithelium within the opercular chamber. The operculum was reflected forward, and a small (~1.5 cm) incision was made parallel to the ventral aorta in the epithelium near the isthmus. Blunt dissection exposed the ventral aorta, and the flow probe was then placed around the vessel using lubricating jelly (K-Y Personal Lubricant; Johnson and Johnson, Montréal, Canada) as an acoustic couplant. With this approach, disruption of the pericardium was avoided. Both incisions were closed, and the cannulae and flow probe lead were secured to the skin, with silk sutures. Ventilation was assessed by suturing brass plates (1 cm^2) to the external surface of each operculum to measure breath-by-breath displacement of the opercula with an impedance converter. Finally, two holes were drilled through the snout between the nostrils using a Dremel tool, and a flared cannula (PE 160) was fed through each hole and secured in place with a cuff. These cannulae were used to deliver CO_2-equilibrated or acidified water into the flow of inspired ventilatory water. After surgery, fish were revived and transferred to individual holding boxes of opaque acrylic provided with flowing, aerated water for at least 24 h of recovery before experimentation.

Experimental protocol

Experiments commenced with a 5 min 'pre' period of recording baseline ventilation and cardiovascular parameters. Tambaqui were then subjected to a series of injections of CO_2-equilibrated or acidified water (into the inspired water stream), and CO_2-equilibrated saline (into the caudal vein), with 4 or 6 min intervals between injections. Water injections (50 ml kg^{-1}) were delivered over a 20 s period into a snout cannula, and included aerated but otherwise untreated water (control) and water pre-equilibrated with 1%, 3%, 5% or 10% CO_2 in air. Measurement of pH for water equilibrated to each of these CO_2 levels revealed values of 6.3, 5.6, 5.3 and 4.9 pH units, respectively. To differentiate between CO_2-induced cardiorespiratory effects and those triggered by the concomitant elevation of H^+, each injection of CO_2-equilibrated water was followed by an injection of water titrated with HCl to the corresponding pH value; vigorous aeration before and after addition of HCl ensured removal of CO_2. Saline (control), or saline equilibrated with 5% or 10% CO_2 in air, was delivered as a bolus (2 ml kg^{-1} over 20 s) into the caudal vein. Because CO_2-enriched saline injections were without effect (see Results), there was no further attempt to dissect the relative roles of CO_2 *vs* H^+.

Following the injection series, the extracorporeal blood circulation (see below) was initiated. Once the extracorporeal blood shunt was established and the measured variables had stabilized, baseline conditions for blood gases and acid–base status as well as ventilation and cardiovascular parameters under normoxic normocarbic conditions were recorded over a 5 min 'pre' period. Blood pressure was monitored at regular (2–5 min) intervals by briefly (for 10–15 s) switching the caudal artery cannula from the extracorporeal blood loop to a pressure transducer using a T-junction and three-way valve. The water supplying the fish box was then rendered progressively hypercarbic by gassing a water equilibration column with 1%, 3%, and then 5% CO_2 in air (Cameron flowmeter model GF-3/MP, Port Aransas, TX, USA) for 15 min at each level. Using this protocol, the final water P_{CO_2} (Pw_{CO_2}) values achieved at each step were 7.2±0.5, 15.5±0.9 and 26.3±2.5 mmHg, respectively (mean ± S.E.M., N=11). At the end of the final hypercarbic exposure, Pw_{CO_2} within the experimental chamber was rapidly returned to normocarbic conditions ('washed out') by increasing the flow of air-equilibrated water to the fish box.

Finally, the carbonic anhydrase inhibitor acetazolamide was used to investigate the cardiorespiratory effects of external *vs* internal hypercarbia. After the 5 min 'pre' period of recording baseline conditions, acetazolamide (30 mg kg^{-1}) was administered *via* the caudal vein cannula and blood acid–base and cardiorespiratory parameters were monitored for 30 min. Fish were then exposed to hypercarbia by gassing the water equilibration column with 3% CO_2 in air (final Pw_{CO_2}=13.6±0.5, N=7), and at the end of the 15 min hypercarbic exposure, CO_2 was again rapidly washed out of the system by increasing the flow of air-equilibrated water to the fish box. Acetazolamide was prepared by dissolving the drug in saline with added NaOH and then slowly titrating the pH down to a level as close as possible to physiological (final pH of the acetazolamide solution was approximately 8.5).

Analytical techniques

An extracorporeal blood circulation (Thomas, 1994) was used to continuously monitor blood gas and acid–base variables. Blood was withdrawn at a rate of 0.5 ml min^{-1} from the caudal artery cannula using a peristaltic pump, and passed through an external circuit (of ~1 ml volume) containing P_{O_2}, P_{CO_2} and pH electrodes before being returned to the fish *via* the caudal vein cannula. To prevent clotting, the circuit was rinsed with heparinised (540 i.u. ml^{-1}) saline for 10–15 min prior to initiating blood flow. Arterial blood pH (pHa), P_{CO_2} (Pa_{CO_2}) and P_{O_2} (Pa_{O_2}) were measured using Metrohm (model 6.0204.100, Brinckman Instruments, Canada, Ltd., Mississuaga, ON, Canada; pH) and Cameron Instruments (CO_2, O_2) electrodes housed in thermostatted cuvettes and connected to a blood gas analyser (BGM 200; Cameron Instruments). A second peristaltic pump was used to withdraw water at a rate of 3.5 ml min^{-1} from the mouth of the fish *via* one of the two buccal cannulae. This water was passed through thermostatted cuvettes containing pH (Metrohm model 6.0204.100) and P_{CO_2} (Cameron Instruments) electrodes connected to a blood gas analyser (Cameron Instruments) for the measurement of water pH (pHw) and Pw_{CO_2}. Before each experiment, the pH electrodes were calibrated by pumping precision buffer solutions through the circuits until stable readings were recorded. A similar procedure was used to calibrate the O_2 and CO_2 electrodes with a zero solution (2 g l^{-1} sodium sulphite; O_2 electrode only) and/or water equilibrated with appropriate gas mixtures (supplied by a GF-3/MP gas mixing flowmeter; Cameron Instruments).

Blood pressure was measured by connecting the caudal artery cannula to a pressure transducer (Model 1050BP, UFI, Morro Bay, CA, USA) linked to an amplifier (Biopac DA 100, Santa Barbara, CA, USA). The pressure transducer was calibrated daily against a static column of water. Blood flow was determined by attaching the factory-calibrated ultrasonic flow probe to a blood flowmeter (model T106; Transonic Systems, Ithaca, NY, USA). The frequency and amplitude of opercular displacements were monitored as indices of ventilation using an impedance converter (model 911, Biocom Inc; Culver City, CA, USA) that detected and quantified the changes in impedance between the brass plates attached to the opercula (Peyraud and Ferret-Bouin, 1960).

A data acquisition system (Biopac Systems) with Acknowledge data acquisition software (sampling rate set at 40 Hz) and a personal computer were used to convert all analogue signals (blood gases and pH, water P_{CO_2} and pH, blood pressure, blood flow and impedance recordings) to digital data. With this system, continuous data recordings were obtained for Pa_{CO_2}, Pa_{O_2}, pHa, Pw_{CO_2}, pHw, mass-specific blood flow ($\dot{V}$b), heart rate (f_H; automatic rate calculation from the pulsatile $\dot{V}$b (trace), mean P_{DA} (arithmetic mean), systemic

