The Journal of Experimental Biology 202, 2177–2190 (1999)
Printed in Great Britain © The Company of Biologists Limited 1999
JEB2009



# THE CONTROL OF BLOOD PRESSURE DURING EXTERNAL HYPERCAPNIA IN THE RAINBOW TROUT (*ONCORHYNCHUS MYKISS)*

S. F. PERRY[1,*], R. FRITSCHE[2], T. M. HOAGLAND[3], D. W. DUFF[4] AND K. R. OLSON[3]

[1]*Department of Biology, University of Ottawa, 30 Marie Curie, Ottawa, Ontario, Canada K1N 6N5,* [2]*Department of Zoophysiology, Göteborg University, Medicinaregatan 18A, Box 463, 405 30 Göteborg, Sweden,* [3]*Indiana University School of Medicine, South Bend Center, University of Notre Dame, Notre Dame, IN 46556-5607, USA* and [4]*Department of Biology, Indiana University at South Bend, South Bend, IN 46565, USA*

*e-mail: sfperry@science.uottawa.ca

*Accepted 24 May; published on WWW 19 July 1999*

## Summary

**Adult freshwater rainbow trout (*Oncorhynchus mykiss*) were exposed acutely (approximately 20 min) in a stepwise manner to increasing levels of environmental carbon dioxide ranging between 1.7 and 9.0 mmHg (0.23–1.2 kPa). Experiments were performed to examine, for the first time, the influence of hypercapnic acidosis on aspects of cardiovascular physiology including blood pressure, cardiac output and vascular resistance. Fish displayed dose (water $CO_2$ partial pressure) -dependent increases in ventral aortic (13–39 %) and dorsal aortic (17–54 %) blood pressures that reflected marked increases in systemic vascular resistance (16–78 %); branchial vascular resistance was unaffected by hypercapnia. At the highest level of hypercapnia (9.0 mmHg), central venous pressure was significantly elevated by 54 %. Although cardiac output remained constant, heart rate was significantly lowered by 4–7 beats min$^{-1}$ at the two highest levels of hypercapnia. To determine whether the cardiovascular responses to hypercapnia were being blunted by the stepwise increase in external $P_{CO_2}$, a separate group of fish was exposed directly to a single step of hypercapnia (water $P_{CO_2}$ 8.0 mmHg). The cardiovascular responses were similar to those exhibited by the more gradually exposed fish except that central venous pressure did not increase and the extent of the bradycardia was greater (13 beats min$^{-1}$).**

**After confirming the effectiveness of yohimbine in blocking the vasoconstrictory α-adrenoreceptors of the systemic vasculature, this antagonist was used as a tool to assess the importance of α-adrenoreceptor stimulation in promoting the cardiovascular responses during hypercapnia. Prior treatment of fish with yohimbine prevented the increased blood pressures and systemic vascular resistance during hypercapnia but did not influence the $CO_2$-induced bradycardia. Plasma levels of catecholamines did not change during hypercapnia, and therefore the stimulation of the systemic α-adrenoreceptors presumably reflected increased sympathetic nerve activity.**

**To determine whether the cardiovascular changes elicited by hypercapnia were related to acidosis-induced hypoxaemia, fish were exposed to hypoxia in a stepwise manner (water $P_{O_2}$ 65–151 mmHg). The cardiovascular responses to hypoxia were markedly different from those to hypercapnia and consisted of pronounced increases in systemic and branchial vascular resistance, but only at the most severe level of hypoxia; ventral and dorsal aortic pressures were unaffected. The differences between the responses to hypercapnia and hypoxia, coupled with the smaller reductions in blood oxygen content during hypercapnia, support the hypothesis that the cardiovascular responses to $CO_2$ are direct and are unrelated to hypoxaemia.**

Key words: *Oncorhynchus mykiss*, hypercapnia, blood pressure, systemic resistance, gill resistance, heart, catecholamine, hypoxia, cardiac output, ventilation.

## Introduction

Environmental hypercapnia, an elevation of ambient $P_{CO_2}$, is exploited routinely in studies of fish physiology as an anaesthetic (Yoshikawa et al., 1991; Bernier and Randall, 1998) or as a tool to elucidate mechanisms of acid–base regulation (Lloyd and White, 1967; Cameron and Randall, 1972; Cameron, 1976), ionic balance (Eddy et al., 1979; Perry et al., 1987; MacKenzie and Perry, 1997), respiratory gas transfer (Thomas et al., 1994), blood gas transport (Eddy, 1976; Eddy et al., 1977; Perry and Kinkead, 1989), metabolism (Walsh et al., 1988; Perry et al., 1988; Mommsen et al., 1988) or ventilatory control (Janssen and Randall, 1975). In many instances, the physiological effects of hypercapnia in teleosts are attributed to hypoxaemia arising indirectly from Bohr and Root effects. For example, the increases in ventilation during moderate hypercapnia in teleost fish have traditionally been linked to a depression of blood $O_2$ content rather than to any

specific effect of $CO_2$ or acidosis (Randall, 1982; Smith and Jones, 1982). Historically, the presence and/or importance of $CO_2/H^+$ chemoreceptors in piscine ventilatory control have been largely discounted. In recent years, however, a growing body of evidence has accumulated implicating a specific role for $CO_2/H^+$ in the stimulation of breathing in elasmobranchs and teleosts (for reviews, see Perry and Wood, 1989; Milsom, 1995a,b). These results reveal the presence of central and/or peripheral $CO_2/H^+$ chemoreceptors in fish that presumably function in conjunction with the well-studied $O_2$ chemoreceptors (for a review, see Fritsche and Nilsson, 1993).

In addition to their effects on ventilation, $O_2$ chemoreceptors are also linked to several cardiovascular reflex adjustments that are mediated by the neuronal (sympathetic and parasympathetic nerves) and humoral (circulating catecholamines) components of the autonomic nervous system (Fritsche and Nilsson, 1993; Bushnell and Jones, 1992; Farrell, 1993; Olson, 1998). Although relatively few species have been examined, generalised responses to ambient hypoxia include an elevation of blood pressure, increased systemic vascular resistance and bradycardia (Satchell, 1961; Holeton and Randall, 1967; Piiper et al., 1970; Wood and Shelton, 1980b; Fritsche and Nilsson, 1989, 1990; Fritsche, 1990; Bushnell and Brill, 1991; Maxime et al., 1995).

Unlike the $O_2$ chemoreceptors, a potential link between $CO_2/H^+$ chemoreceptors and cardiovascular reflexes has not been established in fish. Indeed, the effects of external hypercapnia on cardiovascular function have not yet been investigated in any teleost. Thus, considering the recent implication of $CO_2$ in ventilatory control in fish and the known dual role of hypoxia in eliciting ventilatory and cardiovascular adjustments, the present study was undertaken to assess the cardiovascular responses of freshwater-adapted rainbow trout to environmental hypercapnia and to investigate the underlying control mechanisms.

## Materials and methods

### *Experimental animals*

Rainbow trout [*Oncorhynchus mykiss* (Walbaum)] of either sex, weighing between 366 and 790 g ($N$=46; mass 526±16 g, mean ± S.D.), were obtained from a local hatchery and held indoors in large (volume 2000 l) fibreglass tanks supplied with flowing, aerated well water. The temperature of the holding and experimental tanks varied between 12 and 14 °C; the photoperiod matched the seasonal light:dark cycle. Fish were fed daily to satiation with a commercial trout diet (Purina), but were not fed for 48 h prior to experimentation.

### *Surgical procedures*

#### *Measurement of cardiovascular and ventilatory variables*

Fish were anaesthetised in a 1:12 000 (w/v) solution of benzocaine (ethyl-*p*-aminobenzoate) kept at 4 °C. After the cessation of breathing movements, the fish were transferred to an operating table and the gills were irrigated with a 1:24 000 solution of benzocaine (10 °C) throughout surgery. A polyethylene cannula (Clay Adams, PE 160; 1.14 mm i.d., 1.57 mm o.d.) was implanted into the buccal cavity to facilitate measurement of inspired water $P_{O_2}$, $P_{CO_2}$ and pH. To permit measurement of dorsal aortic blood pressure ($P_{DA}$), drug injections and blood sampling, a polyethylene cannula (Clay Adams, PE 60; 0.76 mm i.d., 1.22 mm o.d.) was implanted into the dorsal aorta *via* percutaneous puncture of the roof of the buccal cavity (Olson et al., 1997). A small (approximately 1 cm) ventral incision was made to expose the pericardial cavity, and the pericardium was dissected to expose the bulbus arteriosus. To allow measurement of central venous pressure ($P_{VEN}$), a non-occlusive silicone cannula (0.51 mm i.d., 0.94 mm o.d.) was implanted into the right horn of the ductus of Cuvier and secured using cyanoacrylate glue (Olson et al., 1997). The cannula was then attached to a length of polyethylene tubing (Clay Adams, PE 90; 0.86 mm i.d., 1.27 mm o.d.) filled with heparinised (100 i.u. $ml^{-1}$ sodium heparin) saline (0.9 % NaCl, w/v). After clamping the ventriculo-bulbar junction, the bulbus arteriosus was cannulated and secured using a protocol similar to that used for the ductus (Olson et al., 1997). The pressure recorded in the bulbus arteriosus is referred to throughout this paper as ventral aortic pressure ($P_{VA}$). Upon removal of the clamp, a 3S or 4S ultrasonic flow probe (Transonics Systems Inc., Ithaca, NY, USA) was placed non-occlusively around the bulbus to enable the measurement of cardiac output ($\dot{V}_b$). The incision was closed with sutures and sealed with cyanoacrylate gel, and the bulbus cannula and flow probe cables were secured to the ventral skin using sutures. Small (1 $cm^2$) brass plates were stitched to the external surface of each operculum to allow the measurement of ventilation amplitude using an impedance converter (Peyraud and Ferret-Bouin, 1960).

After surgery, the fish were revived and allowed to recover for 24 h while constrained in open-ended individual black tubes (10 cm diameter) suspended in holding tanks supplied with aerated well water.

#### *Measurement of blood respiratory variables*

Fish were anaesthetised (see above), and a polyethylene cannula (PE 60) was inserted into the dorsal aorta. The caudal vein was cannulated using polyethylene tubing (PE 50) filled with heparinised (100 i.u. $ml^{-1}$ sodium heparin) saline (0.9 % NaCl). Briefly, a lateral incision was made at the level of the caudal peduncle to expose the haemal arch. The caudal vein was cannulated percutaneously, the wound was closed, and the cannula was secured to the body wall using silk ligatures. This allowed an extracorporeal circuit to be initiated in which the arterial dorsal aortic blood was pumped continuously over a series of electrodes (pH, $P_{O_2}$, $P_{CO_2}$) and then returned to the fish *via* the caudal vein cannula (Thomas, 1994). After surgery, the fish were revived and allowed to recover for 24 h while constrained in open-ended individual black tubes (10 cm diameter) suspended in holding tanks supplied with aerated well water.

The $O_2$ electrodes (Radiometer, model E5047-0) were calibrated by pumping (using the peristaltic pump of the extracorporeal shunt) a zero solution (2 % sodium sulphite) or

air-saturated water continuously through the electrode sample compartments until stable readings were recorded. The $CO_2$ electrodes (Radiometer, model E5037-0) were calibrated in a similar manner using mixtures of 0.5 % and 1.0 % $CO_2$ in air provided by a gas-mixing flowmeter. The pH electrode (Metrohm, model 6.0204.100) was calibrated using precision buffers. The $CO_2$, $O_2$ and pH electrodes were calibrated prior to each experiment.

Immediately prior to experimentation, the extracorporeal shunt was rinsed for 15–20 min with a solution of sodium heparin (1000 i.u. $ml^{-1}$ in saline) to prevent blood from clotting in the tubing and electrode chambers.

