J. exp. Biol. **167**, 179–201 (1992)
Printed in Great Britain © *The Company of Biologists Limited 1992*

CARDIOVASCULAR RESPONSES OF THE RED-BLOODED ANTARCTIC FISHES *PAGOTHENIA BERNACCHII* AND *P. BORCHGREVINKI*

BY M. AXELSSON*, W. DAVISON, M. E. FORSTER AND A. P. FARRELL†

Department of Zoology, University of Canterbury, Christchurch, New Zealand

Accepted 3 February 1992

Summary

The aim of this study was to investigate cardiac performance and cardiovascular control in two red-blooded nototheniid species of antarctic fishes, *Pagothenia bernacchii* (a benthic fish) and *P. borchgrevinki* (a cryopelagic fish), and to make comparisons with existing information on haemoglobin-free antarctic teleosts. In quiescent *P. bernacchii* at 0°C ventral aortic pressure (P_{VA}) was 3.09 kPa and cardiac output ($\dot{Q}$) was 17.6 ml min^{-1} kg^{-1}, with a heart rate (f_H) of 10.5 beats min^{-1} and stroke volume of 1.56 ml kg^{-1}. Following atropine treatment, $\dot{Q}$ was maintained but heart rate increased and stroke volume decreased. Resting heart rate resulted from an inhibitory cholinergic tone of 80.4 % and an excitatory adrenergic tone of 27.5 %. The intrinsic heart rate was 21.7 beats min^{-1} at 0°C.

In quiescent *P. borchgrevinki* at 0°C, P_{VA} was 3.6 kPa, $\dot{Q}$ was 29.6 ml min^{-1} kg^{-1} and stroke volume was 2.16 ml kg^{-1}. The resting heart rate in *P. borchgrevinki* of 11.3 beats min^{-1} resulted from an inhibitory cholinergic tone of 54.5 % and an excitatory adrenergic tone of 3.2 %. The intrinsic heart rate was 23.3 beats min^{-1}.

P. bernacchii maintained $\dot{Q}$ during a progressive decrease in water oxygen tension from 20 to 6.7 kPa, but f_H was increased significantly. Thus, although there is cholinergic control of the heart, no hypoxic bradycardia was observed. Recovery from hypoxia was associated with increases in $\dot{Q}$ and f_H; stroke volume returned to control values. P_{VA} declined in recovery as total vascular resistance decreased. Hypoxic exposure following atropine treatment resulted in progressive increases in P_{VA}, $\dot{Q}$ and stroke volume; f_H decreased during the recovery period. Hypoxic exposure in *P. borchgrevinki* produced similar cardiovascular responses to those observed in *P. bernacchii*.

During an acute increase in water temperature from 0 to 5°C, *P. bernacchii*

* Present address: Comparative Neuroscience Unit, Department of Zoophysiology, University of Göteborg, Box 250 59, S-400 31 Göteborg, Sweden.

† Present address: Department of Biological Sciences, Simon Fraser University, Burnaby, BC, Canada V5A 1S6.

Key words: cardiac output, heart rate, blood pressure, hypoxia, swimming, temperature, adrenaline, cholinergic, *Pagothenia bernacchii*, *Pagothenia borchgrevinki*.

regulated $\dot{Q}$ and total vascular resistance. Stroke volume decreased as f_H increased. The intrinsic heart rate had a Q_{10} of 1.96 over this temperature range. *P. bernacchii* maintained chronotropic inhibition up to a temperature of 2.5–3.0°C. However, by 5°C this chronotropic inhibition of the heart rate was lost.

Infusion of adrenaline into the ventral aorta of *P. bernacchii* resulted in significant increases in $\dot{Q}$, f_H, P_{VA} and total vascular resistance. Infusion of adrenaline after atropine treatment caused similar cardiovascular changes without the change in f_H.

P. borchgrevinki could sustain swimming in a water tunnel at approximately 1 body length per second for 6–10 min. During the bout of swimming there was a doubling of ventilation frequency, a 75% increase in $\dot{Q}$ as f_H doubled and a decrease in total vascular resistance.

Similarities exist between these two red-blooded antarctic teleosts and haemoglobin-free channichthyids. These include a low P_{VA}, associated with a low vascular resistance, and a high cardiac stroke volume. Ventricle mass is somewhat larger than in temperate species of teleosts, especially considering the low aortic pressures developed by the heart. The absence of a sustained bradycardia in *P. bernacchii* during hypoxia or an adrenaline-induced increase in P_{VA} is unusual. This may in part reflect the very high inhibitory cholinergic tone to the heart, the highest value found in resting fish.

Introduction

There are over 200 species of fish that are known to live south of the Antarctic convergence (Andriashev, 1987; Eastman, 1991). These fish inhabit a stenothermal environment in which temperatures typically range from −1.9 to 1.0°C. In this environment, the fish benefit from an abundance of food, limited predation from piscivorous fishes and, in terms of oxygen delivery, a high oxygen solubility in water and plasma. While the metabolic demand for oxygen is reduced at low temperatures in ectothermic animals, there is evidence for partial temperature compensation in notothenioid fish (Holeton, 1970, 1972; Hemmingsen and Douglas, 1970, 1972; Macdonald *et al.* 1987; Forster *et al.* 1987).