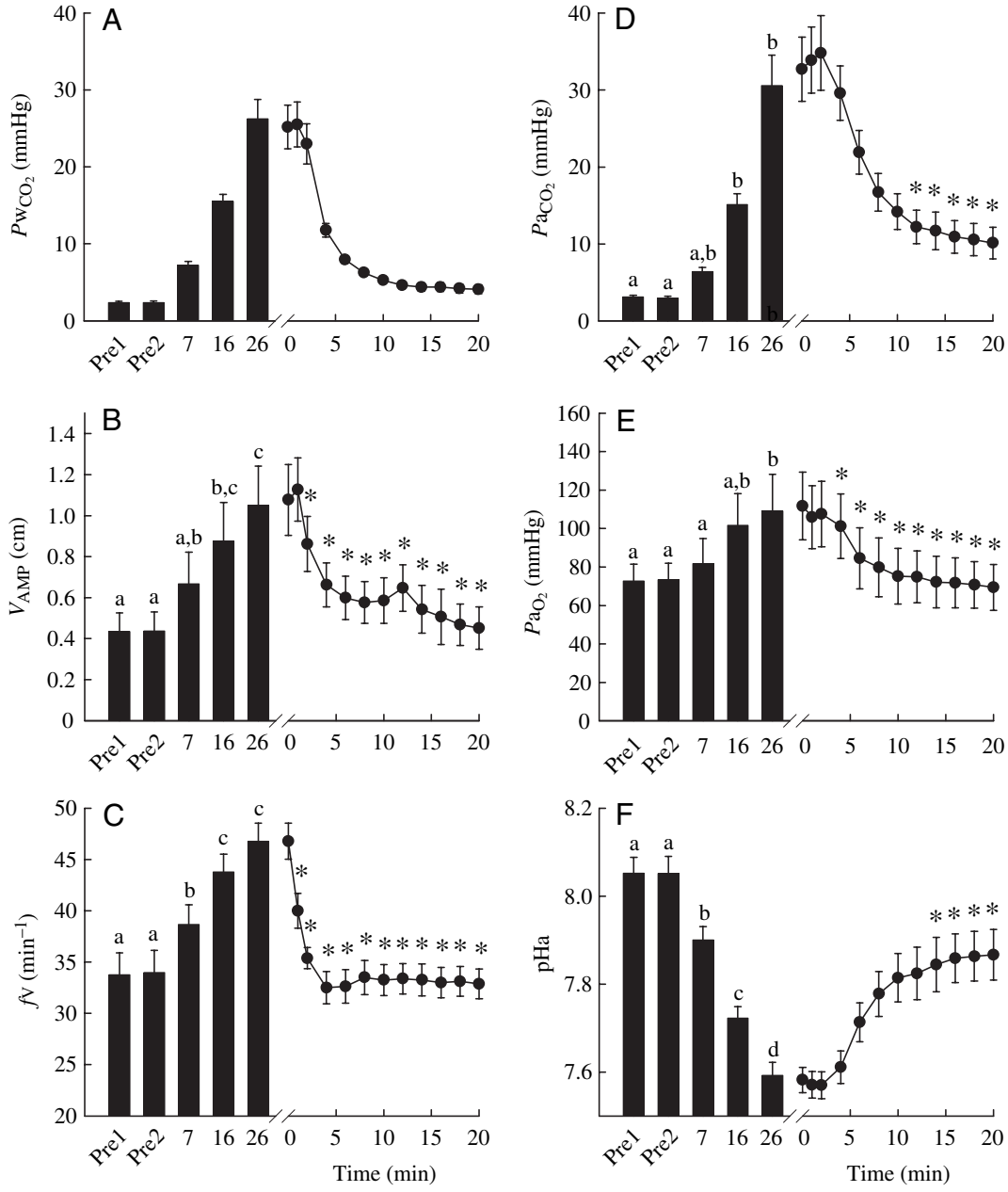

Fig. 1. The effects of stepwise increases in water P_{CO_2} (P_{wCO_2}) followed by the rapid lowering of P_{wCO_2} (A) on ventilation, blood gases, and acid–base status in tambaqui *Colossoma macropomum*, including (B) ventilation amplitude (V_{AMP}; N=9–11), (C) ventilation frequency (f_V; N=8–9), (D) arterial P_{CO_2} (P_{aCO_2}; N=8–9), (E) arterial P_{O_2} (P_{aO_2}; N=7–8) and (F) arterial pH (pHa; N=8–9). Pre1 and Pre2 refer to pre-exposure values compiled 2 min and immediately, respectively, before the onset of hypercarbia; the numbers 7, 16 and 26 denote values compiled for the final 2 min of the three discrete steps of increasing hypercarbia; and the data plotted to the right of the break in the *x*-axis represent values compiled for 2 min intervals after the initiation of rapid washout of CO_2 at 0 min. The data are presented as means ± 1 S.E.M. For stepwise increases in P_{wCO_2}, values that do not share a letter are significantly different from one another (one-way RM-ANOVA, P<0.001 for B–D and F; P=0.001 for E). For the rapid washout of water CO_2, asterisks indicate values that are significantly different from the value at time=0 min (one-way RM-ANOVA, P<0.001 for all).

vascular resistance (R_s; $P_{DA}/\dot{V}b$ and ventilation amplitude (V_{AMP}; the difference between maximum and minimum impedances). Note that P_{DA} and R_s were recorded only intermittently in experiments where the extracorporeal blood shunt was utilised. In addition, cardiac stroke volume (V_S) was calculated by dividing mass-specific blood flow by heart rate, and ventilation frequency (f_V) was determined from the impedance trace.

Statistical analyses

Data are reported as means ± 1 S.E.M. For experiments involving water or saline injections, mean ventilatory and cardiovascular data were compiled for 10 s intervals over the 20 s before and 100 s after the injection, except during the injection itself. For experiments employing the extracorporeal blood circulation, mean blood gas, water gas, acid–base, ventilatory and cardiovascular data were compiled over 2 min periods at selected intervals, apart from P_{DA} and R_s, for which data were compiled only during the 10–15 s periods of recording. Data were analysed for statistical significance by one-way repeated measures analysis of variance (RM-ANOVA) followed by *post hoc* multiple comparisons using the Holm–Sidak method, as appropriate. Where assumptions of normality or equal variance were violated, equivalent non-parametric analyses were employed. The commercial package SigmaStat v3.0 (SPSS Inc.) was used to carry out statistical analyses, and the fiducial limit of significance in all cases was 5%.

Results

Exposure to stepwise hypercarbia

Exposure of tambaqui (N=11) to three stepwise increases in water CO_2 tension to achieve P_{W,CO_2} values of 7.2±0.5 (1% CO_2 delivered to the equilibration column), 15.5±0.9 (3% CO_2 delivered to the equilibration column) and 26.3±2.5 mmHg (5% CO_2 delivered to the equilibration column) within 15 min elicited a significant respiratory acidosis (Fig. 1D,F), together with a marked hyperventilation (Fig. 1B,C) accompanied by bradycardia (Fig. 2E). The changes were in general P_{CO_2}-dependent, with V_{AMP} increasing to a greater extent than f_V (e.g. 128% *vs* 42% increases, respectively, at the highest P_{W,CO_2}). The significant increases in P_{a,O_2} at the higher water CO_2 tensions (Fig. 1E) likely reflected this hyperventilation. Although heart rate was lowered (by 18–35%) during hypercarbia, blood flow was maintained (Fig. 2D) because of a significant rise in cardiac stroke volume (17–62%; Fig. 2F). Neither arterial blood pressure nor systemic vascular resistance were affected by exposure to hypercarbia (Fig. 2B,C).