### *Experimental protocol*

#### *Measurement of cardiovascular and ventilatory variables*

The dorsal aortic, ductus and bulbus cannulae were flushed with heparinised saline (100 i.u. $ml^{-1}$) to prevent clotting and then connected to Argon (041-57050 4A) disposable CDXPRESS pressure transducers precalibrated against a static column of water. Analog blood pressure signals were measured using Hewlett Packard (7853A) patient monitors. Cardiac output was determined by attaching the ultrasonic flow probe to a Transonic T206 dual-channel blood flowmeter. The flow probes were calibrated *in situ* after each experiment by pumping (using a peristaltic pump) saline at known flow rates into the heart of a dead fish immersed in water; saline and water temperatures were maintained at 12–14 °C. Opercular impedance changes were monitored using a custom-built impedance converter and amplifier and were converted to linear opercular deflections (in cm) after appropriate calibration. Water $P_{CO_2}$ and $P_{O_2}$ were measured by continuous pumping of inspired water from the buccal cannula over Radiometer $CO_2$ and $O_2$ electrodes housed within thermostatted cuvettes and connected to a PHM 73 acid–base analyser (Radiometer). In a few cases, water pH was monitored using a pH electrode (Metrohm) housed in a thermostatted chamber. The analog outputs from the impedance converter and acid–base analyser were converted to digital signals by interfacing with a data-acquisition system (Biopac Systems Inc.) using Acknowledge data-acquisition software (sampling rate set at 10 Hz) and a 486 PC.

Cardiovascular signals (blood pressure and cardiac output) were converted to digital data using an A/D converter and LabTech Notebook software using a second 486 PC. Sampling frequency was 10 Hz; 1 s running averages were compiled. The timing of data sampling from the two data-acquisition systems was synchronised. Thus, continuous data recordings were obtained for mass-specific cardiac output ($\dot{V}_b$), heart rate ($f_H$; automatic rate calculation from the pulsatile cardiac output trace), cardiac stroke volume ($V_S$; $\dot{V}_b/f_H$), ventilation frequency ($f_G$; automatic rate calculation from the raw impedance trace), opercular displacement (the difference between maximum and minimum impedance values), mean blood pressures, branchial vascular resistance [$R_g$; (mean $P_{VA}$ minus mean $P_{DA}$)/$\dot{V}_b$] and systemic vascular resistance [$R_s$; (mean $P_{DA}$ minus $P_{VEN}$)/$\dot{V}_b$]. In some instances, measurements of $P_{VEN}$ were not obtained owing to cannula failure; in these cases, $R_s$ was calculated as mean $P_{DA}/\dot{V}_b$. Four separate experimental series were performed.

*Series I: stepwise increases in external* $P_{CO_2}$. When a stable baseline had been established, experiments commenced with a period of normocapnia followed by three phases of progressively increasing hypercapnia and recovery. During normocapnia, the inflowing water (>2.5 l $min^{-1}$) was provided from a gas equilibration column gassed vigorously with air. After a 5 min period of recording, a pre-hypercapnia blood sample (0.6 ml) was withdrawn from the dorsal aortic cannula. After centrifugation (12 000 ***g***), plasma (>250 µl) was added to 10 µl of 5 mmol $l^{-1}$ sodium sulphite, frozen, kept on dry ice for approximately 3 weeks and then shipped while frozen to the University of Ottawa for storage at –80 °C until subsequent analysis of catecholamine levels. Hypercapnia was initiated by gassing (gas flow 2.5 l $min^{-1}$) the equilibration column that provided water to the fish tubes with mixtures of $CO_2$ in air ranging between 1.5 and 4.5 %. The mixtures were provided by a GF-3/MP gas-mixing flowmeter (Cameron Instrument Company, Port Aransas, TX, USA). These levels of $CO_2$ elicited stable levels of inspired $P_{CO_2}$ of 3.5, 6.0 and 9.0 mmHg within 15–20 min. Additional blood samples were withdrawn and processed at the conclusion of each step of hypercapnia. After exposure to the final step of environmental hypercapnia, fish were returned to normocapnic conditions and allowed to recover for a further 25–30 min, at which time a final blood sample was taken.

Plasma catecholamine levels (adrenaline and noradrenaline) were determined on alumina-extracted plasma samples (0.2 ml) using high-performance liquid chromatography (HPLC) with electrochemical detection (Woodward, 1982). The extracted samples were passed through an Ultratechsphere ODS-C18 5 µm column (HPLC Technology Ltd), using a catecholamine and metanephrine mobile phase (Chromosystems, Munich, Germany). Concentrations were calculated relative to appropriate standards and with 3,4-dihydroxybenzylamine hydrobromide (DHBA) as an internal standard in all determinations.

*Series II: single increases in external* $P_{CO_2}$. Because the fish in series I were being exposed to incremental increases in external $P_{CO_2}$, there was a possibility that the cardiovascular responses to the higher levels of $CO_2$ (i.e. the second and third increments) were being blunted as a result of habituation. Therefore, in series II, fish were exposed to a single step increase in external $P_{CO_2}$ designed to match the final level of hypercapnia achieved in series I (9.0 mmHg). In practice, however, the actual $P_{CO_2}$ reached in the single-step experiment (8.0±0.8 mmHg) was slightly lower than in series I. The experimental protocol was identical to that of series I in all other respects except that blood samples were not withdrawn for plasma catecholamine analysis.

*Series III: the effects of α-adrenoceptor blockade on the cardiovascular responses to hypercapnia.* The competitive α-adrenoceptor antagonist yohimbine (Nichols and Ruffolo, 1991) was selected for these experiments because previous studies have established it to be a reliable and well-tolerated blocker of catecholamine-mediated systemic vasoconstriction in rainbow

trout (Holmgren and Nilsson, 1974; Wood, 1976; Wood and Shelton, 1980a,b). In the present study, detailed preliminary experiments were performed to determine the correct dosage of yohimbine and the duration of its α-adrenoceptor blockade. In these experiments, fish were injected *via* the dorsal aortic cannula with 10 nmol $kg^{-1}$ adrenaline (1 ml $kg^{-1}$) (L-adrenaline bitartrate salt; Sigma), and the effects on cardiovascular variables were recorded for 45 min or until values had returned to baseline levels. Fish were then given a bolus intra-arterial injection of yohimbine at doses ranging between 1 and 4 mg $kg^{-1}$. The effects on baseline $R_S$ were recorded, and after a new steady state had been reached (usually within 30 min), a second injection of adrenaline was administered. The lowest dose of yohimbine consistently to block the adrenaline-induced rise in $R_S$ was 2 mg $kg^{-1}$, and this dose was therefore used in all subsequent experiments requiring α-adrenoceptor blockade.

To assess the effects of α-adrenoceptor blockade on the cardiovascular responses to hypercapnia, fish were pre-treated with 2 mg $kg^{-1}$ of yohimbine and, after 30 min, subjected to a single step increase in water $P_{CO_2}$ (to 7.3±0.5 mmHg) that approximately matched the final level achieved in series II. All other aspects of the protocol were identical to those in series II.

*Series IV: stepwise decreases in external* $P_{O_2}$. To assess the potential contribution of acidosis-induced hypoxaemia to the cardiovascular responses to hypercapnia, fish were exposed to stepwise hypoxia. Once a stable baseline value had been established, experiments commenced with a period of normoxia followed by three episodes of progressively increasing hypoxia, and recovery. Hypoxia was initiated by gassing (3.0 l $min^{-1}$) the equilibration column that provided water to the fish tubes with mixtures of $N_2$ in air ranging between 30 % and 100 % $N_2$. These mixtures were provided by a GF-3/MP gas-mixing flowmeter (Cameron Instrument Company, Port Aransas, TX, USA). These levels of hypoxia were chosen because they elicited a range of hypoxaemia spanning the reductions in blood $O_2$ concentration achieved during the hypercapnia exposures. In practice, this protocol elicited stable levels of inspired $P_{O_2}$ of 122, 95 and 65 mmHg within 15–20 min. Blood samples (0.6 ml) were withdrawn and processed as in series I prior to hypoxia, at the conclusion of each step of hypoxia and after return to normoxia.

*Measurement of blood respiratory variables*

To relate cardiovascular changes elicited by external hypercapnia or hypoxia to changing blood respiratory status, arterial blood $P_{O_2}$ ($Pa_{O_2}$), $P_{CO_2}$ ($Pa_{CO_2}$) and pH (pHa) were monitored in separate experimental series during incremental and single-step hypercapnia (protocols identical to series I and II) and incremental hypoxia (protocol identical to series IV). Blood respiratory status was assessed continuously using an extracorporeal blood shunt (see above). Cardiovascular measurements were not made during these experiments nor was any blood sampled.

*Statistical analyses and presentation of data*

Data are presented as means ± 1 standard error of the mean (S.E.M.). The data were analysed statistically by parametric repeated-measures one-way analysis of variance (ANOVA). When the ANOVA indicated a significant difference, a *post-hoc* multiple-comparison test (Bonferroni *t*-test) was used to identify data points that were significantly different from each other (all pairwise multiple comparison used in stepwise experiments) or from a single control point (i.e. the final value of the pre-hypercapnic period in single-step experiments). When parametric test assumptions were violated, the data were analysed using Friedman's repeated-measures ANOVA on ranks followed by Dunn's all pairwise multiple-comparison test or Dunnett's comparison with a single control point. In experiments designed to validate the effectiveness of yohimbine as an α-adrenoceptor antagonist, pre- and post-adrenaline injection data were analysed using a two-tailed paired Student's *t*-test; differences between the control and yohimbine-treated fish were determined by ANOVA. All statistical tests, including determinations of normality and variance, were performed using commercial software (SigmaStat version 2.03). The fiducial limit of significance was set at 5 %.

## Results

*Cardiovascular and ventilatory responses to hypercapnia*

*Series I: stepwise increases in external* $P_{CO_2}$

Fish ($N$=11) were exposed to three stepwise increases in ambient $P_{CO_2}$ to achieve (within 20 min) levels of hypercapnia of 3.5±0.3, 6.0±0.3 and 9.0±0.3 mmHg (Table 1). Water pH

Table 1. *The effects of a stepwise increase in external* $P_{CO_2}$ *on water/blood gases and ventilation variables in rainbow trout* Oncorhynchus mykiss

| | $Pw_{CO_2}$ (mmHg) | $Pw_{O_2}$ (mmHg) | $Pa_{CO_2}$ (mmHg) | $Pa_{O_2}$ (mmHg) | pHa | $f_G$ ($min^{-1}$) | $V_{amp}$ (cm) |
|---|---|---|---|---|---|---|---|
| Pre | 1.66±0.16$^{a}$ | 152.7±0.9$^{a,c}$ | 2.84±0.11$^{a}$ | 119.6±6.2$^{a,c}$ | 7.83±0.02$^{a}$ | 93.6±3.9$^{a}$ | 0.77±0.19$^{a,d}$ |
| Step 1 | 3.53±0.29$^{b}$ | 151.2±0.8$^{a,c}$ | 3.99±0.24$^{b}$ | 137.1±2.7$^{b}$ | 7.76±0.02$^{b}$ | 89.1±3.0$^{a}$ | 0.95±0.22$^{a,c,d}$ |
| Step 2 | 5.99±0.29$^{c}$ | 149.9±0.9$^{b}$ | 6.31±0.24$^{c}$ | 140.4±1.2$^{b}$ | 7.64±0.02$^{c}$ | 87.7±3.1$^{a}$ | 1.21±0.29$^{a,c}$ |
| Step 3 | 9.04±0.26$^{d}$ | 149.9±0.9$^{b}$ | 9.42±0.40$^{d}$ | 141.6±1.4$^{b}$ | 7.53±0.02$^{d}$ | 87.5±3.8$^{a}$ | 1.27±0.26$^{b,c}$ |
| Post | 1.70±0.08$^{a}$ | 151.2±1.3$^{a,c}$ | 3.24±0.16$^{a}$ | 121.3±5.7$^{a,c}$ | 7.84±0.02$^{a}$ | 95.4±3.4$^{a}$ | 0.57±0.09$^{d}$ |

For each variable, values that do not share a common letter are statistically different from each other ($P$<0.05).