The cardiovascular adaptations that permit antarctic fish to live in these frigid waters are of particular interest. Without any compensating mechanism, the hearts of antarctic fish might be expected to perform proportionately more work, since blood, in common with other fluids, has an increased viscosity at low temperatures (Graham and Fletcher, 1985). Antarctic fish reduce the effective blood viscosity in two ways: they have a high lipid content in the plasma (Rickenbach, 1990) and a reduction in the number of circulating red blood cells. A small number of antarctic fish species (17 species of channichthyids) have haemoglobin-free blood which reduces blood viscosity to 0.3–0.4 Pa s at 0°C (Hemmingsen and Douglas, 1972; Wells *et al.* 1990). In other antarctic species haematocrit values (8–18%) are lower than in temperate species of teleosts (Macdonald *et al.* 1987). One of these species, *Pagothenia borchgrevinki*, shows an unusually large increase in haematocrit when stressed (Davison *et al.* 1988; Franklin *et al.* 1991), suggesting that there

is an energetic advantage to sequestering large numbers of cells in the spleen to be released only when needed. In contrast, haematocrit values between 25 and 39 % were reported for two species of antarctic nototheniids (*Notothenia neglecta* Nybelin and *N. gibberifrons* Lönnberg) and for a bathydraconid (*Parachaenichthys charcoti* Vaillant) (Holeton, 1970).

Studies of two species of channichthyids, *Chaenocephalus aceratus* and *Pseudochaenichthys georgianus*, have described some of the cardiovascular adaptations associated with the absence of haemoglobin from the blood. *C. aceratus* has a high blood volume, estimated to be 2–3 times greater than that of other teleosts (Hemmingsen and Douglas, 1970). Because the oxygen content of the blood is low while oxygen uptake is not, it was predicted that cardiac output ($\dot{Q}$) would be extremely high (Holeton, 1970). Most recorded values of $\dot{Q}$ in haemoglobin-free fish are high, but unfortunately they show a large variation and may not be completely free of measurement error. Reported values of $\dot{Q}$ determined by the Fick principle are $61\,ml\,min^{-1}\,kg^{-1}$ (Holeton, 1970, 1972), $119\,ml\,min^{-1}\,kg^{-1}$ (Hemmingsen *et al.* 1972) and $94–104\,ml\,min^{-1}\,kg^{-1}$ (Hemmingsen and Douglas, 1977). However, determinations of $\dot{Q}$ using the Fick principle may be erroneously high in fish because of cutaneous gas exchange, gill oxygen consumption and blood shunts within the gills (Randall, 1985). In the case of *C. aceratus*, skin oxygen consumption appears to be high (Hemmingsen and Douglas, 1970). The reported $\dot{Q}$ values for *C. aceratus* based on measurements using electromagnetic flow probes are $20–30\,ml\,min^{-1}\,kg^{-1}$ [from Hemmingsen *et al.* (1972), and assuming equal flow distribution to all gill arches] and $67\,ml\,min^{-1}\,kg^{-1}$ (Hemmingsen and Douglas, 1977); for *Pseudochaenichthys georgianus* $\dot{Q}$ is $68\,ml\,min^{-1}\,kg^{-1}$ (Hemmingsen and Douglas, 1977) at heart rates of $15–20\,beats\,min^{-1}$. The relatively large ventricles of channichthyids, 0.3 % of the body mass in *C. aceratus* (Holeton, 1970) and 0.39 % in *Chionodraco hamatus* (Tota *et al.* 1991*b*), compared with those of temperate teleost species (Santer, 1985; Farrell, 1991) are also consistent with a high $\dot{Q}$, especially since ventral aortic blood pressure (P_{VA}) is relatively low (mean=2.0 kPa).

In view of the high $\dot{Q}$ and low P_{VA} in *C. aceratus*, Holeton (1970) also predicted a cardiovascular adaptation in the resistance vessels. The dimensions of the resistance vessels (arterioles) are not known, but the diameter of the capillaries in the axial muscle of *C. aceratus* was found to be three times greater than that in other teleosts (Fitch *et al.* 1984).

The few cardiovascular studies of antarctic fish possessing red blood cells indicate that routine oxygen consumption is similar to that of the channichthyids, but that the former tolerate aquatic hypoxia better, because they have a higher venous oxygen reserve. Their ventricle is apparently not unusually large (0.06–0.10 % of body mass) (Holeton, 1970; Macdonald *et al.* 1987).

Very little is known about cardiovascular control in red-blooded antarctic fishes, and there are conflicting reports on the haemoglobin-free fishes. Our objective was to make measurements of $\dot{Q}$ and P_{VA} in two species of nototheniid fishes that possess red blood cells, *Pagothenia bernacchii* and *P. borchgrevinki*. Their

responses to environmental hypoxia, swimming, acute temperature change and pharmacological intervention were studied to obtain information on cardiovascular control.

Materials and methods

Two species of nototheniid fish, *Pagothenia bernacchii* (Boulenger, 1902; also *Notothenia bernacchii, Trematodus bernacchii* and *Pseudotrematomus bernacchii*) and *P. borchgrevinki* (Boulenger, 1902; also *Notothenia hodgsoni*), were collected from McMurdo Sound in the Ross Sea, Antarctica, during the 1990 summer season. The cryopelagic *P. borchgrevinki* were caught over deep water by lowering handlines through a hole in the annual ice. *P. bernacchii* were caught in traps placed on the sea floor in 30 m of water. Fish were kept in sea water (−0.5 °C) at Scott Base until they were transported by air to the University of Canterbury, Christchurch, New Zealand. Prior to experimentation, fish were maintained for 2 months in an aquarium system at 0 °C and in continuous light, as this corresponded to the summer light regime at Scott Base. All animals were fed once per week.