Rapid removal of CO_2 from the water resulted in the equally rapid return of ventilation (Fig. 1B,C), heart rate (Fig. 2E) and stroke volume (Fig. 2F) towards pre-exposure levels. Blood flow increased significantly during the rapid washout, by 16–23% (Fig. 2D), owing to the persistent elevation of cardiac stroke volume. The greater blood flow was accompanied by a significant lowering of systemic resistance (Fig. 2C). Although blood gas and acid–base variables also recovered during the lowering of water CO_2 (Fig. 1D–F), these changes were slower to occur, such that even 20 min after initiation of the rapid washout, P_{a,CO_2} remained significantly (Wilcoxon

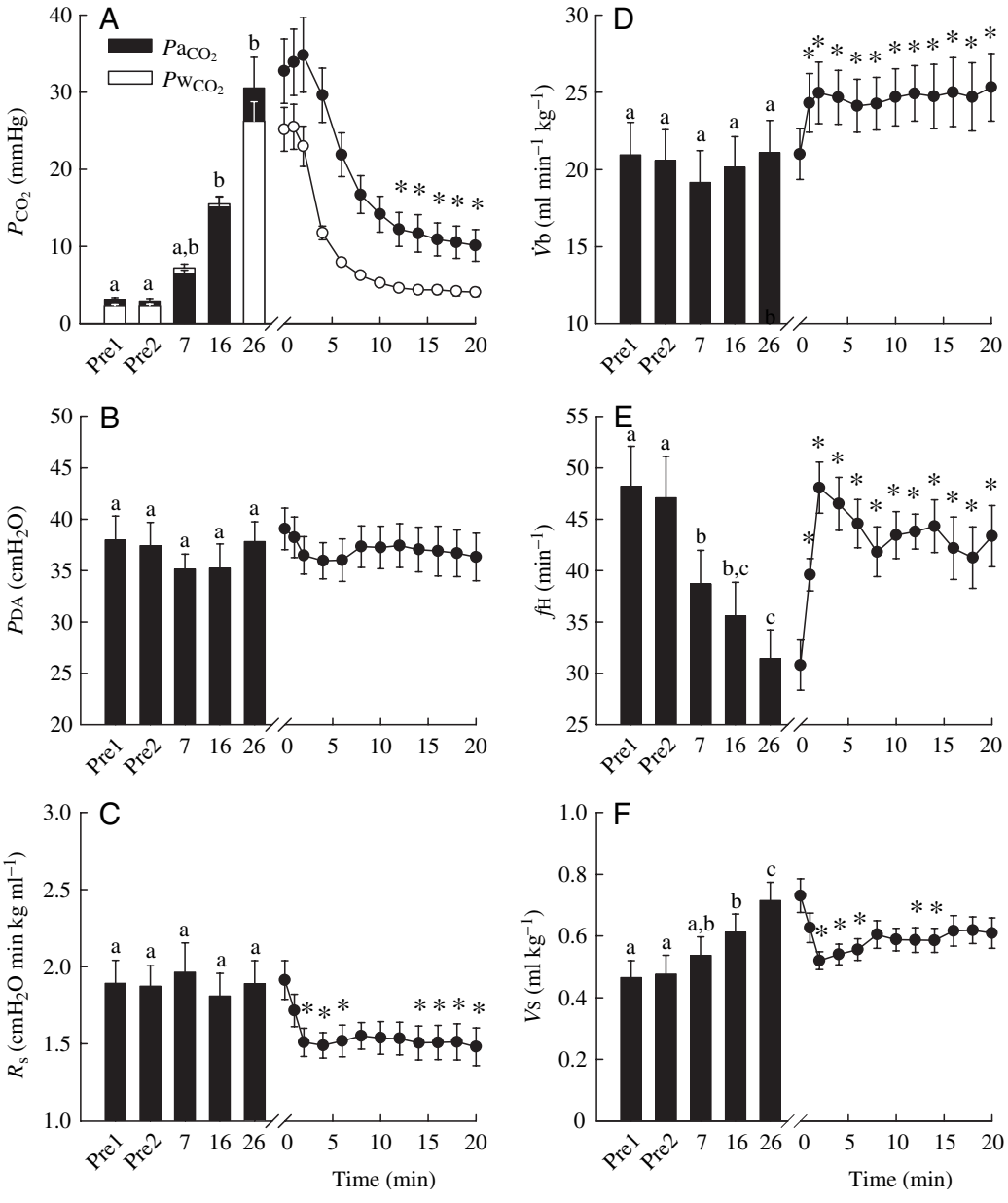

Fig. 2. The effects of stepwise increases in water P_{CO_2} (P_{W,CO_2}) followed by the rapid lowering of P_{W,CO_2} (A) on cardiovascular variables in tambaqui *Colossoma macropomum* including (B) arterial blood pressure (P_{DA}; N=10–11), (C) systemic vascular resistance (R_S; N=9–10), (D) cardiac output ($\dot{V}b$; N=8–10), (E) heart rate (f_H; N=9–11) and (F) cardiac stroke volume (V_S; N=9–10). Data for P_{a,CO_2} are replotted from Fig. 1 in A for ease of comparison. Pre1 and Pre2 refer to pre-exposure values compiled 2 min and immediately, respectively, before the onset of hypercarbia; the numbers 7, 16 and 26 denote values compiled for the final 2 min of the three discrete steps of increasing hypercarbia; and the data plotted to the right of the break in the x-axis represent values compiled for 2 min intervals after the initiation of rapid washout of CO_2 at 0 min. The data are presented as means ± 1 S.E.M. For stepwise increases in P_{W,CO_2}, values that do not share a letter are significantly different from one another (one-way RM-ANOVA; P values: B, 0.386; C, 0.892; D, 0.144; E and F, <0.001). For the rapid washout of water CO_2, asterisks indicate values that are significantly different from the value at time=0 min (one-way RM-ANOVA, P values: B, 0.444; C, 0.002; D–F, <0.001).

signed rank test, P=0.008) elevated over the pre-exposure value by 6.62±2.20 mmHg (N=8), while pHa was depressed by 0.18±0.04 units (N=8). Notably, neither ventilation amplitude (Wilcoxon signed rank test, P=0.469) nor frequency (paired Student's t-test, P=0.094) was significantly different from the baseline value at this time, suggesting that ventilation, at least, was more sensitive to changes in $P_{w\mathrm{CO_2}}$ than to those in $P_{a\mathrm{CO_2}}$.

Acetazolamide treatment

This observation was confirmed by administration of the carbonic anhydrase inhibitor acetazolamide, a treatment that generated a significant respiratory acidosis (Fig. 3D,F) in the absence of any change in $P_{w\mathrm{CO_2}}$ (Fig. 3A). A single bolus injection of acetazolamide caused $P_{a\mathrm{CO_2}}$ to increase approximately threefold over the subsequent 30 min, at the same time lowering pHa by 0.26±0.02 units (N=8), yet was without significant effect on ventilation (Fig. 3B,C) or most cardiovascular variables (Fig. 4); systemic resistance (Fig. 4C) and cardiac stroke volume (Fig. 4F) did differ statistically from the pre-injection value at single points in each case. By contrast, exposure of acetazolamide-treated fish to 15 min of hypercarbia (first broken line), in which $P_{w\mathrm{CO_2}}$ reached 13.6±0.5 mmHg, produced changes in ventilation and cardiovascular variables that were virtually identical to those observed in untreated fish exposed to a similar level of hypercarbia, even though the respiratory acidosis was more profound in treated fish; V_{AMP} and f_V rose by 110% and 24%, respectively, and heart rate fell by 20% (Fig. 4E), but blood flow was maintained (Fig. 4D) owing to the concomitant 19%