Pre, Post, pre- and post-hypercapnia recording periods; $Pw_{CO_2}$, external $P_{CO_2}$; $Pw_{O_2}$, external $P_{O_2}$; $Pa_{CO_2}$, arterial $P_{CO_2}$; $Pa_{O_2}$, arterial $P_{O_2}$; $f_G$, gill ventilation rate; $V_{amp}$, ventilatory 1 mmHg=0.133 kPa.

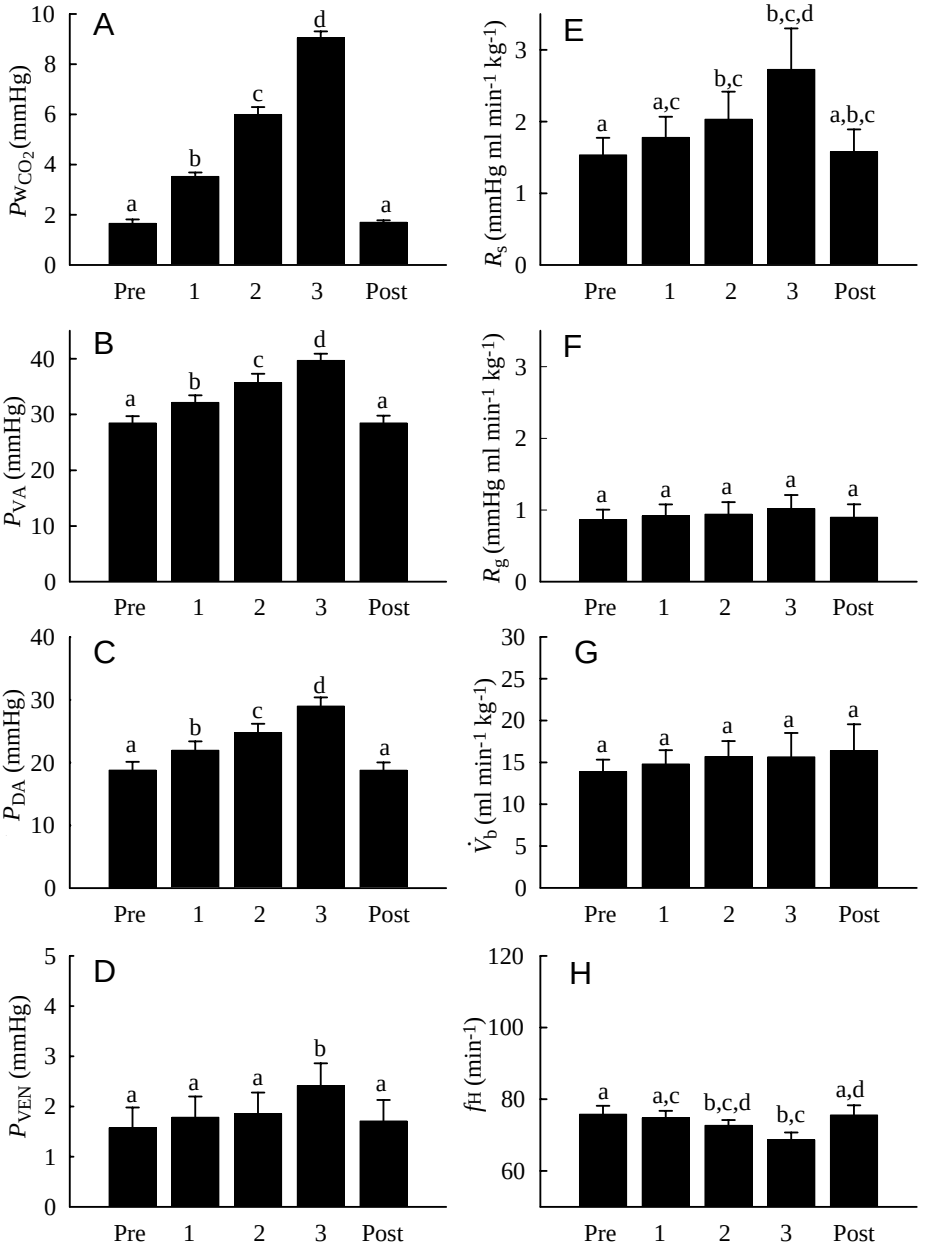


Fig. 1. The effects of stepwise increases in external $P_{CO_2}$ ($Pw_{CO_2}$) (A) on cardiovascular variables in rainbow trout (*Oncorhynchus mykiss*) including (B) ventral aortic blood pressure ($P_{VA}$; $N$=8), (C) dorsal aortic blood pressure ($P_{DA}$; $N$=11), (D) central venous blood pressure ($P_{VEN}$; $N$=9), (E) systemic vascular resistance ($R_s$; $N$=11), (F) gill vascular resistance ($R_g$; $N$=8), (G) cardiac output ($\dot{V}_b$; $N$=11) and (H) heart rate ($f_H$; $N$=11). Pre and Post refer to the pre- and post-hypercapnia recording periods; the numbers 1–3 denote the three discrete steps of increasing hypercapnia (3.5, 6.0 and 9.0 mmHg). The data are presented as means +1 S.E.M.; values that do not share identical letters are significantly different ($P$<0.05). 1 mmHg=0.1333 kPa.

(pHw) was monitored in two experiments and averaged 7.64 during normocapnia and 7.37, 7.16 and 7.06 during the three steps of hypercapnia, respectively. The effects on the measured and calculated cardiovascular variables are summarised in Fig. 1. $P_{VA}$ and $P_{DA}$ were increased at all levels of hypercapnia in a $P_{CO_2}$-dependent manner (Fig. 1B,C). At the highest level of hypercapnia, $P_{VA}$ and $P_{DA}$ were increased by 39 % and 54 %, respectively. $P_{VEN}$ was increased by 54 %, but only during the final episode of hypercapnia (Fig. 1D). Systemic resistance was also increased up to 78 % in a $P_{CO_2}$-dependent fashion, although the change was not statistically significant at the lowest level of hypercapnia (Fig. 1E); $R_g$ was unaffected by hypercapnia (Fig. 1F). Although heart rate was lowered during hypercapnia (Fig. 1H), cardiac output was maintained (Fig. 1G) because of a significant elevation of stroke volume (15–26 %) during the latter two periods of hypercapnia (data not shown). The ventilatory response to incremental hypercapnia was extremely variable and, consequently, ventilatory amplitude (as determined by opercular displacement) was statistically elevated from pre-hypercapnia values only during the final stage of hypercapnia (Table 1). Breathing frequency was unaltered during hypercapnia (Table 1).

The effects of hypercapnia on plasma levels of catecholamines are summarised in Table 2. The concentrations of adrenaline or noradrenaline were low and constant at all levels of hypercapnia. The total catecholamine levels (adrenaline plus noradrenaline) in the plasma never exceeded 5 nmol l$^{-1}$.

### *Series II: single increase in external* $P_{CO_2}$

Exposing rainbow trout ($N$=9) to a single increase in external $P_{CO_2}$ ($Pw_{CO_2}$), designed to match the highest level of hypercapnia achieved in step 3 of series I, caused similar

Table 2. *The effects of a stepwise increase in external* $P_{CO_2}$ *on plasma catecholamine levels in rainbow trout* Oncorhynchus mykiss

| | $P_{wCO_2}$ (mmHg) | [Noradrenaline] (nmol $l^{-1}$) | [Adrenaline] (nmol $l^{-1}$) | [Total catecholamines] (nmol $l^{-1}$) |
|---|---|---|---|---|
| Pre | 1.66±0.16[a] | 0.5±0.3 | 1.7±1.0 | 2.2±1.3 |
| Step1 | 3.53±0.29[b] | 0.5±0.2 | 0.8±0.3 | 1.3±0.5 |
| Step 2 | 5.99±0.29[c] | 1.3±0.7 | 0.8±0.2 | 2.1±0.7 |
| Step 3 | 9.04±0.26[d] | 1.0±0.3 | 2.3±0.6 | 3.3±0.9 |
| Post | 1.70±0.08[a] | 2.2±1.8 | 2.6±0.6 | 4.8±3.2 |

For each variable, values that do not share a common letter are statistically different from each other ($P$<0.05).

Pre, Post, pre- and post-hypercapnia recording periods; $P_{wCO_2}$, external $P_{CO_2}$.

1 mmHg=0.133 kPa.

cardiovascular responses (Fig. 2). Two notable differences were that $P_{VEN}$ did not increase (Fig. 2D) and that $f_H$ was lowered to a greater extent (Fig. 2H). Because of the high degree of variance, apparent increases (approximately 15 %) in cardiac stroke volume were not statistically significant ($P$=0.06; data not shown). Ventilatory amplitude was increased, whereas breathing frequency was unchanged by hypercapnia (Table 3). In two experiments in which pHw was measured, it decreased from 7.64 to 7.23 during hypercapnia.

*Series III: the effects of α-adrenoceptor blockade on the cardiovascular responses to hypercapnia*

Initial experiments were designed to confirm the effectiveness of yohimbine as an α-adrenoceptor antagonist in trout. The results of these experiments are depicted in Fig. 3. The injection of adrenaline into untreated fish caused expected increases in $P_{VA}$, $P_{DA}$, $P_{VEN}$, $R_s$ and $\dot{V}_b$; $R_g$, $f_H$ and $V_S$ were unaffected. Injection of yohimbine (2 mg $kg^{-1}$) caused a lowering of baseline $R_s$ (Fig. 3D) and abolished the pressor and $R_s$ responses to adrenaline injection.

Treatment of fish with yohimbine resulted in an elevated $\dot{V}_b$ during normocapnia (Fig. 4G) resulting in an increased $V_S$ (data not shown) and eliminated the pressor and systemic resistance increases normally associated with hypercapnia (Fig. 4). However, the bradycardia elicited by hypercapnia was not affected by α-adrenoceptor blockade (Fig. 4H).

*Series IV: stepwise decreases in external* $P_{O_2}$

Fish ($N$=7) were exposed to three stepwise decreases in ambient $P_{O_2}$ to achieve (within 20 min) levels of hypoxia of 122.3±2.6, 95.1±3.1 and 64.9±4.7 mmHg (Table 4). The effects of the stepwise hypoxia on the recorded and calculated cardiovascular variables are illustrated in Fig. 5. Unlike in the hypercapnic fish, $P_{VA}$ (Fig. 5B) and $P_{DA}$ (Fig. 5C) remained constant during hypoxia, whereas $P_{VEN}$ was elevated during the most severe stage of hypoxia (Fig. 5D). Systemic and gill vascular resistances remained constant at the two first levels of hypoxia, but were significantly increased during the final episode (Fig. 5E,F). Cardiac output and $f_H$ decreased significantly during the final stage of hypoxia (Fig. 5G,H); $V_S$ was unaffected (data not shown).