Surgery

All surgery was performed in a cold room with an air temperature of 5 °C. The anaesthetics were maintained at 0 °C. Following anaesthetization in a 1:10 000 solution of MS-222 (Sandoz) in sea water, the animals were placed on the operating table and the gills were continuously irrigated with a lighter dose of anaesthetic (1:20 000 MS-222). The ventral aorta was chronically cannulated with PE30 tubing *via* the afferent branchial artery of the third right gill arch (Axelsson and Nilsson, 1986). The tubing was filled with heparinized (50 i.u. ml^{-1}) 1.4 % NaCl.

The ventral aorta was exposed through a medial incision, and a cuff-type Doppler flow probe was fitted around the vessel. Care had to be taken to avoid a large vein running immediately ventral to the aorta. Miniature Doppler flow probes were preferred both to measurements by the Fick principle and to electromagnetic flow probes as a means of measuring $\dot{Q}$ in these animals. There are uncertainties using the Fick principle in fish (see Introduction), and electromagnetic flow probes are much too large for fish as small as these. While Doppler flow probes provide reliable information on zero flow and the relative changes in flow, they are difficult to calibrate. To improve the accuracy of our determinations, we carefully sized the probes to the dimensions of the ventral aorta (diameter approximately 1 mm) and calibrated each probe *in situ* using the range setting employed in the experiment.

Calibration of Doppler flow probes

To estimate absolute values for blood flow in the ventral aorta, a careful *in situ* calibration of each flow probe was performed. Each animal was killed by over-

anaesthesia and placed ventral side up on a tray of ice. The heart was exposed and the ventricle was cannulated with PE50 tubing for perfusion of the ventral aorta. Perfusion was effected with a Watson Marlow peristaltic pump and heparinized 1.4% NaCl to which blood had been added to give a haematocrit of around 4–5%. Flow rates were determined gravimetrically for at least four different flow levels. The relatively small numbers of absolute values for $\dot{Q}$, stroke volume and total vascular resistance given in Table 1 represent animals in which linear regression analysis of the data showed a good correlation within the range that was recorded *in vivo* ($r>0.9$ in all cases). In cases where the calibration was unsuccessful, only relative changes in $\dot{Q}$ are presented. However, because relative changes in $\dot{Q}$ are independent of the calibration of the flow probe, we are confident of the relative changes in $\dot{Q}$, stroke volume and total vascular resistance that are presented and the information that these values provide about cardiovascular control.

Pagothenia bernacchii

Holding conditions

After surgery, each animal was transferred to an experimental chamber where it was allowed to recover for at least 24 h. The experimental chamber consisted of a small closed-box respirometer (1.9 l) containing an air-stone to aerate the water continuously (or supply gas mixtures during the hypoxia experiments). The cannula and flow probe leads were passed out of the chamber through a small hole in the lid. The chamber was contained in a constant-temperature waterbath which maintained its temperature at 0°C.

During the experiment the ventral aortic cannula was attached to a Bell & Howell pressure transducer (model 4–327) which was calibrated against a static water column. The Doppler flow probe was connected to an Iowa University flowmeter. Oxygen tension in the water was monitored using an IL1302 oxygen electrode connected to a Strathkelvin meter. Blood flow and pressure and oxygen partial pressure of the sea water were continuously monitored on a Devices four-channel recorder and data were continuously sampled with a computerized data acquisition system.

Drug injection experiments

P. bernacchii were allowed to recover from surgery for at least 24 h. Each experiment was started with a recording of the resting variables, which are presented in Table 1 (untreated). Sequential injections of adrenergic and cholinergic agonists and antagonists were used to assess the adrenergic and cholinergic control of the heart and systemic vasculature. The following sequence of injections was used for all animals: adrenaline (10 nmol kg^{-1}) (this is both an α- and a β-adrenoceptor agonist and usually produces a systemic vasoconstriction in fish, Nilsson, 1983); atropine (1.2 mg kg^{-1}) to block the muscarinic receptors; adrenaline (10 nmol kg^{-1}); propranolol (2.5 mg kg^{-1}) to block β-adrenoceptor responses.

All drugs were injected *via* the ventral aortic cannula through a four-way valve. Sham injections of saline were made in order to determine the effect of injected volumes of saline: these were found to have no effect on the recorded variables.

The choice of doses of drugs to be administered was based on experiments on other species of teleosts (Axelsson and Nilsson, 1986; Axelsson *et al.* 1988; Axelsson, 1988). However, the dose of adrenaline given was approximately ten times lower than that used previously. We employed this lower dose ($10\,nmol\,kg^{-1}$) as it provoked a significant response without causing a long-lasting increase in blood pressure. Following atropine and propranolol administration, the fish were given at least 1 h to recover from any direct actions of the drugs before the blocking activity of the drug was assessed.

Hypoxic exposure experiments

Animals were exposed to hypoxic water before administration of any drugs and then again after injection of atropine. The initial P_{O_2} of the sea water in the experimental chamber was about 20–21 kPa. Nitrogen and air mixtures were introduced into the experimental chamber using a Wösthoff gas-mixing pump (Bochum, Germany) to achieve a final Pw_{O_2} of approximately 6.7 kPa (50 mmHg). The total duration of each hypoxia exposure was approximately 40 min, with the time taken to reach the nadir being about 25 min, and a further 15 min elapsing before normoxia was restored. Recordings were made continuously during hypoxia and recovery. The hypoxic level of 6.7 kPa was chosen to allow comparison with previous studies of icefish (*C. aceratus*). Hemmingsen and Douglas (1970) showed that these animals became agitated at this Pw_{O_2}, while Holeton's (1970) fish died below 6.7 kPa. Davison *et al.* (1990) have shown that oxygen becomes limiting below about 3.9 kPa in *P. borchgrevinki*.