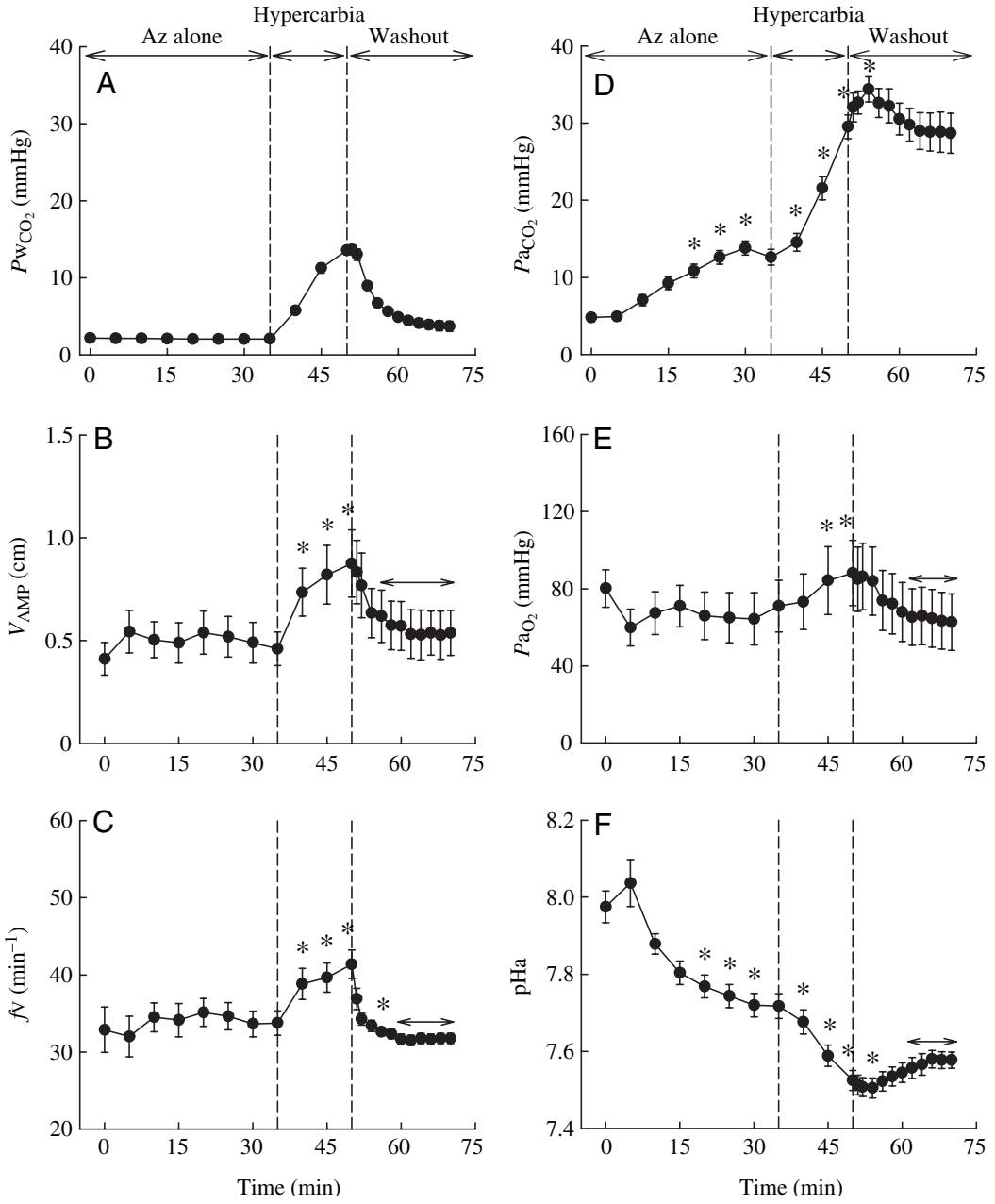

Fig. 3. The effects of acetazolamide (Az) injection followed by hypercarbia ($P_{w\mathrm{CO_2}}$=13.6 mmHg) and the rapid lowering of $P_{w\mathrm{CO_2}}$ (A) on ventilation and blood gas and acid–base status in tambaqui *Colossoma macropomum*, including (B) ventilation amplitude (V_{AMP}; N=8), (C) ventilation frequency (f_V; N=8), (D) arterial $P_{\mathrm{CO_2}}$ ($P_{a\mathrm{CO_2}}$; N=8), (E) arterial $P_{\mathrm{O_2}}$ ($P_{a\mathrm{O_2}}$; N=6–7), and (F) arterial pH (pHa; N=8). Acetazolamide was injected at time=0 min, while the onset of hypercarbia and rapid water CO_2 washout are designated by the vertical broken lines. Values are means ± 1 S.E.M. Significant differences from the pre-injection (time=0 min) or pre-hypercarbic exposure (time= 35 min) values are indicated by an asterisk (one-way RM-ANOVA; P values for acetazolamide treatment and hypercarbia, respectively: B, 0.127 and 0.002; C, 0.569 and <0.001; D, <0.001 for both; E, 0.241 and 0.028; F, <0.001 for both). For the rapid lowering of $P_{w\mathrm{CO_2}}$, significant differences from the initial value (time=50 min) are indicated by an asterisk or double-arrowhead line (one-way RM-ANOVA with P values <0.001 for all).

increase in stroke volume. A small and transient increase in blood pressure also occurred (Fig. 4B); this response was not observed in untreated fish.

The recovery of ventilation and cardiovascular variables in response to the rapid lowering of $P_{W_{CO_2}}$ (second broken line) was particularly striking in acetazolamide-treated fish because it occurred on the backdrop of a sustained, severe, respiratory acidosis (Figs 3 and 4). The significant decreases in ventilation variables (Fig. 3B,C) and stroke volume (Fig. 4F) as well as the rise in heart rate (Fig. 4E) tracked changes in $P_{W_{CO_2}}$ rather than $P_{a_{CO_2}}$ (Fig. 4A). The lack of correspondence between V_{AMP} and $P_{a_{CO_2}}$ is illustrated by a representative data recording for an individual fish (Fig. 5). As in untreated fish, blood flow increased significantly with the onset of rapid water CO_2 washout (Fig. 4D), but the extent of the blood flow increase (7–13%) was smaller in acetazolamide-treated tambaqui and was not accompanied by a significant decrease in systemic resistance (Fig. 4C).

Injections of CO_2-enriched saline

As a final test of the potential for changes in blood CO_2 tension to elicit cardiorespiratory responses, tambaqui were injected with saline equilibrated with 5% or 10% CO_2 in air. Assuming complete mixing of the saline bolus (2 ml kg^{-1} delivered over 20 s) with venous blood, estimating venous P_{CO_2} to be approximately 4 mmHg (based on the measured $P_{a_{CO_2}}$ of ~3mmHg), and using the cardiac output measured under resting conditions (21 ml min^{-1} kg^{-1}), these internal injections of 5% or 10% CO_2-equilibrated saline would be expected to yield transient P_{CO_2} values of 11.5 or 20mmHg,