As in the hypercapnia series, the ventilatory response to stepwise hypoxia exhibited a high degree of variability, and ventilatory amplitude was therefore statistically elevated from

Table 3. *The effects of a single increase in external* $P_{CO_2}$ *on water/blood gases and ventilation variables in rainbow trout,* Oncorhynchus mykiss

| | $P_{wCO_2}$ (mmHg) | $P_{wO_2}$ (mmHg) | $P_{aCO_2}$ (mmHg) | $P_{aO_2}$ (mmHg) | pHa | $f_G$ ($min^{-1}$) | $V_{amp}$ (cm) |
|---|---|---|---|---|---|---|---|
| Pre | 1.06±0.14 | 152.2±1.5 | 2.70±0.40 | 118.0±8.5 | 7.93±0.03 | 87.7±5.4 | 0.43±0.05 |
| Hypercapnia | 7.98±0.75* | 145.8±4.4 | 8.10±0.62* | 123.5±10.1 | 7.59±0.07* | 87.9±5.1 | 1.00±0.17* |
| Post | 1.46±0.26 | 149.8±3.4 | 3.74±0.70 | 116.5±13.1 | 7.88±0.05 | 83.5±4.6 | 0.55±0.07 |

* indicates a significant difference from the pre-hypercapnia value ($P$<0.05).

Abbreviations are explained in Table 1.

1 mmHg=0.133 kPa.

Table 4. *The effects of a stepwise decrease in external* $P_{O_2}$ *on water/blood gases and ventilation variables in rainbow trout,* Oncorhynchus mykiss

| | $P_{wO_2}$ (mmHg) | $P_{wCO_2}$ (mmHg) | $P_{aCO_2}$ (mmHg) | $P_{aO_2}$ (mmHg) | pHa | $f_G$ ($min^{-1}$) | $V_{amp}$ (cm) |
|---|---|---|---|---|---|---|---|
| Pre | 151.3±1.3[a] | 1.48±0.07[a] | 2.25±0.23[a] | 109.6±6.1[a] | 7.90±0.03[a,c] | 96.9±5.0[a] | 0.31±0.06[a,c] |
| Step 1 | 122.3±2.6[b] | 1.53±0.06[a] | 2.07±0.20[a] | 91.8±4.3[b] | 7.94±0.03[a,c] | 93.3±6.2[a] | 0.39±0.08[a,c] |
| Step 2 | 95.1±3.1[c] | 1.49±0.06[a] | 1.98±0.19[a] | 64.0±4.6[c] | 7.96±0.03[b,c] | 99.0±5.9[a] | 0.60±0.15[a,c] |
| Step 3 | 64.9±4.7[d] | 1.47±0.04[a] | 1.93±0.21[a] | 19.6±1.8[d] | 7.87±0.03[a] | 90.9±3.9[a] | 0.98±0.28[b] |
| Post | 143.8±3.3[a] | 1.51±0.07[a] | 2.64±0.24[b] | 107.4±3.9[a] | 7.78±0.03[a] | 91.2±5.1[a] | 0.32±0.06[c] |

For each variable, values that do not share a common letter are statistically different from each other ($P$<0.05).

Abbreviations are defined in Table 1.

1 mmHg=0.133 kPa.

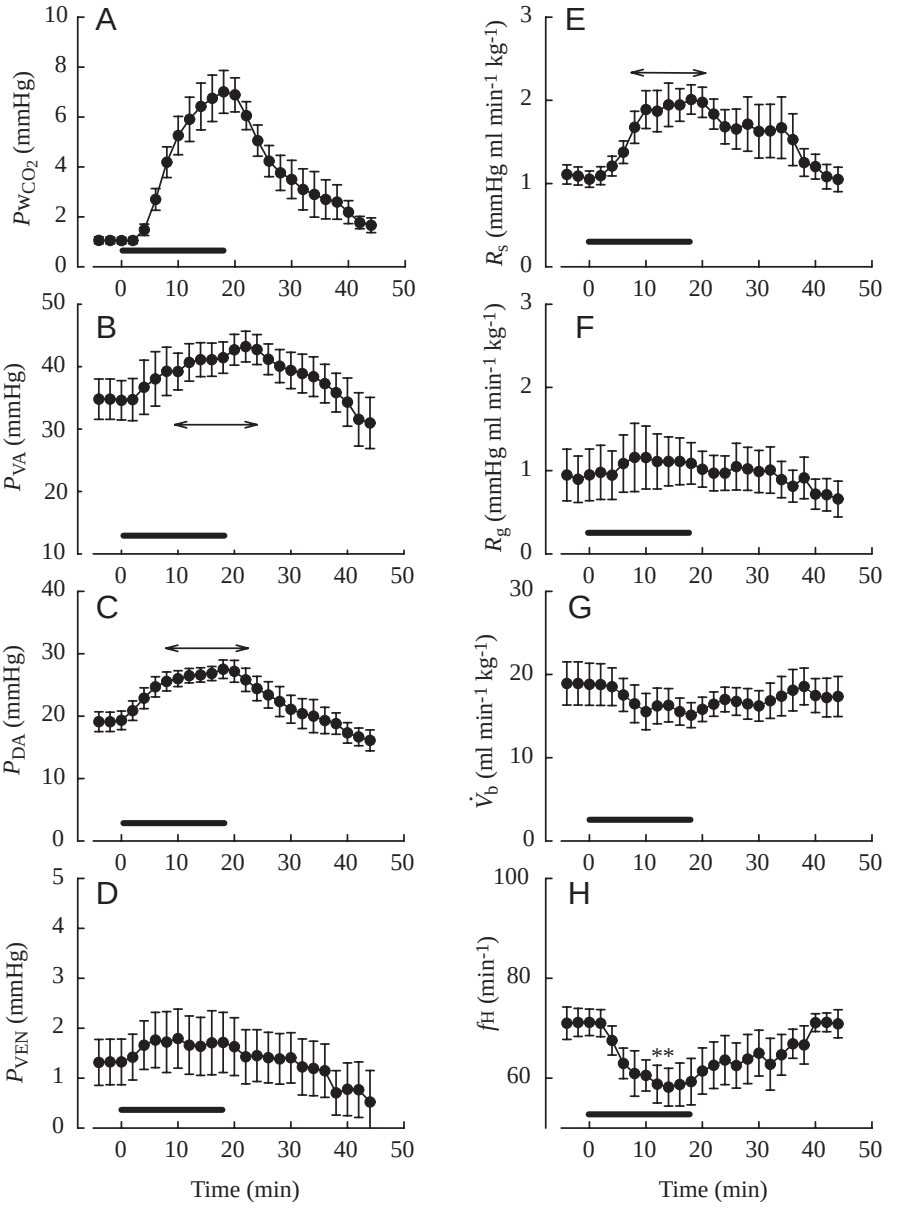


Fig. 2. The effects of a single exposure of rainbow trout (*Oncorhynchus mykiss*) to hypercapnia on (A) water carbon dioxide tension ($P_{wCO_2}$ $N$=9), (B) ventral aortic blood pressure ($P_{VA}$, $N$=6), (C) dorsal aortic blood pressure ($P_{DA}$, $N$=9), (D) central venous pressure ($P_{VEN}$; $N$=8), (E) systemic vascular resistance ($R_s$; $N$=9), (F) gill vascular resistance ($R_g$; $N$=6), (G) cardiac output ($\dot{V}_b$; $N$=9) and (H) heart rate ($f_H$; $N$=9). The solid horizontal bars in each panel represent the duration of the exposure to hypercapnia. The double-headed arrows indicate extended periods when statistically significant differences between the pre-hypercapnic and hypercapnic values occurred. The data are presented as means +1 S.E.M. An asterisk indicates specific points that were statistically different from the pre-hypercapnic values. 1 mmHg=0.1333 kPa.

pre-hypoxia values only during the final stage of hypoxia (Table 4). Breathing frequency was unaltered during hypoxia (Table 4).

The effects of hypoxia on plasma levels of catecholamines are summarised in Table 5. The circulating levels of adrenaline and noradrenaline remained constant until the final stage of hypoxia ($P_{wO_2}$=65 mmHg) was reached, at which time levels of both catecholamines were significantly elevated.

### *Blood respiratory variables during hypercapnia and hypoxia*

Exposure of fish to stepwise hypercapnia caused predictable $P_{wCO_2}$-dependent changes in blood respiratory and acid–base status, including a reduction in pHa and an increase in $P_{aCO_2}$ (Table 1). $P_{aO_2}$ was increased at the first level of hypercapnia and thereafter remained constant until the return to normocapnia. However, during the single-step hypercapnia experiment (series II), $P_{aO_2}$ remained constant (Table 3). During stepwise hypoxia, $P_{aO_2}$ decreased in accordance with the fall in external $P_{O_2}$ ($P_{wO_2}$) (Table 4). Arterial pH was elevated during the second stage of hypoxia ($P_{wO_2}$=95 mmHg) and probably reflected the tendency for a reduction in $P_{aCO_2}$ during hyperventilation (albeit the changes were not statistically significant).

## Discussion

### *Cardiovascular responses to environmental hypercapnia*

The effects of external hypercapnia on ventilation and blood acid–base balance are well documented in fish (e.g. Janssen and Randall, 1975; Thomas and Le Ruz, 1982; Thomas, 1983; Perry et al., 1987). The present study, however, is the first to investigate the effects of external hypercapnia on