Effect of temperature

P. bernacchii were exposed to temperatures of up to 5 °C by heating the water surrounding the experimental chamber. The rate of increase within the chamber was approximately $0.1\,°C\,min^{-1}$. Aeration of the water within the chamber ensured complete mixing. Animals were tested at the end of all other experimental work (i.e. following atropine and propranolol injections). The fish were then allowed to recover from the drugs for several days after which time the heart rate had returned to control values, and then they were subjected to the temperature experiment again. Several fish that had not been previously exposed to drugs were also tested.

Pagothenia borchgrevinki

Swimming and hypoxia experiments

Following surgery, these fish were allowed to recover for 24 h in a Blazka-type swimming tunnel. Recordings of resting values were made as for *P. bernacchii*. Ventilation rate was also noted. Fish were then exercised at approximately

20 cm s^{-1} (1 body length s^{-1}). Continuous recordings were made during the exercise bout and during recovery. Following recovery, each fish was injected with adrenaline. After a suitable recovery time (1–2 h) atropine was administered, and after 1 h the fish were exercised again. Propranolol was then injected, and after a further hour an attempt was made to exercise the fish for a third time.

Fish were subsequently allowed to recover for several days in the aquarium. Heart rate was monitored to assess recovery from atropine. Following this, the animals were transferred to the chamber used for *P. bernacchii* and their responses to hypoxia and increased temperature were tested as described above.

Calculations and statistical evaluation

A computerized data acquisition system (Toshiba model 3200SX running Labtech Notebook) was used for automatic sampling of the data. Further processing and statistical treatment were performed with an analysis program written by P. Thorén (Hässle AB, Sweden).

Evaluation of the statistical significance of differences between observations was made using a Wilcoxon signed-rank test (two-tailed). Results were considered significant if P was less than 0.05. To correct for repeated tests, a sequential rejective Bonferroni test (Holm, 1979) was used to eliminate, as far as possible, the chance of discarding any true null hypothesis (Type I error).

The relative cholinergic and adrenergic tone on the heart was calculated using a simplification of the method used by Lin and Horvath (1972) (see Axelsson *et al.* 1988). The heart rate after adrenergic and cholinergic tone had been blocked is referred to as intrinsic heart rate. Total vascular resistance (R_{tot}) was estimated from $P_{VA}/\dot{Q}$ assuming a venous blood pressure of zero. Stroke volume (V_S) was calculated from $\dot{Q}/f_H$.

Results

Pagothenia bernacchii

Twelve fish with average body mass of 50.5±5.1 g (mean±S.E.M.) and ventricle mass 54±8 mg were used. The relative mass of the *P. bernacchii* ventricle was 0.106±0.004 %. The atrium (25±4 mg) was approximately half the size of the ventricle (relative atrial mass=0.05±0.003 %). The haematocrit was 15.4±0.9 %.

Control $\dot{Q}$ was 17.6±7.9 ml min^{-1} kg^{-1}, f_H was 10.5±0.9 beats min^{-1} and P_{VA} was 3.09±0.23 kPa (Table 1). We were careful to allow sufficient time for fish to acclimate to the experimental chamber (as indicated by a low and steady heart rate) before measurements were made. Heart rates in disturbed fish were much higher than the control value.

Atropine increased heart rate to 27.4±1.0 beats min^{-1}, but $\dot{Q}$ and P_{VA} were unchanged (Table 1). Consequently, stroke volume was 60 % lower than the control stroke volume. Subsequent treatment with propranolol reduced heart rate to 21.7±0.9 beats min^{-1}.

Table 1. *Cardiovascular variables in two notothenid fish,* Pagothenia bernacchii *and* Pagothenia borchgrevinki

	P. bernacchii			*P. borchgrevinki*		
	Untreated	Post-atropine	Post-propranolol	Untreated	Post-atropine	Post-propranolol
Cardiac output, $\dot{Q}$ ($ml\,min^{-1}\,kg^{-1}$)	17.6±7.9 (5)	18.6±8.3 (5)	25.0±7.5 (5)	29.6±6.8 (3)	38.9±7.4 (3)	40.5±11.4 (3)
Heart rate, f_H ($beats\,min^{-1}$)	10.5±0.9 (12)	27.4±1.0* (12)	21.7±0.9* (12)	11.3±2.9 (3)	24.0±0.5* (3)	23.3±0.9* (3)
Stroke volume, V_S ($ml\,kg^{-1}$)	1.56±0.28 (5)	0.63±0.11* (11)	1.03±0.28 (5)	2.16±0.53 (3)	1.62±0.35 (3)	1.80±0.48 (3)
Ventral aortic pressure, P_{VA} (kPa)	3.09±0.23 (11)	3.18±0.13 (11)	3.12±0.15 (11)	3.60±0.20 (3)	2.73±0.12* (3)	3.13±0.38 (3)
Total vascular resistance, R_{tot} ($Pa\,min\,kg\,ml^{-1}$)	197±49 (5)	186±28 (5)	219±70 (5)	159±68 (3)	93±12 (3)	138±72 (3)

* Denotes values statistically significant from control (Untreated) ($P<0.05$; paired *t*-test). Mean values are presented±S.E.M. for (N) animals.