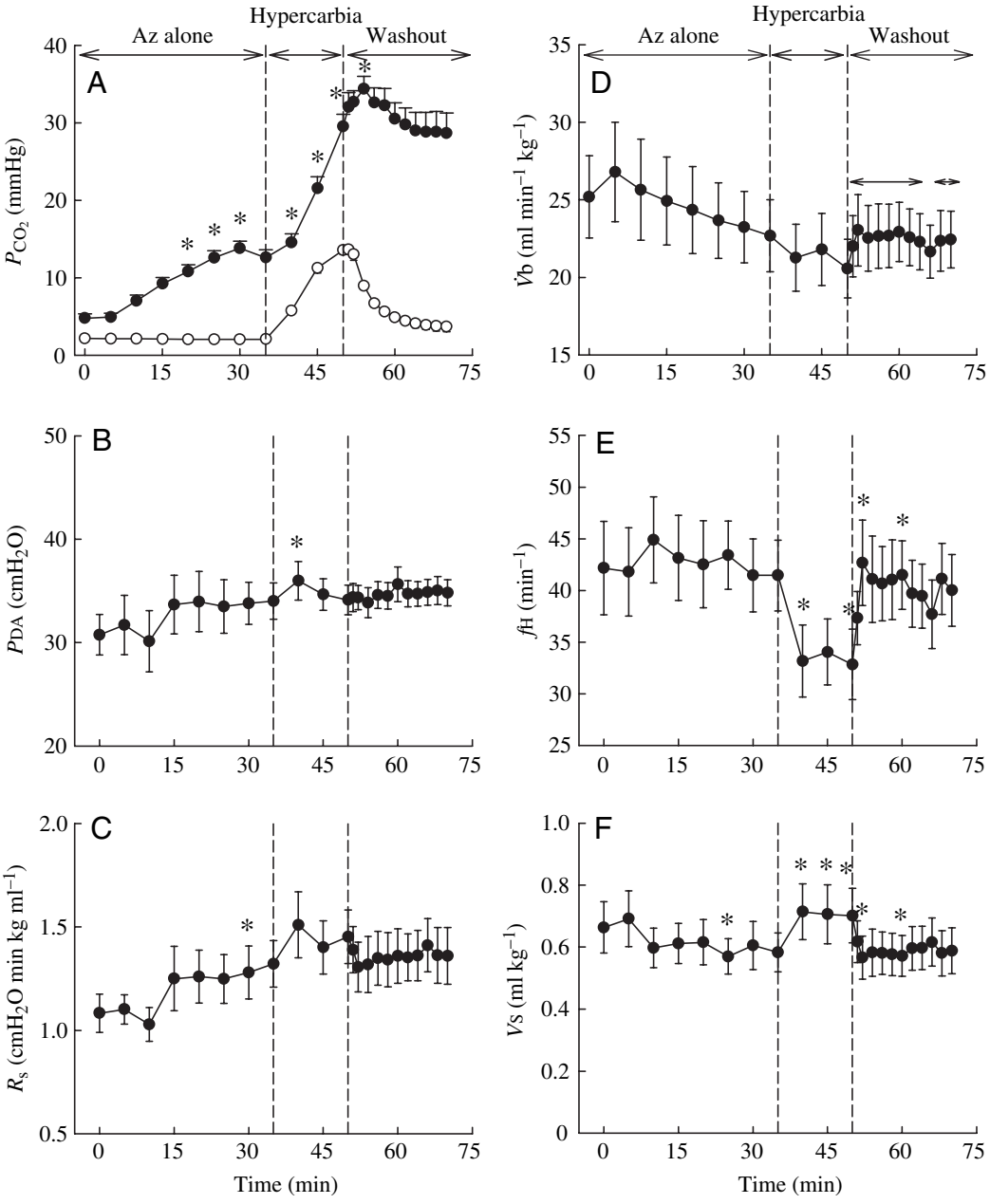

Fig. 4. The effects of acetazolamide (Az) injection followed by hypercarbia ($P_{W_{CO_2}}$=13.6 mmHg) and the rapid lowering of $P_{W_{CO_2}}$ (A) on cardiovascular variables in tambaqui *Colossoma macropomum*, including (B) arterial blood pressure (P_{DA}; N=7–8), (C) systemic vascular resistance (R_S; N=6–7), (D) cardiac output ($\dot{V}b$; N=7), (E) heart rate (f_H; N=8), and (F) cardiac stroke volume (V_S; N=7). Data for $P_{a_{CO_2}}$ are replotted from Fig. 3 in A for ease of comparison. Acetazolamide was injected at time=0 min, while the onset of hypercarbia and rapid water CO_2 washout are designated by the vertical broken lines. Values are means ± 1 S.E.M. Significant differences from the pre-injection (time= 0 min) or pre-hypercarbic exposure (time=35 min) values are indicated by an asterisk (one-way RM-ANOVA; P values for acetazolamide treatment and hypercarbia, respectively: B, 0.248 and 0.045; C, 0.050 and 0.116; D, 0.016 and 0.134; E, 0.291 and 0.012; F, 0.038 and 0.006). For the rapid lowering of $P_{W_{CO_2}}$, significant differences from the initial value (time= 50 min) are indicated by an asterisk or double-arrowhead line (one-way RM-ANOVA with P values: B, 0.281; C, 0.107; D, 0.011; E, 0.042; F, 0.017).

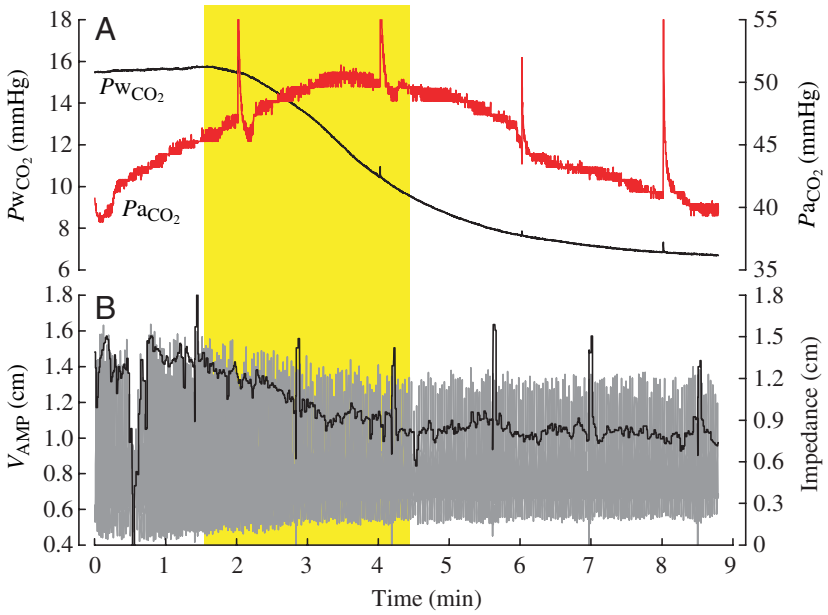

Fig. 5. Representative data acquisition traces illustrating the lack of correspondence between changes in arterial P_{CO_2} (Pa_{CO_2}; red trace, A), and those in ventilation, presented as the raw impedance trace (grey, B) overlain by the calculated ventilation amplitude (V_{AMP}) trace (black line, B). Rather, as the yellow area emphasizes, ventilation tracked changes in water P_{CO_2} (Pw_{CO_2}; black line, B).

respectively, in the blood at the gill. Increases in blood pressure (7–11%), blood flow (20–24%) and systemic resistance (6–11%) occurred in response to these injections but were attributable to volume loading, since similar increases (9%, 31% and 4%, respectively) were also observed upon injection of air-equilibrated saline (Table 1). No specific effect of internally injected CO_2 was detected for any measured variable (Table 1).

Injections of CO_2-enriched or acidified water

The effects of injecting CO_2-enriched water into the flow of ventilatory water were compared with reactions to the injection of acidified water to distinguish between the roles of CO_2 and H^+ in eliciting cardiorespiratory responses to hypercarbia. A marked, P_{CO_2}-dependent bradycardia accompanied the injection of CO_2-enriched water into the buccal cavity of tambaqui (Fig. 6A–D). At the peak of the response (~20–30 s after beginning the injection), f_H was decreased by 20–49% for injections of water equilibrated with 1–10% CO_2. Because cardiac stroke volume was maintained or increased only slightly (data not shown), the bradycardia resulted in significant 14–45% reductions in cardiac output with injection of all levels of CO_2-enriched water (Table 2). The fish also exhibited significant P_{CO_2}-dependent increases in systemic resistance (Fig. 6E–H), which were reflected in significant increases in blood pressure (Table 2). The different responses of systemic resistance to bolus injections of CO_2-enriched water (Fig. 6E–H) *vs* exposure to hypercarbic water (Figs 2C, 4C) were striking. By contrast with the effect of CO_2, injection of acidified water was generally without significant effect, apart from a small (11%) and transient depression of heart rate that occurred only in response to injection of the most acidic (pH 4.9) water (Fig. 6C), and a correspondingly slight 5% depression of cardiac output (Table 2). Ventilatory responses to CO_2-enriched water injections were more sporadic; V_{AMP} increased significantly only with injection of 10% CO_2, while significant frequency responses were limited to injections of 5% and 10% CO_2 (Table 2). Responses to the parallel injections of acidified water were either insignificant or of substantially lower magnitude (Table 2). Injection of air-equilibrated water was without significant effect in all cases (data not shown).

Table 1. *The effects in tambaqui* Colossoma macropomum *of internal injections of CO_2-enriched saline or saline alone on selected cardiorespiratory variables*

	Saline (N=7)			5% CO_2 (N=6)			10% CO_2 (N=6)		
	Pre	Peak	P	Pre	Peak	P	Pre	Peak	P
P_{DA} (cmH_2O)	35.5±1.6	38.7±1.7	0.021	35.6±1.4	39.4±1.6	0.09	35.5±1.4	38.1±1.6	0.32
$\dot{V}b$ (ml min^{-1} kg^{-1})	20.4±2.0	26.9±2.8	<0.001	23.9±2.9	28.4±3.2	0.015	24.1±2.5	30.1±3.6	0.016
R_s (cm H_2O min kg ml^{-1})	1.82±0.13	1.87±0.16	0.012	1.61±0.20	1.77±0.19	0.013	1.50±0.13	1.59±0.15	0.008
f_H (min^{-1})	34.5±3.9	26.9±2.8	0.003	33.1±4.7	25.9±3.2	0.74	32.0±4.1	27.9±3.6	0.67
V_{AMP} (cm)	0.30±0.06	0.40±0.07	0.008	0.35±0.08	0.41±0.08	0.11	0.34±0.08	0.39±0.05	0.43

P_{DA}, arterial blood pressure; $\dot{V}b$, cardiac output; R_s, systemic vascular resistance; f_H, heart rate; V_{AMP}, ventilation amplitude.