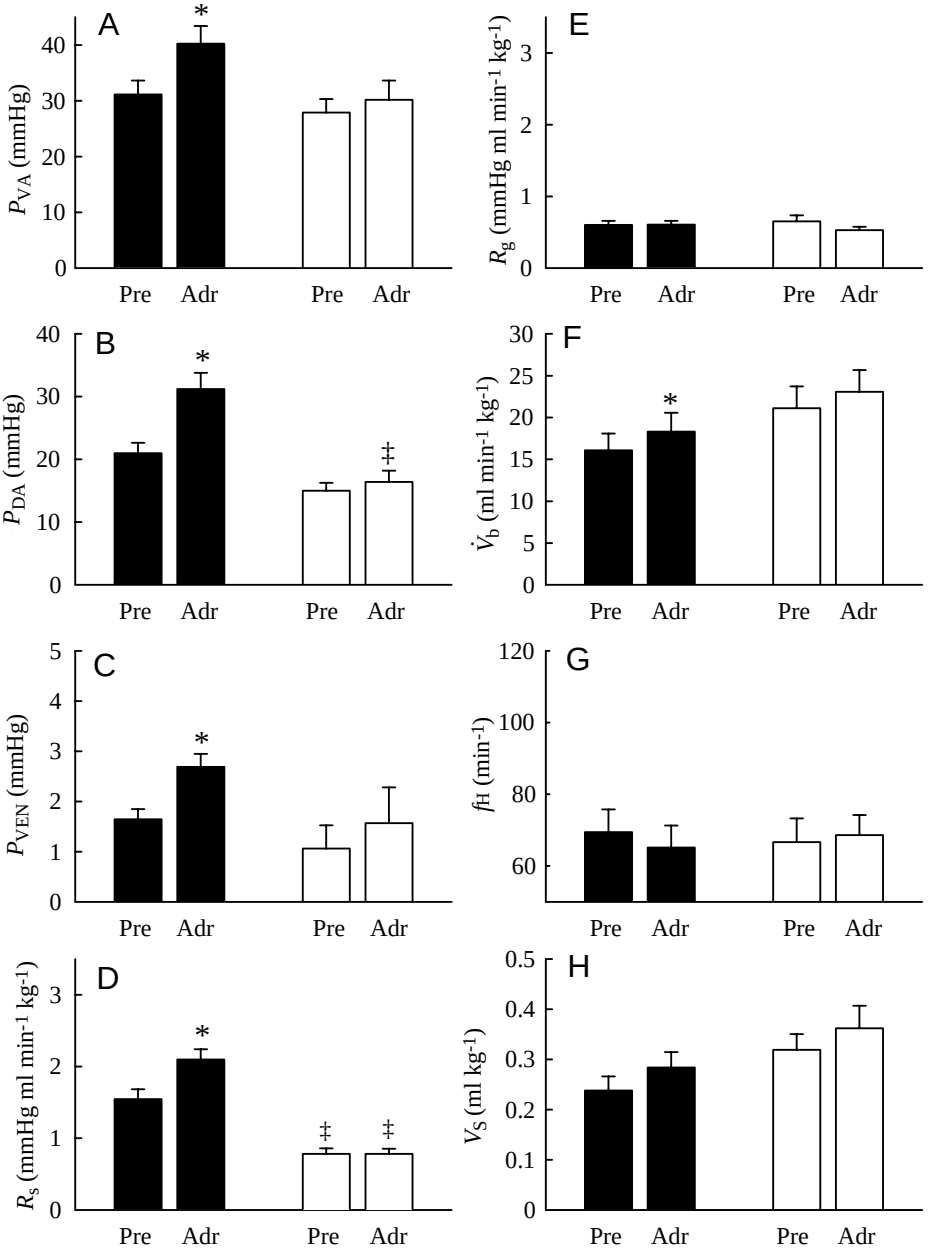


Fig. 3. The effects of intra-arterial injections of adrenaline (Adr; 10 nmol kg$^{-1}$) in rainbow trout (*Oncorhynchus mykiss*) before (filled columns) and after (open columns) treatment with yohimbine (2 mg kg$^{-1}$) on (A) ventral aortic blood pressure ($P_{VA}$, $N$=4), (B) dorsal aortic blood pressure ($P_{DA}$, $N$=7), (C) central venous pressure ($P_{VEN}$; $N$=5), (D) systemic vascular resistance ($R_S$; $N$=7), (E) gill vascular resistance ($R_g$; $N$=4), (F) cardiac output ($\dot{V}_b$; $N$=7), (G) heart rate ($f_H$; $N$=7) and (H) cardiac stroke volume ($V_S$; $N$=7). The data are presented as means +1 S.E.M. An asterisk indicates statistically significant differences arising from adrenaline treatment; a double dagger indicates statistically significant differences from the pre-yohimbine value ($P$<0.05). Pre, before adrenaline treatment. 1 mmHg=0.1333 kPa.

cardiovascular physiology in any teleost. The results clearly demonstrated that exposing trout to hypercapnia causes marked cardiovascular adjustments consisting of elevated blood pressures and bradycardia. The origin of the increase in arterial blood pressures ($P_{VA}$ and $P_{DA}$) was a large elevation of systemic vascular resistance.

In teleosts, systemic vascular resistance is controlled predominantly by the dual actions of constrictory α- and dilatory β-adrenoceptors (Nilsson, 1983). In most species that have been examined, the α-constrictory response predominates, and thus an increase in sympathetic nerve activity to the systemic vasculature or an elevation of circulating catecholamine levels generally causes an increase in systemic resistance (Wood and Shelton, 1980a,b). In the present study, the increase in systemic resistance during hypercapnia was clearly caused by the stimulation of α-adrenoceptors. This was established by comparing the responses of untreated fish with those pre-treated with the α-adrenoceptor antagonist yohimbine. Previous studies in fish have demonstrated that yohimbine is able to abolish adrenergic increases in systemic resistance *in vivo* (Wood and Shelton, 1980a) and in perfused trunk preparations (Wood, 1976) as well as eliminating adrenergic increases in vascular smooth muscle tension *in vitro* (Holmgren and Nilsson, 1974). Other conventional α-blockers, such as phentolamine (Smith, 1978) or phenoxybenzamine (Stevens et al., 1972; Wood, 1976; Xu and Olson, 1993), were not used in the present study because of the difficulty of achieving complete blockade (phentolamine) or the requirement for multiple injections spanning several hours (phenoxybenzamine). The

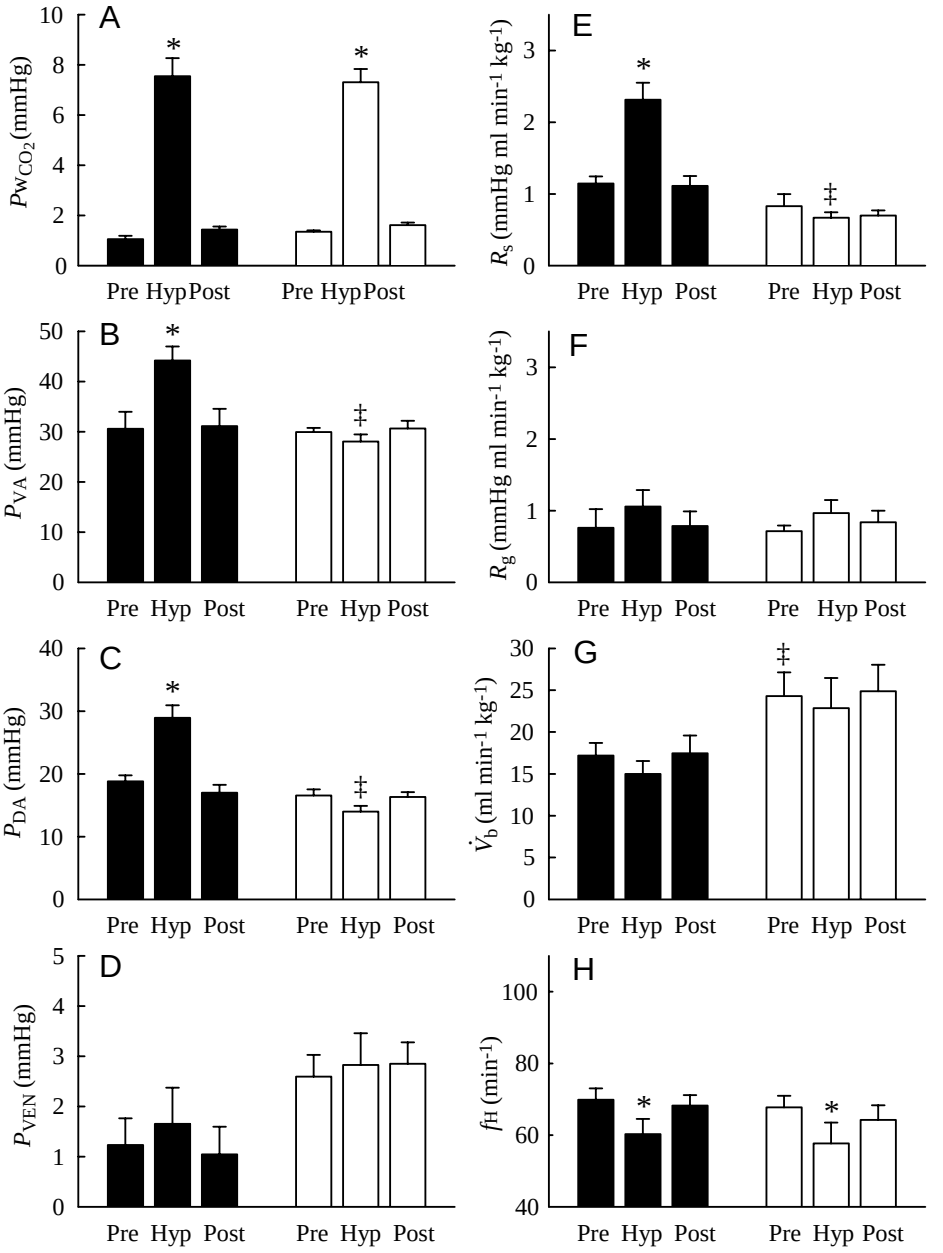


Fig. 4. The effects of a single step increase in water $P_{CO_2}$ ($P_{wCO_2}$) (A) in rainbow trout (*Oncorhynchus mykiss*) before (filled columns) and after (open columns) treatment with yohimbine (2 mg kg$^{-1}$) on (B) ventral aortic blood pressure ($P_{VA}$, $N$=5), (C) dorsal aortic blood pressure ($P_{DA}$, $N$=8), (D) central venous pressure ($P_{VEN}$; $N$=7), (E) systemic vascular resistance ($R_s$; $N$=7), (F) gill vascular resistance ($R_g$; $N$=5), (G) cardiac output ($\dot{V}_b$; $N$=8) and (H) heart rate ($f_H$; $N$=7). The data are presented as means +1 S.E.M. An asterisk indicates a statistically significant difference from the pre-hypercapnia (Pre) value; a double dagger indicates a statistically significant differences from the untreated (no yohimbine) value ($P$<0.05). Hyp, hypercapnia; Post, post-hypercapnia. 1 mmHg=0.1333 kPa.

effectiveness of yohimbine as an α-antagonist was verified in a detailed series of preliminary experiments.

### *Circulating catecholamine levels* versus *sympathetic nerve activity*

Plasma catecholamine levels did not increase at any level of hypercapnia (Table 2). Thus, the stimulation of systemic α-adrenoceptors and resultant increase in $R_s$ during hypercapnia were presumably caused exclusively by an increase in sympathetic nerve activity. Although previous studies have demonstrated an elevation of circulating catecholamine levels during hypercapnia in trout (Perry et al., 1987, 1989; Kinkead et al., 1993; Thomas et al., 1994; Perry and Gilmour, 1996), the response is highly variable and apparently dependent upon the severity of hypercapnia imposed on the fish. Several authors have reported stable plasma catecholamine levels in trout exposed to mild or moderate hypercapnia (Kinkead and Perry, 1991; Julio et al., 1998). The widely different catecholamine secretory responses to hypercapnia in trout may reflect the extent of hypoxaemia elicited by the respiratory acidosis (see Discussion in Julio et al., 1998). In comparison with other studies, however, it is surprising that plasma catecholamine levels were not elevated at the most severe level of hypercapnia ($P_{wCO_2}$=9 mmHg). This apparent blunting of the catecholamine secretory response to hypercapnia may reflect the fact that the trout in this facility are chronically acclimated to relatively high levels of $P_{wCO_2}$ in the normocapnic holding water (e.g. approximately 1.7 mmHg during pre-hypercapnia measurements).

Prior blockade of α-adrenoceptors eliminated the pressor responses to hypercapnia but did not eliminate the bradycardia. This indicates that the bradycardia was a direct consequence of the environmental hypercapnia and not a secondary barostatic reflex response. The origin of the bradycardia was not investigated in the present study, but presumably involved an increase in parasympathetic (cholinergic) vagal tone (Farrell and Jones, 1992), which is the predominant mechanism of controlling heart rate in most teleosts. Despite the significant reductions in $f_H$, cardiac output was maintained during hypercapnia owing to simultaneous increases in cardiac stroke volume. In the present study, the fish were subjected to extensive cardiovascular surgery. Previous studies have shown that similar surgery (Gamperl et al., 1994a) and, in particular, opening of the pericardium (Farrell et al., 1988) can influence cardiac performance. Thus, we cannot exclude the possibility that the cardiac responses to hypercapnia were underestimated in the present study.

A modest (0.85 mmHg), but significant, increase in $P_{VEN}$ was observed during the final stage of stepwise exposure to hypercapnia (Fig. 1), whereas $P_{VEN}$ was not significantly affected by a single exposure to nearly the same $P_{wCO_2}$ (although it also appeared to increase slightly). The reason for this discrepancy is not clear, but it may be explained by the different treatment regimes that resulted in a slightly more severe and greatly prolonged hypercapnia during stepwise treatment. Nevertheless, the increase in $P_{VEN}$ in the stepwise hypercapnia experiments is consistent with previous observations of a sympathetic control of venous tone in trout (Zhang et al., 1998).