Control of heart rate

The resting heart rate of *P. bernacchii* at 0°C was 10.5±0.9 beats min^{-1} ($N=12$; Table 1). This was the result of an inhibitory cholinergic tone of 80.4±6.4% and an excitatory adrenergic tone of 27.5±5.1%. The intrinsic heart rate was 21.7±0.9 beats min^{-1}.

Response to hypoxia

During hypoxia, $\dot{Q}$ was maintained down to a $P_{W_{O_2}}$ of 6.7 kPa (Fig. 1). There was no sustained hypoxic bradycardia, a response that is typically observed in temperate species of teleosts (Randall, 1970; Satchell, 1991). In fact, mean heart rate was 2 beats min^{-1} higher at 6.7 kPa than in normoxia. In some fish, heart rate had a phasic pattern where the beat-to-beat interval was prolonged for 1–3 beats and then followed by a period with shorter than average beat-to-beat intervals

Fig. 1. Cardiovascular responses to hypoxia and return towards normoxia in *Pagothenia bernacchii* at 0°C. The measured and calculated variables are ventral aortic pressure (P_{VA}), heart rate (f_H), cardiac output ($\dot{Q}$), stroke volume (V_S) and total vascular resistance (R_{tot}). The partial pressure of oxygen is shown on the abscissa. Sample sizes (N) are shown for each variable. (●) untreated animals; (○) atropinized fish. Asterisks indicate values that are significantly different from the starting values ($P<0.05$). Values were only compared at two times. Dashed horizontal lines indicate the initial value in the untreated animals. The dashed vertical lines indicate the beginning of the recovery period.

(Fig. 2). P_{VA} and total vascular resistance (R_{tot}) were unchanged down to 8.0 kPa, but increased at a P_{WO_2} of 6.7 kPa (Fig. 1).

During the recovery from hypoxia, f_H increased to a maximum of 18 beats min^{-1}, stroke volume was gradually restored to control values and $\dot{Q}$ was

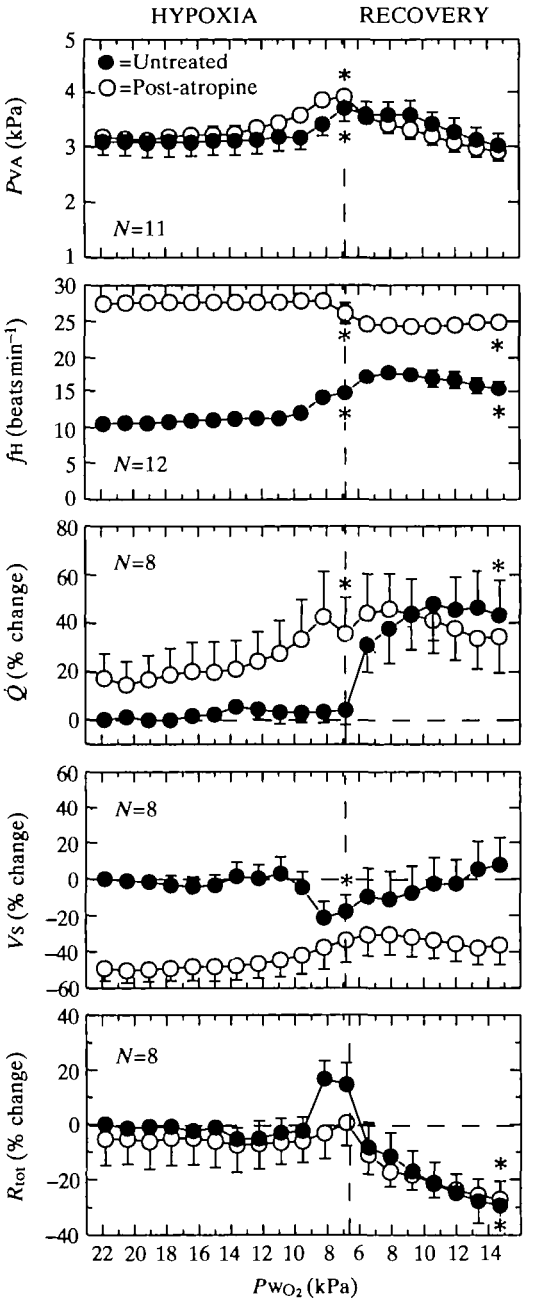

Fig. 1.

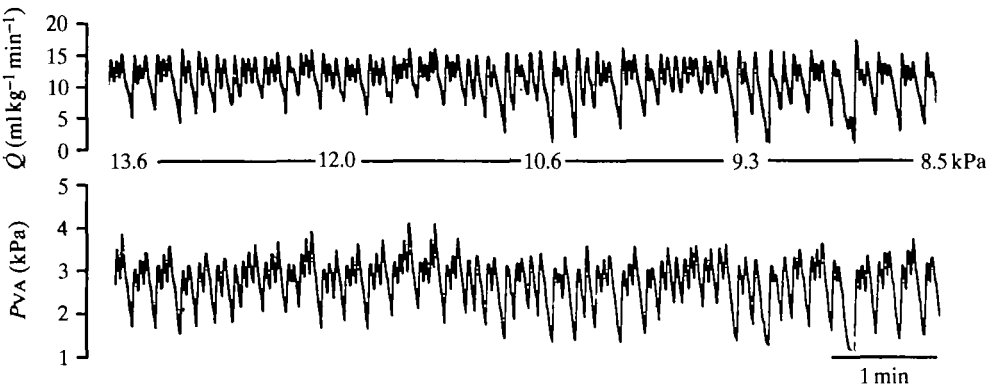

Fig. 2. The signal from the Doppler flow probe (cardiac output, $\dot{Q}$) and ventral aortic pressure (P_{VA}) in a single non-atropinized specimen of *Pagothenia bernacchii* to show the irregular heart beat. The fish was exposed to a decreasing partial pressure of oxygen (the values of $P_{W_{O_2}}$ are indicated on the trace at 2-min intervals).