Saline was enriched with either 5% or 10% CO_2.

'Pre', data compiled for 10 s prior to the injection; 'Peak', the 10 s interval during the 100 s post injection, for which the greatest response was detected; '*P*', the *P* value for the one-way RM-ANOVA carried out on the full data set (i.e. the values for the 20 s prior to and 100 s post saline injection).

Values are means ± 1 S.E.M.

Discussion

Previous studies of CO_2/pH chemoreception in tambaqui focused on determining the location and innervation of the receptors that mediate the cardiorespiratory responses to hypercarbia (Sundin et al., 2000; Milsom et al., 2002; Florindo et al., 2004). Tambaqui, like other exclusively water-breathing fish (reviewed by Milsom, 2002), appear to lack central CO_2/pH chemoreceptors (Milsom et al., 2002). Using the elimination of hypercarbic cardiorespiratory responses upon denervation as the criterion for involvement of a particular population of peripheral receptors, a predominantly branchial location for CO_2/pH chemoreceptors was revealed (Sundin et al., 2000; Milsom et al., 2002; Florindo et al., 2004). Cardiac and ventilation rate responses, in particular, were attributed to receptors that were exclusively branchial, with the hypercarbic bradycardia mediated by receptors confined to the first gill arch, and the increase in breathing frequency by receptors distributed across all gill arches (Sundin et al., 2000). In contrast, the persistence of blood pressure and, to some extent, ventilation amplitude responses to hypercarbia in tambaqui subjected to total gill denervation suggested the involvement of extrabranchial receptors (Sundin et al., 2000; Florindo et al., 2004). The findings of the present study add to this existing information on receptor location by characterizing the orientation of the CO_2/pH chemoreceptors in tambaqui as well as their specificity for CO_2 *vs* H^+.

The cardiorespiratory responses elicited by hypercarbia may be initiated by branchial chemoreceptors that

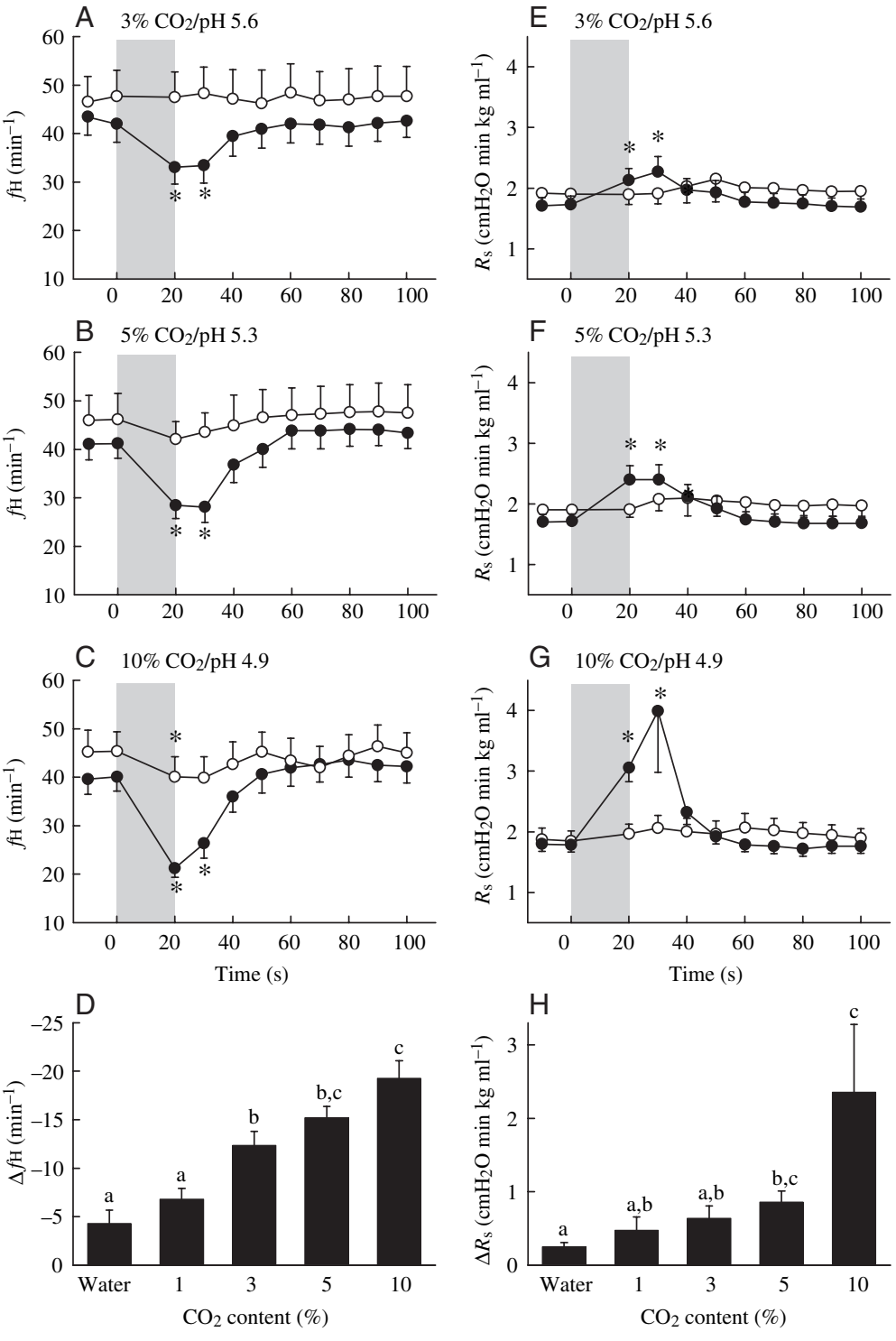

Fig. 6. (A–C,E–G) The effects of external injections of CO_2-enriched water (black circles) or CO_2-free acidified water (white circles) on heart rate (f_H; A–C; N=16–17 for CO_2-enriched water and N=7 for acidified water) and systemic vascular resistance (R_s; E–G; N=14 for CO_2-enriched water and N=6 for acidified water) in tambaqui *Colossoma macropomum*. The shaded areas represent the 20 s period of injection. (D,H) The peak changes in f_H (Δf_H; D) and R_s (ΔR_s; H) for injections of air-equilibrated water and water equilibrated with 1, 3, 5 or 10% CO_2 in air. Values are means ± 1 S.E.M. An asterisk denotes a statistically significant difference from the initial pre-injection value (one-way RM-ANOVA; P<0.001 for all CO_2 injections; P=0.016 for injection of acidified water on f_H in C). For peak responses, groups that do not share a letter are significantly different from one another (one-way RM-ANOVA; P<0.001 for both).