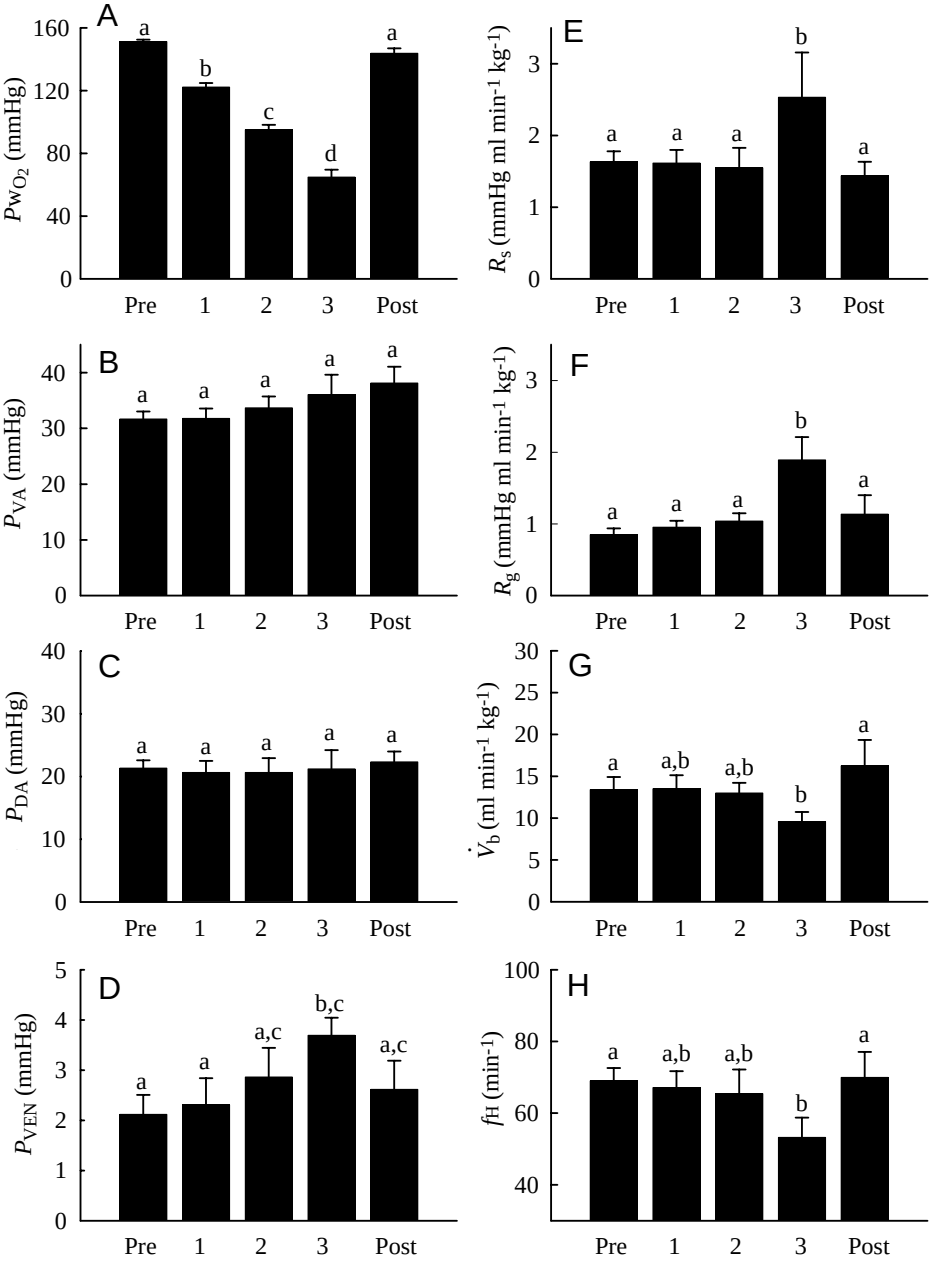


Fig. 5. The effects of stepwise decreases in external $P_{O_2}$ ($P_{wO_2}$) (A) on selected cardiovascular variables in rainbow trout (*Oncorhynchus mykiss*) including (B) ventral aortic blood pressure ($P_{VA}$; $N$=6), (C) dorsal aortic blood pressure ($P_{DA}$; $N$=6), (D) central venous blood pressure ($P_{VEN}$; $N$=4), (E) systemic vascular resistance ($R_s$; $N$=6), (F) gill vascular resistance ($R_g$; $N$=6), (G) cardiac output ($\dot{V}_b$; $N$=6) and (H) heart rate ($f_H$; $N$=6). Pre and Post refer to the pre- and post-hypoxia recording periods; the numbers 1–3 denote the three discrete steps of decreasing hypoxia (122, 95 and 65 mmHg). The data are presented as means +1 S.E.M.; values that do not share identical letters are significantly different ($P$<0.05). 1 mmHg=0.1333 kPa.

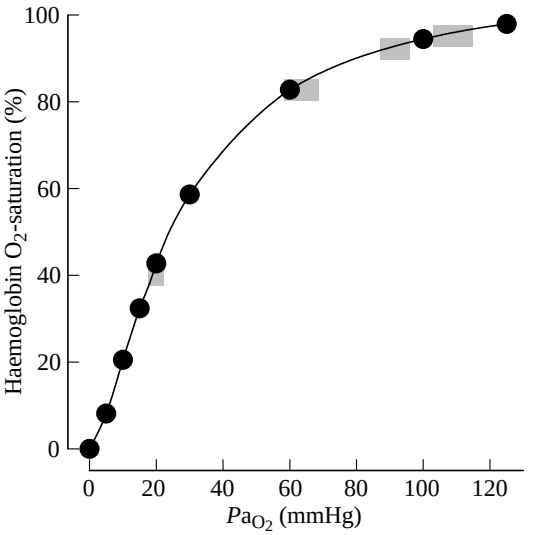


Fig. 6. An *in vitro* oxygen equilibrium curve for rainbow trout blood (data from Montpetit and Perry, 1998) showing the expected approximate reductions in haemoglobin oxygen-saturation associated with the falling arterial blood $P_{O_2}$ ($Pa_{O_2}$) during the stepwise hypoxia experiments (series IV). The horizontal length of each shaded box represents the mean $Pa_{O_2}$ value ±1 S.E.M. prior to hypoxia and during the three stages of increasing hypoxia. 1 mmHg=0.1333 kPa.

### *Does hypoxaemia contribute to the cardiovascular effects of environmental hypercapnia?*

As a result of Bohr and Root effects, environmental hypercapnia causes a reduction in arterial blood $O_2$ content. In trout, changes in blood $O_2$ content are believed to play an important role in the control of ventilation during hypoxia and hypercapnia (Randall, 1982; Smith and Jones, 1982). Thus, we investigated the possible contribution of hypoxaemia in promoting the cardiovascular responses to elevated ambient $CO_2$. The results clearly exclude a significant role for hypoxaemia in mediating these responses to hypercapnia in rainbow trout. First, the cardiovascular responses to hypercapnia were markedly different from the responses to hypoxia. Unlike in the hypercapnic fish, $P_{DA}$ and $P_{VA}$ remained constant at all levels of hypoxia. Even when systemic resistance was increased at the most severe level of hypoxia, arterial blood pressures did not rise, presumably as a result of concomitant decreases in $f_H$ and $\dot{V}_b$. Furthermore, during hypoxia, $R_s$ and $R_g$ both increased, whereas $R_g$ was unaffected during hypercapnia. Second, and perhaps more importantly, the expected reduction in blood $O_2$ content (approximately 20 %; see Fig. 6) during the second stage of hypoxia ($Pw_{O_2}$=95 mmHg) was almost certainly greater than the degree of hypoxaemia experienced at any level of hypercapnia. Although not measured in the present study, previous experiments utilising similar levels of ambient hypercapnia have reported reductions in blood $O_2$ content of approximately 15 % (Perry and Gilmour, 1996; Julio et al., 1998). Thus, given the absence of any cardiovascular adjustments during the second stage of hypoxia (predicted reduction in blood $O_2$ content 20 %), it is difficult to envisage a contributing role of hypoxaemia during exposure to $CO_2$. Indeed, even at the most severe level of hypercapnia, blood $O_2$ content was probably reduced by only 15 %. Finally, dorsal and ventral aortic pressor responses were initiated at low levels of hypercapnia (e.g. $Pw_{CO_2}$=3.5 mmHg). Such levels of hypercapnia would have negligible effects on blood $O_2$ content considering the minor changes in blood pH (from 7.83 to 7.76; Table 1).

Table 5. *The effects of a stepwise decrease in external* $P_{O_2}$ *on plasma catecholamine levels in rainbow trout* Oncorhynchus mykiss

| | $Pw_{O_2}$ (mmHg) | [Noradrenaline] (nmol $l^{-1}$) | [Adrenaline] (nmol $l^{-1}$) | [Total catecholamines] (nmol $l^{-1}$) |
|---|---|---|---|---|
| Pre | 151.3±1.3 | 0.3±0.2 | 1.0±0.7 | 1.3±0.8 |
| Step1 | 122.3±2.6* | 0.3±0.1 | 0.7±0.3 | 1.0±0.3 |
| Step 2 | 95.1±3.1* | 0.4±0.1 | 1.1±0.2 | 1.4±0.3 |
| Step 3 | 64.9±4.7* | 7.7±4.4* | 22.4±12.7* | 30.1±17.0* |
| Post | 143.8±3.3* | 1.3±0.3 | 3.7±1.5 | 5.0±1.7 |

* indicates a significant difference from the pre-hypoxia value ($P$<0.05).

Pre, Post, pre- and post-hypercapnia recording periods; $Pw_{O_2}$, external $P_{O_2}$.

1 mmHg=0.133 kPa.

### *External* versus *internal $CO_2/H^+$ receptors and possible direct actions of $CO_2/H^+$*

The presence of external and/or internal gill oxygen receptors is well established (Bamford, 1974; Smith and Jones, 1978; Fritsche and Nilsson, 1989, 1993; Burleson and Smatresk, 1990a,b). Stimulation of these receptors initiates hyperventilation as well as at least two cardiovascular reflexes, bradycardia (Wood and Shelton, 1980b; Smith and Davie, 1984; Fritsche and Nilsson, 1989; Fritsche, 1990; Sundin, 1995) and hypertension (Holeton and Randall, 1967; Saunders and Sutterlin, 1971; Wood and Shelton, 1980b; Fritsche and Nilsson, 1990).

The exact mechanism(s) by which $CO_2$ exerts its effects on ventilation and cardiovascular function is not known, but the results of the present study suggest the involvement of external $CO_2$ receptors linked to the autonomic nervous system. It seems most likely that the primary cardiovascular response (increased systemic resistance) was initiated by external $CO_2$ receptors because increases in dorsal aortic pressure have not been observed in fish experiencing endogenous (hyperoxic) hypercapnia (e.g. white sucker *Catostomus commersoni*, Wilkes et al., 1981; rainbow trout, S. F. Perry, unpublished observations). $CO_2$ and $H^+$ could also affect the vascular resistance by a direct action on the blood vessels. However, this seems unlikely as the sole explanation given (i) the rapidity of the blood pressure response, (ii) the quick recovery of the response after hypercapnia and (iii) the blockade of the response by yohimbine. Furthermore, recent results obtained from a perfused trunk preparation (J. McKendry and S. F.

Perry, unpublished data) have demonstrated that increasing $P_{CO_2}$ (up to 15 mmHg) does not cause vasoconstriction of the systemic vasculature in rainbow trout.