significantly elevated (Fig. 1). P_{VA} decreased during the later phase of recovery as total vascular resistance (R_{tot}) decreased significantly.

Response to hypoxia after atropine pretreatment

After atropine treatment, $\dot{Q}$, V_S and P_{VA} increased progressively as $P_{W_{O_2}}$ decreased to 6.7 kPa. The increase in $\dot{Q}$ occurred even though the experiment began with an already elevated $\dot{Q}$ as a result of the atropine pretreatment (Fig. 1). This response contrasts with that of the non-atropinized fish, in which there was no change in $\dot{Q}$. Despite this difference in the response to hypoxia, it is interesting that in both the atropinized and non-atropinized fish $\dot{Q}$ peaked during recovery (Fig. 1). f_H was constant as $P_{W_{O_2}}$ decreased except at 6.7 kPa $P_{W_{O_2}}$. Heart rate was much more regular in the atropinized fish, with no prolonged beat-to-beat intervals. As with the non-atropinized fish, R_{tot} decreased significantly during recovery.

Response to an acute increase in water temperature

The water temperature was increased from 0 to 5°C over a period of 40 min (Fig. 3). There was no marked increase in the heart rate of untreated fish until the temperature reached approximately 2.5°C. Above this temperature, heart rate rose rapidly to reach values similar to those recorded in the atropine/propranolol-treated animals. As a result, the Q_{10} for resting f_H was greater than 4 over this temperature range. After the pretreatment with propranolol and atropine, the heart was beating at an intrinsic rate of 21.7 beats min^{-1} at 0°C. Heart rate increased progressively to a value of 31.5 beats min^{-1} at 5°C (Fig. 3). The Q_{10} for the intrinsic heart rate was 1.96. The increase in f_H was accompanied by a progressive decrease in V_S, and $\dot{Q}$ increased by only 10%. P_{VA} was relatively unchanged in atropinized fish up to 3°C but declined significantly thereafter along with R_{tot}.

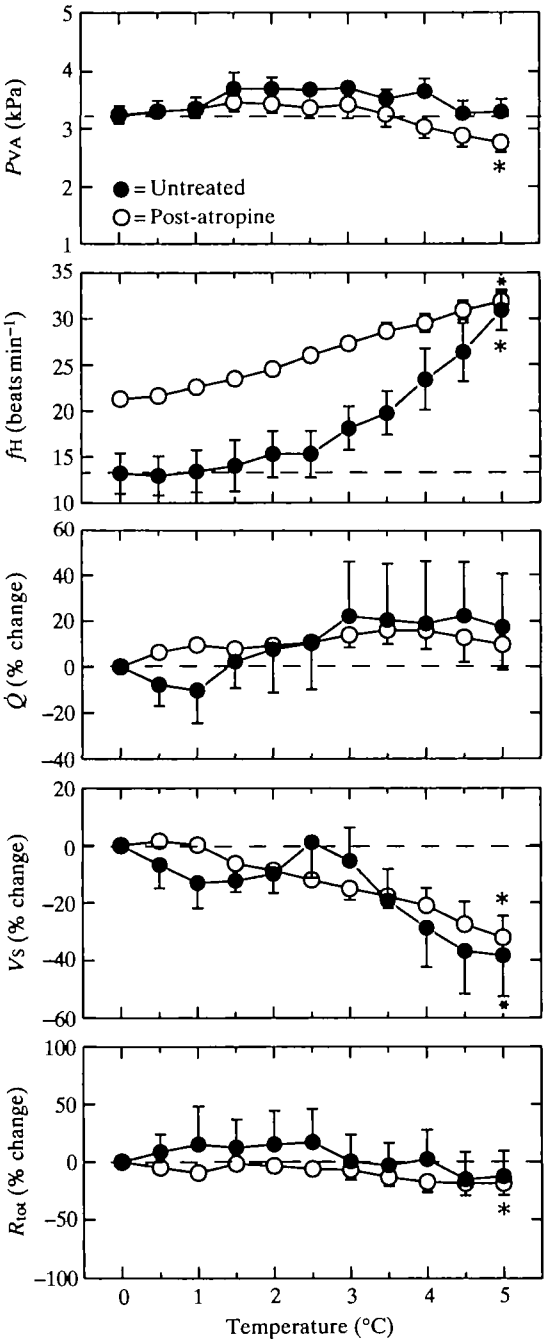

Fig. 3. Cardiovascular responses of *Pagothenia bernacchii* to a progressive rise in water temperature from 0 to 5°C over 40 min. Measured variables as for Fig. 1, N=10 animals. Dashed horizontal lines indicate the values for non-atropinized fish at time zero.