Table 2. *The effects in tambaqui* Colossoma macropomum *of external injections of CO_2-enriched water or CO_2-free acidified water on selected cardiorespiratory variables*

	Hypercarbic water			Acidified water			
Treatment	Pre	Peak	*P*	Treatment pH	Pre	Peak	*P*
$\dot{V}$b (ml min^{-1} kg^{-1})							
3% CO_2/pH 5.6	22.9±2.1 (14)	18.5±2.1 (14)	<0.001	5.6	20.8±3.6 (6)	20.2±3.7 (6)	0.73
5% CO_2/pH 5.3	22.5±1.7 (15)	16.6±1.8 (15)	<0.001	5.3	20.8±3.2 (6)	19.2±3.1 (6)	0.54
10% CO_2/pH 4.9	22.2±1.9 (14)	12.4±1.5 (14)	<0.001	4.9	20.6±3.3 (6)	19.7±3.3 (6)	0.032
P_{DA} (cmH_2O)							
3% CO_2/pH 5.6	35.9±1.4 (16)	38.7±1.8 (16)	<0.001	5.6	37.9±3.0 (7)	39.2±2.7 (7)	0.69
5% CO_2/pH 5.3	35.6±1.3 (16)	39.0±1.7 (16)	<0.001	5.3	38.3±3.1 (7)	40.5±3.7 (7)	0.16
10% CO_2/pH 4.9	37.6±2.0 (16)	41.9±2.2 (16)	<0.001	4.9	37.3±3.1 (7)	39.7±2.1 (7)	0.29
V_{AMP} (cm)							
3% CO_2/pH 5.6	0.47±0.09 (13)	0.57±0.09 (13)	0.91	5.6	0.43±0.19 (6)	0.51±0.19 (6)	0.97
5% CO_2/pH 5.3	0.42±0.09 (15)	0.57±0.10 (15)	0.45	5.3	0.49±0.19 (6)	0.52±0.20 (6)	0.04
10% CO_2/pH 4.9	0.45±0.10 (14)	0.70±0.11 (14)	0.004	4.9	0.52±0.19 (7)	0.63±0.21 (7)	0.53
fV (min^{-1})							
3% CO_2/pH 5.6	26.1±3.3 (14)	28.6±3.7 (14)	0.85	5.6	26.2±3.2 (6)	30.2±4.9 (6)	0.63
5% CO_2/pH 5.3	28.3±2.9 (15)	32.3±3.4 (15)	0.011	5.3	28.7±3.8 (6)	33.0±4.2 (6)	0.65
10% CO_2/pH 4.9	26.9±3.2 (14)	35.9±4.2 (14)	0.02	4.9	30.0±4.4 (6)	32.7±4.2 (6)	0.026

$\dot{V}$b, cardiac output; P_{DA}, arterial blood pressure; V_{AMP}, ventilation amplitude; fV, ventilation frequency.

'Pre', data compiled for 10 s prior to the injection; 'Peak', the 10 s interval during the 100 s post injection, for which the greatest response was detected; '*P*', the *P* value for the one-way RM-ANOVA carried out on the full data set (i.e. the values for the 20 s prior to and 100 s post saline injection). The fact that the full time-course data set, not the peak responses, were analysed statistically accounts for some apparent discrepancies in the data presented in this table, such as the (apparently) larger but insignificant V_{AMP} change with 5% CO_2 *vs* the smaller but significant change with acidified (pH 5.3) water.

Values are means ± 1 S.E.M. (*N*).

detect changes in water CO_2/pH, and/or by receptors that monitor blood CO_2/pH levels, because exposure to elevated ambient CO_2 causes a rise in blood P_{CO_2} and a concomitant fall in blood pH (Fig. 1). Thus, three experimental approaches were employed to discern between external and internal orientation of the branchial CO_2/pH receptors. First, tambaqui were treated with acetazolamide to inhibit red blood cell carbonic anhydrase activity. Assuming that CO_2 excretion in tambaqui follows the pathway mapped out for teleost fish in general (e.g. Perry, 1986; Tufts and Perry, 1998), carbonic anhydrase will catalyze the interconversion of CO_2 and HCO_3^- within the red blood cell, a step that is critical to the transfer of CO_2 from tissues to blood, and from blood to ventilatory water. In acetazolamide-treated fish, this step would be slowed to the uncatalyzed rate, thereby causing CO_2 retention (Henry and Heming, 1998). As expected, treatment of tambaqui with acetazolamide evoked the classic response (e.g. Hoffert and Fromm, 1973) of a profound respiratory acidosis, in which Pa_{CO_2} approximately tripled in 30 min while pHa fell by 0.26 units (Fig. 3D,F). Yet despite this marked internal hypercapnia, the typical cardiorespiratory responses to CO_2/pH of hyperventilation and bradycardia (Figs 1B,C, 2E) were not observed until the acetazolamide-treated tambaqui were exposed to external hypercarbia (Figs 2B,C, 3E). These findings argue strongly in favour of branchial CO_2/pH chemoreceptors with a solely external orientation. Observations from the two additional experimental approaches were in agreement with this conclusion, allaying concerns about any non-specific side effects of the drug treatment. Chemoreceptor impairment, in particular, was considered a possibility because carbonic anhydrase plays a role in CO_2 chemoreception in both invertebrates and vertebrates (e.g. Iturriaga et al., 1991; Swenson and Hughes, 1993; Erlichman et al., 1994; Coates et al., 1998; see also review by Iturriaga, 1993), although the very similar responses of acetazolamide-treated and untreated tambaqui to Pw_{CO_2} values of 14–15 mmHg rendered this prospect unlikely.

Externally oriented branchial CO_2/pH chemoreceptors would account for the close correspondence between cardiorespiratory adjustments and changes in water P_{CO_2} during the rapid washout of CO_2 from the water following hypercarbic exposures, as well as the independence of these responses from arterial P_{CO_2}. The CO_2 electrode response time was likely faster for water than for blood measurements, owing to the different viscosities of these liquids. However, any concern that the apparent tracking of cardiorespiratory responses to water rather than blood P_{CO_2} simply reflected different response times was alleviated by the particularly marked differences in the time courses of Pw_{CO_2} and cardiorespiratory variable changes during washout in acetazolamide-treated tambaqui (Fig. 5).

The observation that injection of CO_2-laden water into the buccal cavity triggered cardiorespiratory reactions (Fig. 6, Table 2) that were not detected in response to the injection of a bolus of CO_2-enriched saline into the caudal vein (Table 1) was also consistent with an external orientation for the branchial CO_2/pH chemoreceptors. In the latter experiment, it was assumed that externally oriented receptors would be preferentially stimulated by injecting CO_2-enriched water into the mouth, whereas CO_2-enriched saline injections would preferentially stimulate internally oriented receptors. Although exclusive stimulation of internally or externally oriented receptors is likely to be impossible with this approach owing to the potential for CO_2 diffusion across the gill epithelium, the extent of activation of blood-oriented receptors by external (water) relative to internal (saline) injections was probably trivial. Similarly, the absence of response to internal injection suggested that water-oriented receptors were activated to a trivial extent by CO_2-enriched saline. Alternative explanations for the lack of response to CO_2-enriched saline injection are that the P_{CO_2} increase achieved by the injection of CO_2-equilibrated saline was insufficient (in length or magnitude) to trigger internally oriented CO_2 receptors, or that the CO_2 was converted to HCO_3^- and/or excreted during transit through the circulation and gills. These possibilities cannot be ruled out, but the most parsimonious explanation of the data in the context of the results for the two other experimental approaches is that the cardiorespiratory responses to hypercarbia in tambaqui are linked to the activation of branchial CO_2/pH chemoreceptors that are oriented only towards the external (water) *milieu*. In dogfish (Perry and McKendry, 2001), Atlantic salmon (Perry and McKendry, 2001) and rainbow trout (McKendry and Perry, 2001; Perry and Reid, 2002), externally oriented branchial CO_2/pH chemoreceptors were also deduced from data generated using experimental approaches similar to those of the present study. The existence of internally oriented receptors that detect changes in blood P_{CO_2} and/or pH was suggested by earlier studies in which indirect correlative relationships between ventilation and blood P_{CO_2} or acid–base status were constructed (e.g. Heisler et al., 1988; Graham et al., 1990; Wood and Munger, 1994; see also review by Gilmour, 2001). Increasingly, however, the weight of evidence from experiments designed to distinguish directly between internal and external stimuli suggests that reflex cardiorespiratory responses to hypercarbia are mediated by externally oriented chemoreceptors. In this regard, the situation for CO_2/pH sensing differs from that for O_2, in that a population of internally oriented O_2 chemoreceptors exists; these receptors are distributed over all gill arches and linked specifically to ventilatory reflexes (Burleson et al., 1992; Burleson, 1995).