The hyperventilatory response during hypercapnia probably resulted from the specific effects of $CO_2/H^+$ on stimulating internal central or peripheral $CO_2/H^+$ chemoreceptors (Heisler et al., 1988; Graham et al., 1990; Wood et al., 1990; Kinkead and Perry, 1991; Wood and Munger, 1994; Perry and Gilmour, 1996) as well as the indirect stimulating influence of hypercapnic hypoxaemia (Smith and Jones, 1982). Further, we cannot exclude the possible contribution of external $CO_2/H^+$ receptors. Although the role of circulating catecholamines in promoting hyperventilatory responses in fish is under debate (Randall and Taylor, 1991; Perry et al., 1992), the results of the present study (hyperventilation in the absence of elevated catecholamine levels) reinforce the view that increased levels of plasma catecholamines are not a prerequisite for hyperventilation to occur (Kinkead and Perry, 1991; Perry and Gilmour, 1996).

### *Are the cardiovascular responses to hypercapnia physiologically significant?*

During hypercapnia, $Pa_{O_2}$ may increase (see Table 1) or remain constant (see Table 3; Eddy et al., 1977) despite a lowering of venous $P_{O_2}$ ($Pv_{O_2}$) (Thomas et al., 1994). This apparent enhancement of gas transfer may reflect the combination of ventilatory and cardiovascular adjustments. It has been suggested that hypertension enhances branchial gas transfer *via* 'lamellar recruitment'. The increase in ventral aortic pressure is believed to cause 'lamellar recruitment' by initiating perfusion of the more distal lamellae on the filaments as well as by altering the flow pattern within each lamella (Booth, 1978, 1979a,b; Farrell et al., 1979; Soivio and Tuurala, 1981). Further experiments are required to elucidate the physiological significance (if any) of the increased systemic vascular resistance during hypercapnia. In particular, it would be informative to compare gas transfer during hypercapnia in untreated fish (large increase in $R_s$) with that in fish treated with α-adrenoceptor antagonists (no increase in $R_s$).

Trout generally inhabit well-aerated waters that are likely to exhibit low and constant levels of $CO_2$. However, external hypercapnia could potentially occur in large bodies of water subject to thermal stratification or in smaller lakes or rivers fed by carbonate- or bicarbonate-rich water derived from underground springs. In fish farms, holding trout under crowded conditions in spring-fed ponds can lead to external hypercapnia. Regardless of whether trout experience elevated $CO_2$ levels in the natural environment, their responsiveness to external hypercapnia (as for hypoxia) may reflect a phylogenetic lineage in which the selective advantage conferred by the ability to respond to elevated $CO_2$ and/or reduced $O_2$ levels was significant.

### *A comparison of the cardiovascular responses to hypercapnia and hypoxia*

The two common responses shared by trout exposed to hypercapnia with trout and other teleosts exposed to hypoxia are an elevation of systemic vascular resistance and bradycardia. However, it is important to point out that the cardiovascular responses of teleosts to hypoxia are highly variable. For example, $R_s$ responses to hypoxia in teleosts range from little or no change (Peyreaud-Waitzenegger and Soulier, 1989; Axelsson and Fritsche, 1991; Bushnell and Brill, 1992; Gamperl et al., 1994b) to large increases (Fritsche and Nilsson, 1989, 1990; Axelsson and Farrell, 1993; Sundin, 1995). The mechanisms underlying the influence of hypoxia on $R_s$ have been examined thoroughly in the Atlantic cod *Gadus morhua* (Fritsche and Nilsson, 1990; Axelsson and Fritsche, 1991). Essentially, the net effect of hypoxia on $R_s$ in cod reflects the stimulation of systemic α-adrenoreceptors by sympathetic nerves and by circulating catecholamines that is opposed by unidentified vasodilator substances; generally, the vasoconstrictor effect predominates. Thus, reflex stimulation of systemic α-adrenoceptors appears to be a common response to both hypercapnia and hypoxia. The degree of bradycardia experienced by fish during hypoxia is also highly variable and may depend upon the severity of the hypoxia and the rapidity with which it is imposed. A similar situation may exist in fish exposed to hypercapnia because the reduction in $f_H$ in the present study was proportional to the degree of $P_{CO_2}$ elevation and was greatest in fish rapidly exposed to hypercapnia (single step experiment).

In summary, hypercapnia elicits profound cardiovascular and ventilatory adjustments in rainbow trout. Although the physiological significance of the cardiovascular changes remains unclear, it is nevertheless apparent from this and previous (Heisler et al., 1988; Wood et al., 1990; Graham et al., 1990; Kinkead and Perry, 1991; Wood and Munger, 1994; Perry and Gilmour, 1996) studies that $CO_2/H^+$ is emerging as an important modulator of cardiorespiratory function in fishes.

This research was supported by NSERC Research and Equipment grants to S.F.P. and by NSF grants (grant No. IBN 9723306) to K.O. We are grateful to Drs Serge Thomas and Mark Powell for their contributions to earlier versions of this work. We also thank Dr Peter Bushnell for his valuable advice and assistance throughout this study.

## References

**Axelsson, M. and Farrell, A. P.** (1993). Coronary blood flow *in vivo* in the coho salmon (*Oncorhynchus kisutch*). *Am. J. Physiol.* **264**, R963–R971.

**Axelsson, M. and Fritsche, R.** (1991). Effects of exercise, hypoxia and feeding on the gastrointestinal blood flow in the Atlantic cod *Gadus morhua*. *J. Exp. Biol.* **158**, 181–198.

**Bamford, O. S.** (1974). Oxygen reception in the rainbow trout (*Salmo gairdneri*). *Comp. Biochem. Physiol.* **48**A, 69–76.

**Bernier, N. J. and Randall, D. J.** (1998). Carbon dioxide anaesthesia in rainbow trout: effects of hypercapnic level and stress on induction and recovery from anaesthetic treatment. *J. Fish Biol.* **52**, 621–637.

**Booth, J. H.** (1978). The distribution of blood flow to the gills of fish: Application of a new technique to rainbow trout (*Salmo gairdneri*). *J. Exp. Biol.* **73**, 119–129.

**Booth, J. H.** (1979a). Circulation in trout gills: the relationship

between branchial perfusion and the width of the lamellar blood space. *Can. J. Zool.* **57**, 2183–2185.

**Booth, J. H.** (1979b). The effects of oxygen supply, epinephrine and acetylcholine on the distribution of blood flow in trout gills. *J. Exp. Biol.* **83**, 31–39.

**Burleson, M. L. and Smatresk, N. J.** (1990a). Effects of sectioning cranial nerves IX and X on cardiovascular and ventilatory reflex responses to hypoxia and NaCN in channel catfish. *J. Exp. Biol.* **154**, 407–420.

**Burleson, M. L. and Smatresk, N. J.** (1990b). Evidence for two oxygen-sensitive loci in channel catfish, *Ictalurus punctatus*. *Physiol. Zool.* **63**, 208–221.

**Bushnell, P. G. and Brill, R. W.** (1991). Responses of swimming skipjack (*Katsuwonus pelamis*) and yellowfin (*Thunnus albacares*) tunas to acute hypoxia and a model of their cardiorespiratory function. *Physiol. Zool.* **64**, 787–811.

**Bushnell, P. G. and Brill, R. W.** (1992). Oxygen transport and cardiovascular responses in skipjack tuna (*Katsuwonus pelamis*) and yellowfin tuna (*Thunnus albacares*) exposed to acute hypoxia. *J. Comp. Physiol.* **162**, 131–143.

**Bushnell, P. G. and Jones, D. R.** (1992). The arterial system. In *The Cardiovascular System* (ed. W. S. Hoar, D. J. Randall and A. P. Farrell), pp. 89–139. New York: Academic Press.

**Cameron, J. N.** (1976). Branchial ion uptake in Arctic grayling: resting values and the effects of acid–base disturbance. *J. Exp. Biol.* **64**, 711–725.

**Cameron, J. N. and Randall, D. J.** (1972). The effect of increased ambient $CO_2$ on arterial $CO_2$ tension, $CO_2$ content and pH in rainbow trout. *J. Exp. Biol.* **57**, 673–680.

**Eddy, F. B.** (1976). Acid–base balance in rainbow trout (*Salmo gairdneri*) subjected to acid stresses. *J. Exp. Biol.* **64**, 159–171.

**Eddy, F. B., Lomholt, J. P., Weber, R. E. and Johansen, K.** (1977). Blood respiratory properties of rainbow trout (*Salmo gairdneri*) kept in water of high $CO_2$ tension. *J. Exp. Biol.* **67**, 37–47.

**Eddy, F. B., Smart, G. R. and Bath, R. N.** (1979). Ionic content of muscle and urine in rainbow trout, *Salmo gairdneri* Richardson kept in water of high $CO_2$ content. *J. Fish Diseases* **2**, 105–110.

**Farrell, A. P.** (1993). Cardiovascular system. In *The Physiology of Fishes* (ed. D. H. Evans), pp. 219–250. Boca Raton, FL: CRC Press.

**Farrell, A. P., Daxboeck, C. and Randall, D. J.** (1979). The effect of input pressure and flow on the pattern and resistance to flow in the isolated perfused gill of a teleost fish. *J. Comp. Physiol.* **133**, 233–240.

**Farrell, A. P., Johansen, J. A. and Graham, M. S.** (1988). The role of the pericardium in cardiac performance of the trout (*Salmo gairdneri*). *Physiol. Zool.* **61**, 213–221.

**Farrell, A. P. and Jones, D. R.** (1992). The heart. In *The Cardiovascular System* (ed. W. S. Hoar, D. J. Randall and A. P. Farrell), pp. 1–88. New York: Academic Press.

**Fritsche, R.** (1990). Effects of hypoxia on blood pressure and heart rate in three marine teleosts. *Fish Physiol. Biochem.* **8**, 85–92.

**Fritsche, R. and Nilsson, S.** (1989). Cardiovascular responses to hypoxia in the Atlantic cod, *Gadus morhua*. *Exp. Biol.* **48**, 153–160.

**Fritsche, R. and Nilsson, S.** (1990). Autonomic nervous control of blood pressure and heart rate during hypoxia in the cod, *Gadus morhua*. *J. Comp. Physiol.* B **160**, 287–292.

**Fritsche, R. and Nilsson, S.** (1993). Cardiovascular and ventilatory control during hypoxia. In *Fish Ecophysiology* (ed. J. C. Rankin and F. B. Jensen), pp. 180–206. London: Chapman & Hall.

**Gamperl, A. K., Pinder, A. W. and Boutilier, R. G.** (1994a). Effect of coronay ablation and adrenergic stimulation on *in vivo* cardiac performance in trout (*Oncorhynchus mykiss*). *J. Exp. Biol.* **186**, 127–143

**Gamperl, A. K., Pinder, A. W., Grant, R. R. and Boutilier, R. G.** (1994b). Influence of hypoxia and adrenaline administration on coronary blood flow and cardiac performance in seawater rainbow trout (*Oncorhynchus mykiss*). *J. Exp. Biol.* **193**, 209–232.

**Graham, M. S., Turner, J. D. and Wood, C. M.** (1990). The control of ventilation during hypercapnia in the skate, *Raja ocellata*. I. Blood and extradural fluid chemistry. *Respir. Physiol.* **80**, 259–277.

**Heisler, N., Toews, D. P. and Holeton, G. F.** (1988). Regulation of ventilation and acid–base status in the elasmobranch *Scyliorhinus stellaris* during hyperoxia induced hypercapnia. *Respir. Physiol.* **71**, 227–246.

**Holeton, G. F. and Randall, D. J.** (1967). Changes in blood pressure in the rainbow trout during hypoxia. *J. Exp. Biol.* **46**, 297–305.

**Holmgren, S. and Nilsson, S.** (1974). Drug effects on isolated artery strips from two teleosts, *Gadus morhua* and *Salmo gairdneri*. *Acta Physiol. Scand.* **90**, 431–437.

**Janssen, R. G. and Randall, D. J.** (1975). The effects of changes in pH and $P_{CO_2}$ in blood and water on breathing in rainbow trout, *Salmo gairdneri*. *Respir. Physiol.* **25**, 235–245.