Response to adrenaline infusion

Injection of 10 nmol kg^{-1} adrenaline into the ventral aorta produced immediate increases in R_{tot}, P_{VA} and f_H (Fig. 4). At first, $\dot{Q}$ was unchanged as V_S decreased. After the first 2 min, stroke volume began to recover while heart rate continued increasing so that $\dot{Q}$ had increased by 30 % after 3.5 min. Total vascular resistance peaked between 1.0 and 1.5 min after injection, and decreased thereafter (Fig. 4). Therefore, the pressor response to adrenaline initially resulted from a vasoconstriction and was prolonged by an increase in $\dot{Q}$.

When adrenaline was injected after atropine pretreatment, the changes in P_{VA} and R_{tot} were qualitatively similar but quantitatively slightly larger than the corresponding responses measured before the injection of the blocker (Fig. 4). However, the increase in $\dot{Q}$ was due to a change in stroke volume 2 min after adrenaline injection as f_H was unchanged (Fig. 4).

Pagothenia borchgrevinki

Three fish with a mean body mass of 64 g and a ventricle mass of 100 mg were used. The mean relative ventricle mass of the *P. borchgrevinki* heart was 0.156 %. The atrium (30 mg) was approximately one-third of the size of the ventricle (relative atrial mass=0.042 %). In resting fish prior to swimming, the ventilation rate was 26 ventilations min^{-1} and $\dot{Q}$ was 29.6 ml min^{-1} kg^{-1} with a heart rate of 11.3 beats min^{-1} (Table 1). The haematocrit was 17.7±2.3 %.

Response to swimming

Three fish were swum at 20 cm s^{-1} (approximately 1 body length s^{-1}) for 6–10 min, after which they appeared exhausted. *P. borchgrevinki* are labriform swimmers. The sculling rate of the pectoral fins during swimming ranged from 70 to 105 strokes min^{-1}. Ventilation frequency increased approximately twofold to 45–52 ventilations min^{-1}. $\dot{Q}$ increased immediately at the onset of swimming and plateaued after 3.25 min at a value 75 % higher than the control value (Fig. 5). f_H also increased immediately and had almost doubled after 2.25 min of swimming. V_S did not show any changes during swimming. Total vascular resistance (R_{tot}) decreased, which ensured that there was little change in P_{VA} during swimming (Fig. 5). In two of the fish, the swimming challenge was repeated after a 24 h recovery period. The cardiovascular responses were similar to those during the first swim (not shown).

At the end of the exercise period, haematocrit was slightly higher in each of the three fish (mean value=20.4±2.3 %). Venous oxygen content was measured in two fish before and immediately after swimming. It averaged 1.58 vol% before and 0.48 vol% afterwards, a more than threefold decrease.

Recovery from the bout of swimming was characterized by a return of all measured cardiovascular variables towards their resting values. By 15 min post-exercise, f_H was still above the pre-exercise level and $\dot{Q}$ was still above the control level.

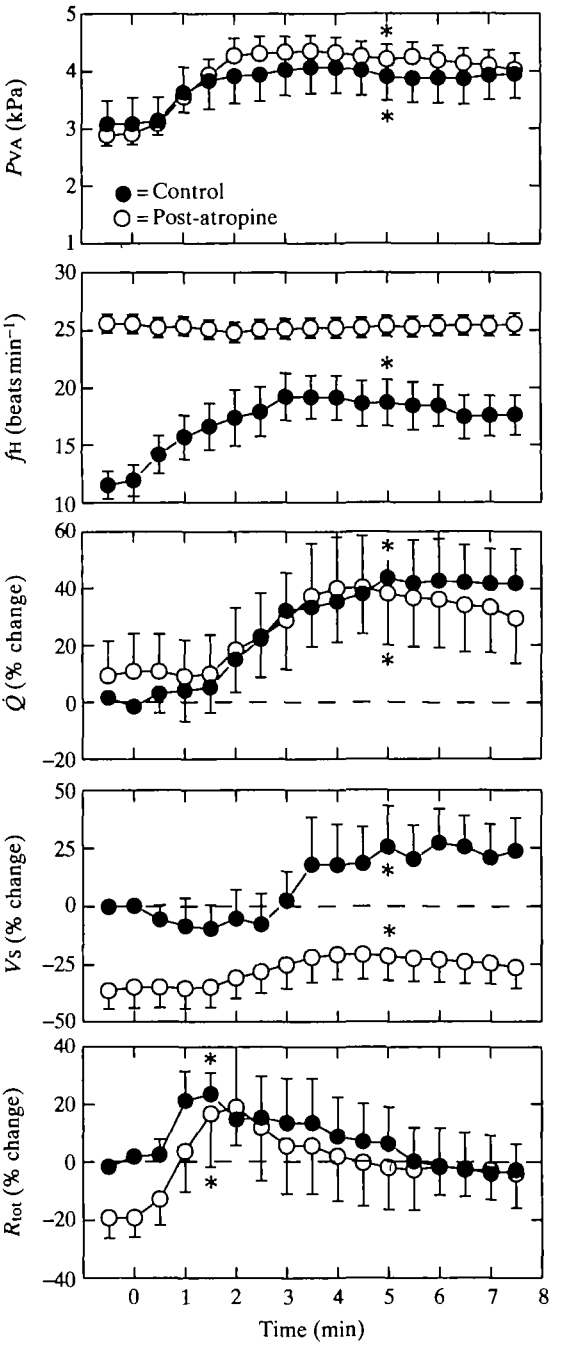

Fig. 4. Cardiovascular responses of *Pagothenia bernacchii* to a single injection of adrenaline (10 nmol kg^{-1}) given at time zero. Measured variables as for Fig. 1, N=12 animals. Asterisks indicate values that are significantly different from the starting values (P<0.05). Dashed horizontal lines indicate the values for non-atropinized fish at time zero.