The present study also included an experiment to discern between the specific effects of CO_2 *vs* H^+ in the initiation of cardiorespiratory responses to hypercarbia; namely, a comparison of responses to the injection of CO_2-enriched water into the buccal cavity with those elicited by CO_2-free water acidified to the pH of the corresponding CO_2-laden water injection. The results clearly demonstrated that CO_2 itself was the key factor controlling cardiorespiratory function (Fig. 6, Table 2). Although the injection of acidified water was accompanied by significant changes in heart rate, blood flow, ventilation amplitude and ventilation frequency, these effects were in general restricted to injection of the most acidic water (pH 4.9) and were in all cases of much smaller magnitude (11%, 5%, 15% and 16%, respectively) than those produced by injection of the corresponding CO_2-laden water (49%, 45%, 44% and 34%, respectively). The data imply that minor cardiorespiratory reactions may occur with strongly acidic stimuli, but that the chemoreceptors respond predominantly to CO_2 rather than to protons. The small magnitude of the effects, coupled with the need for intense acidic stimuli, probably accounted for the absence of response to acid injection in a previous study on tambaqui (Sundin et al., 2000). Acid injections into the inspired water were also found to be without significant effect in traira (Reid et al., 2000) and dogfish (Perry and McKendry, 2001), while the slight impact of acid injection on ventilation amplitude in Atlantic salmon was attributed to CO_2 formed when HCO_3^- ions in seawater were titrated by the added H^+ (Perry and McKendry, 2001). Earlier studies similarly reported that environmental acidification in the absence of elevated $P_{W_{CO_2}}$ had little effect on ventilation in rainbow trout (Janssen and Randall, 1975; Neville, 1979; Thomas and Le Ruz, 1982) and taken as a whole, these data suggest that the cardiorespiratory responses to hypercarbia are mediated by externally oriented branchial chemoreceptors that respond specifically to CO_2. Nevertheless, protons produced by the hydration of CO_2 that diffuses into the cell probably play a role in signal transduction within chemoreceptor cells in fish, as in mammals (e.g. Iturriaga et al., 1991, 1993; Gonzalez et al., 1994).

In addition to contributing information on receptor orientation and stimulus modality to the existing data on chemoreceptor localization in tambaqui, the present study more fully characterizes the cardiovascular reflexes of tambaqui to hypercarbia, as previous work focused on changes in heart rate and ventilation (Sundin et al., 2000; Milsom et al., 2002; Florindo et al., 2004). In the presence of ambient CO_2 tensions elevated to at least 7 mmHg (lower CO_2 tensions were not tested), tambaqui consistently exhibited bradycardia, greater cardiac stroke volume and hyperventilation, marked by increases of both frequency and amplitude. Arterial blood pressure rose in some cases, presumably in response to increased systemic resistance, and despite simultaneous reductions in cardiac output. This pattern of cardiorespiratory reflexes to hypercarbia emphasises equally the relatively conserved nature of some responses and the highly variable nature of others. For example, hyperventilation is a common response to hypercarbia, both among the studies on tambaqui (Sundin et al., 2000; Milsom et al., 2002; Florindo et al., 2004) and within fish in general (see review by Gilmour, 2001). In some species, including tambaqui (this study; Florindo et al., 2004) and the elasmobranchs examined to date (Randall et al., 1976; Graham et al., 1990; Perry and Gilmour, 1996;

McKendry et al., 2001; Perry and McKendry, 2001), increases in ventilation amplitude are more important contributors to the hyperventilatory response than are frequency adjustments, but overall there is a high degree of interspecific variation in the relative importance of frequency *vs* amplitude changes (Gilmour, 2001). Bradycardia also is a common response to hypercarbia (see review by Perry and Gilmour, 2002), yet while tambaqui responded to higher CO_2 tensions (5%) in all studies with bradycardia (Sundin et al., 2000; Milsom et al., 2002; Florindo et al., 2004), conflicting results were obtained using lower levels of CO_2. For example, Florindo et al. (2004) observed increases in heart rate at 1–2.5% CO_2, in contrast to the bradycardia observed in the present study (Fig. 2E) and the lack of heart rate response observed by Sundin et al. (2000) at similar CO_2 tensions. It is possible that this discrepancy reflects differences in the length of hypercarbic exposure, as the first measurement time utilized by Florindo et al. (2004) was 60 min, while the hypercarbic period in the present study was limited to 15 min.

Although hyperventilation and bradycardia are common responses to hypercarbia, they are by no means universal. White sturgeon *Acipenser transmontanus*, for example, responded to hypercarbia with a tachycardia (Crocker et al., 2000), while a number of species, including channel catfish (Burleson and Smatresk, 2000) and brown bullhead (see table 2 in Gilmour, 2001; table 1 in Perry and Gilmour, 2002) were resistant to hypercarbia, either failing to change heart rate or ventilation, or exhibiting very attenuated responses. To some extent, this variation may reflect species differences in sensitivity to CO_2. For example, neither the European eel *Anguilla anguilla* nor the closely related American eel *Anguilla rostrata* responded to CO_2 tensions of 5–6mmHg (McKenzie et al., 2002; see table 2 in Gilmour, 2001; table 1 in Perry and Gilmour, 2002), but adjustments of both heart rate and ventilation were exhibited by European eels exposed to P_{wCO_2} values of 10–80 mmHg (McKenzie et al., 2002). Similarly, it was necessary to raise water P_{CO_2} to 14 mmHg before hyperventilatory responses appeared in carp *Cyprinus carpio* (Soncini and Glass, 2000), and to ~38 mmHg to observe significant hypercarbic responses in traira (Reid et al., 2000). Like eel, carp and traira, the results of earlier studies indicated that tambaqui are relatively insensitive to changes in water CO_2 tension (Sundin et al., 2000). The results of the present study and that of Florindo et al. (2004), however, indicated that tambaqui are more sensitive to CO_2 than originally thought. Variation in the methods used to expose fish to hypercarbia may account for this difference.

The remaining cardiovascular adjustments to hypercarbia, including blood pressure, systemic resistance, cardiac output and stroke volume, exhibit a high degree of interspecific variation among fish, encompassing essentially all possible response patterns (see table 2 in Perry and Gilmour, 2002). The increases in arterial blood pressure and systemic resistance, coupled with constant or slightly reduced cardiac output observed for tambaqui under some conditions in the present study (Fig. 6, Table 2), were reminiscent of the responses of the salmonid fish that have been examined to date (Perry et al., 1999; McKendry and Perry, 2001; Perry and McKendry, 2001). The appearance of these responses, however, was dependent upon the method of CO_2 delivery (injection of a bolus of CO_2-enriched water into the mouth *vs* exposure to hypercarbic water), perhaps because of preferential stimulation of different receptor populations. In addition, Milsom et al. (2002) reported the existence in tambaqui of receptors sensitive to CO_2 that had an inhibitory influence on ventilation frequency. The chemoreceptor control of cardiorespiratory function during hypercarbia in fish is clearly complex, likely involving multiple receptor populations in a variety of locations (although with a branchial concentration) that are linked to different cardiorespiratory parameters with positive and/or negative influences. Resolving this complexity is an ongoing challenge, particularly because the patterns of chemoreceptor control as well as the cardiorespiratory responses to hypercarbia vary among fish species for reasons that remain elusive. Despite the variability, CO_2 has emerged as an important modulator of cardiorespiratory function in fish, with responses mediated, in all species that have been examined to date, by receptors that are oriented towards the external environment with sensitivity specifically to CO_2.

List of symbols

f_H	heart rate
f_V	ventilation frequency
P_{aCO_2}	arterial P_{CO_2}
P_{CO_2}	partial pressure of CO_2
P_{DA}	pressure in dorsal aorta
pHa	arterial blood pH
pHw	water pH
P_{O_2}	partial pressure of oxygen
P_{wCO_2}	water P_{CO_2}
R_s	systemic vascular resistance
V_{AMP}	ventilation amplitude
$\dot{V}_b$	mass-specific blood flow
V_S	stroke volume

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