**Julio, A. E., Montpetit, C. and Perry, S. F.** (1998). Does blood acid–base status modulate catecholamine secretion in the rainbow trout (*Oncorhynchus mykiss*)? *J. Exp. Biol.* **201**, 3085–3095.

**Kinkead, R., Aota, S., Perry, S. F. and Randall, D. J.** (1993). Propranolol impairs the hyperventilatory response to acute hypercapnia in rainbow trout. *J. Exp. Biol.* **175**, 115–126.

**Kinkead, R. and Perry, S. F.** (1991). The effects of catecholamines on ventilation in rainbow trout during external hypoxia or hypercapnia. *Respir. Physiol.* **84**, 77–92.

**Lloyd, R. and White, W. R.** (1967). Effect of high concentration of carbon dioxide on the ionic composition of rainbow trout blood. *Nature* **216**, 1341–1342.

**MacKenzie, W. M. and Perry, S. F.** (1997). The effects of hypercapnia on branchial and renal calcium fluxes in the rainbow trout (*Oncorhynchus mykiss*). *J. Comp. Physiol.* B **167**, 52–60.

**Maxime, V., Nonnotte, G., Peyraud, C., Williot, P. and Truchot, J. P.** (1995). Circulatory and respiratory effects of an hypoxic stress in the Siberian sturgeon. *Respir. Physiol.* **100**, 203–212.

**Milsom, W. K.** (1995a). Regulation of respiration in lower vertebrates: Role of $CO_2$/pH chemoreceptors. In *Advances in Comparative and Environmental Physiology* (ed. R. Gilles), vol. 21, *Gas Exchange and Regulation of Respiration* (ed. N. Heisler), pp. 62–104. Berlin: Springer-Verlag.

**Milsom, W. K.** (1995b). The role of $CO_2$/pH chemoreceptors in ventilatory control. *Braz. J. Med. Biol. Res.* **28**, 1147–1160.

**Mommsen, T. P., Walsh, P. J., Perry, S. F. and Moon, T. W.** (1988). Interactive effects of catecholamines and hypercapnia on glucose production in isolated trout hepatocytes. *Gen. Comp. Endocr.* **70**, 63–73.

**Montpetit, C. and Perry, S. F.** (1998). The effects of chronic hypoxia on the acute adrenergic stress response in the rainbow trout (*Oncorhynchus mykiss*). *Physiol. Zool.* **71**, 377–386.

**Nichols, A. J. and Ruffolo, R. R.** (1991). Structure–activity relationships for α-adrenoceptor agonists and antagonists. In *α-Adrenoceptors: Molecular Biology, Biochemistry and Physiology* (ed. R. R. Rufollo), *Progress in Basic Clinical Pharmacology*, vol. 8 (ed. O. Lomax and E. S. Vesell), pp. 75–114. Basel: Karger.

**Nilsson, S.** (1983). *Autonomic Nerve Function in the Vertebrates. Zoophysiology*, vol. 13. Berlin, Heidelberg, New York: Springer-Verlag. 253pp.

**Olson, K. R.** (1998). The cardiovascular system. In *The Physiology of Fishes* (ed. D. H. Evans), pp. 129–154. Boca Raton, FL: CRC Press.

**Olson, K. R., Conklin, D. J., Weaver, L., Duff, D. W., Herman, C. A., Wang, X. Y. and Conlon, J. M.** (1997). Cardiovascular effects of homologous bradykinin in rainbow trout. *Am. J. Physiol.* **41**, R1112–R1120.

**Perry, S. F. and Gilmour, K. M.** (1996). Consequences of catecholamine release on ventilation and blood oxygen transport during hypoxia and hypercapnia in an elasmobranch (*Squalus acanthias*) and a teleost (*Oncorhynchus mykiss*). *J. Exp. Biol.* **199**, 2105–2118.

**Perry, S. F. and Kinkead, R.** (1989). The role of catecholamines in regulating arterial oxygen content during hypercapnic acidosis in rainbow trout (*Salmo gairdneri*). *Respir. Physiol.* **77**, 365–378.

**Perry, S. F., Kinkead, R. and Fritsche, R.** (1992). Are circulating catecholamines involved in the control of breathing by fishes? *Rev. Fish Biol. Fish.* **2**, 65–83.

**Perry, S. F., Kinkead, R., Gallaugher, P. and Randall, D. J.** (1989). Evidence that hypoxemia promotes catecholamine release during hypercapnic acidosis in rainbow trout (*Salmo gairdneri*). *Respir. Physiol.* **77**, 351–364.

**Perry, S. F., Malone, S. and Ewing, D.** (1987). Hypercapnic acidosis in the rainbow trout (*Salmo gairdneri*). I. Branchial ionic fluxes and blood acid–base status. *Can. J. Zool.* **65**, 888–895.

**Perry, S. F., Walsh, P. J., Mommsen, T. P. and Moon, T. W.** (1988). Metabolic consequences of hypercapnia in the rainbow trout, *Salmo gairdneri*: beta adrenergic effects. *Gen. Comp. Endocr.* **69**, 439–447.

**Perry, S. F. and Wood, C. M.** (1989). Control and co-ordination of gas transfer in fishes. *Can. J. Zool.* **67**, 2961–2970.

**Peyraud, C. and Ferret-Bouin, P.** (1960). Methode electronique pour l'analyse de la mechanique respiratoire des poissons. *Bull. Soc. Hist. Nat. Toulouse* **95**, 385–390.

**Peyreaud-Waitzenegger, M. and Soulier, P.** (1989). Ventilatory and cardiovascular adjustments in the European eel (*Anguilla anguilla*) exposed to short term hypoxia. *Exp. Biol.* **48**, 107–122.

**Piiper, J., Baumgarten, D. and Meyer, M.** (1970). Effects of hypoxia upon respiration and circulation in the dogfish *Scyliorhinus stellaris*. *Comp. Biochem. Physiol.* **36**A, 513–520.

**Randall, D. J.** (1982). The control of respiration and circulation in fish during exercise and hypoxia. *J. Exp. Biol.* **100**, 275–288.

**Randall, D. J. and Taylor, E. W.** (1991). Evidence of a role for catecholamines in the control of breathing in fish. *Rev. Fish Biol. Fish.* **1**, 139–157.

**Satchell, G. H.** (1961). The response of the dogfish to anoxia. *J. Exp Biol.* **38**, 531–543.

**Saunders, R. L. and Sutterlin, A. M.** (1971). Cardiac and respiratory responses to hypoxia in the sea raven, *Hemitripterus americanus* and an investigation of possible control mechanisms. *J. Fish Res. Bd Can.* **28**, 491–503.

**Smith, D. G.** (1978). Neural regulation of blood pressure in rainbow trout (*Salmo gairdneri*). *Can. J. Zool.* **56**, 1678–1683.

**Smith, F. M. and Davie, P. S.** (1984). Effects of sectioning cranial nerves IX and X on the cardiac response to hypoxia in the coho salmon, *Oncorhynchus kisutch*. *Can. J. Zool.* **62**, 766–768.

**Smith, F. M. and Jones, D. R.** (1978). Localization of receptors causing hypoxic bradycardia in trout (*Salmo gairdneri*). *Can. J. Zool.* **56**, 1260–1265.

**Smith, F. M. and Jones, D. R.** (1982). The effect of changes in blood oxygen carrying capacity on ventilation volume in the rainbow trout (*Salmo gairdneri*). *J. Exp. Biol.* **97**, 325–334.

**Soivio, A. and Tuurala, H.** (1981). Structural and circulatory responses to hypoxia in the secondary lamellae of *Salmo gairdneri* gills at two temperatures. *J. Comp. Physiol.* **145**, 37–43.

**Stevens, E. D., Bennion, G. R., Randall, D. J. and Shelton, G.** (1972). Factors affecting arterial pressures and blood flow from the heart in intact, unrestrained lingcod, *Ophiodon elongatus*. *Comp. Biochem. Physiol.* **43**A, 681–695.

**Sundin, L. I.** (1995). Responses of the branchial circulation to hypoxia in the Atlantic cod, *Gadus morhua*. *Am. J. Physiol.* **37**, R771–R778.

**Thomas, S.** (1983). Changes in blood acid–base balance in trout (*Salmo gairdneri* Richardson) following exposure to combined hypoxia and hypercapnia. *J. Comp. Physiol.* B **152**, 53–57.

**Thomas, S.** (1994). Extracorporeal circulation. In *Biochemistry and Molecular Biology of Fishes* (ed. P. W. Hochachka and T. P. Mommsen), pp. 161–167. Amsterdam: Elsevier.

**Thomas, S., Fritsche, R. and Perry, S. F.** (1994). Pre- and post-branchial blood respiratory status during acute hypercapnia or hypoxia in rainbow trout (*Oncorhynchus mykiss)*. *J. Comp. Physiol.* **164**, 451–458.

**Thomas, S. and Le Ruz, H.** (1982). A continuous study of rapid changes in blood acid–base status of trout during variations of water $P_{CO_2}$. *J. Comp. Physiol.* **148**, 123–130.

**Walsh, P. J., Mommsen, T. P., Moon, T. W. and Perry, S. F.** (1988). Effects of acid–base variables on *in vitro* hepatic metabolism in rainbow trout. *J. Exp. Biol.* **135**, 231–241.

**Wilkes, P. R. H., Walker, R. L., McDonald, D. G. and Wood, C. M.** (1981). Respiratory, ventilatory, acid–base and ionoregulatory physiology of the white sucker, *Catostomus commersoni*: the influence of hyperoxia. *J. Exp. Biol.* **91**, 239–254.

**Wood, C. M.** (1976). Pharmacological properties of the adrenergic receptors regulating systemic vascular resistance in the rainbow trout. *J. Comp. Physiol.* **107**, 211–228.

**Wood, C. M. and Munger, R. S.** (1994). Carbonic anhydrase injection provides evidence for the role of blood acid–base status in stimulating ventilation after exhaustive exercise in rainbow trout. *J. Exp. Biol.* **194**, 225–253.

**Wood, C. M. and Shelton, G.** (1980a). Cardiovascular dynamics and adrenergic responses of the rainbow trout *in vivo*. *J. Exp. Biol.* **87**, 247–270.

**Wood, C. M. and Shelton, G.** (1980b). The reflex control of heart rate and cardiac output in the rainbow trout: interactive influences of hypoxia, haemorrhage and systemic vasomotor tone. *J. Exp. Biol.* **87**, 271–284.

**Wood, C. M., Turner, J. D., Munger, S. and Graham, M. S.** (1990). Control of ventilation in the hypercapnic skate *Raja ocellata*. II. Cerebrospinal fluid and intracellular pH in the brain and other tissues. *Respir. Physiol.* **80**, 279–297.

**Woodward, J. J.** (1982). Plasma catecholamines in resting rainbow trout, *Salmo gairdneri* Richardson, by high pressure liquid chromatography. *J. Fish Biol.* **21**, 429–432.

**Xu, H. Y. and Olson, K. R.** (1993). Evidence for multiple adrenoceptor sites in rainbow trout vasculature. *J. Comp. Physiol.* B **163**, 470–476.

**Yoshikawa, H., Yokoyama, Y., Ueno, S. and Mitsuda, H.** (1991). Changes of blood gas in carp, *Cyprinus carpio*, anesthetized with carbon dioxide. *Comp. Biochem. Physiol.* **98**A, 431–436.

**Zhang, Y., Weaver, L., Jr, Ibeawuchi, A. and Olson, K. R.** (1998). Catecholaminergic regulation of venous function in the rainbow trout. *Am. J. Physiol.* **274**, R1195–R1202.