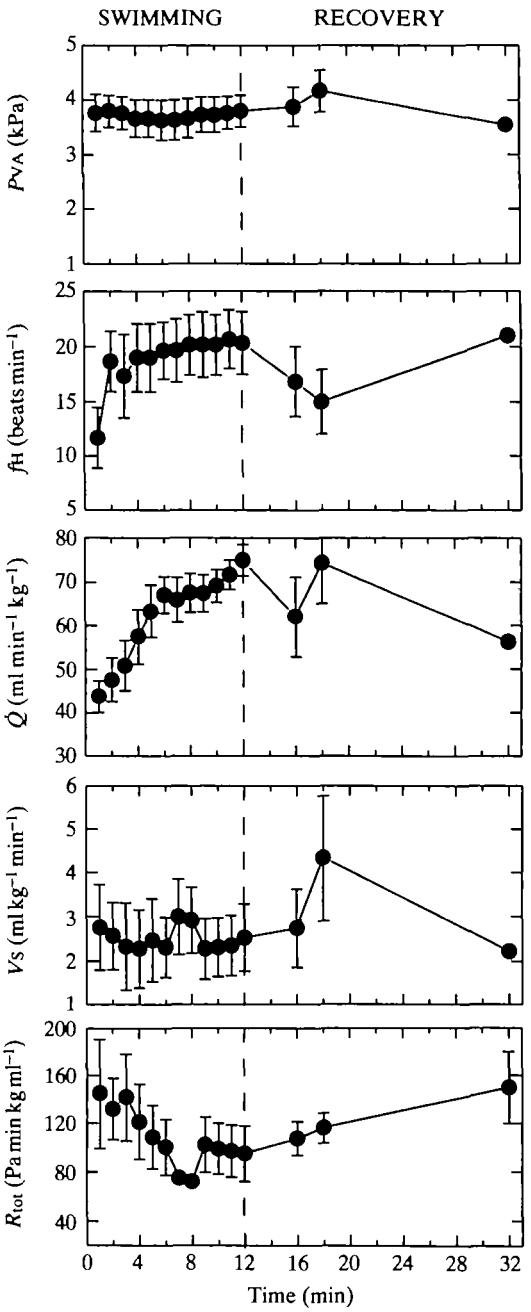

Fig. 5. Cardiovascular responses of *Pagothenia borchgrevinki* to forced swimming (non-atropinized fish). Measured variables as for Fig. 1, N=5 swimming trials. The dashed vertical line indicates the start of the recovery period.

Swimming after pretreatment with atropine and propranolol

Pretreatment of *P. borchgrevinki* with atropine increased heart rate and decreased P_{VA} (Table 1). The fish swam for shorter periods after atropine treatment (3–6 min). Resting heart rate was 11.3±2.9 beats min^{-1} at 0°C. The intrinsic heart rate was twice this value (23.3±0.9 beats min^{-1}) (Table 1). Resting heart rate was maintained by an inhibitory cholinergic tone of 54.5±12 % and an excitatory adrenergic tone of 3.2±2.6 %. The fish would not swim after propranolol pretreatment.

Response to hypoxia

Three *P. borchgrevinki* were exposed to progressive hypoxia after a 2- to 4-day recovery from the swimming experiment. Recovery from the previous experiments was indicated by the low heart rates (15 beats min^{-1}). The major difference between *P. borchgrevinki* and *P. bernacchii* was the effect of hypoxia on f_H. Two fish showed a bradycardia and only one showed a tachycardia. The individual with the highest resting f_H showed the biggest fall in f_H (41 %) (Fig. 6). Recovery from the hypoxic exposure was accompanied by a 50 % increase in $\dot{Q}$, with both heart rate and stroke volume increasing.

Discussion

Previous studies of cardiovascular physiology in antarctic fish have been performed in the Antarctic. Our approach was to bring the fish to an environment where we had greater access to instrumentation. This meant that the fish were transported considerable distances and held in recirculating sea water collected at Lyttleton Harbour near Christchurch, New Zealand, and cooled to 0°C. Specimens of *P. bernacchii* have lived for more than 1 year in this system and will take food. It has proved more difficult to keep *P. borchgrevinki* alive and only three fish were able to swim following surgery.

Because the fish were small, efforts were made to minimize disturbances. The various measuring devices undoubtedly encumbered the animal, but the leads from the Doppler probes were thin and the tubing used for ventral aortic cannulation was the smallest possible that would permit accurate records of pulsatile pressure. Also, both species, including the cryopelagic *P. borchgrevinki*, spend most of their time lying quietly on the bottom of the aquaria. Thus, the leads and cannulae were not usually a hindrance to the animals. However, during swimming, the drag effects of the leads needed to be taken into account as they would have necessitated a greater swimming effort than that predicted by the water velocity. The frequency and volume of blood samples were kept to a minimum to avoid a major haemodilution. This precluded sampling for catecholamine analysis, which would have been useful to assess whether the animals were stressed.

The measurement of total vascular resistance does not allow any distinction to be made between changes in gill and systemic resistances. A change in R_{tot} could

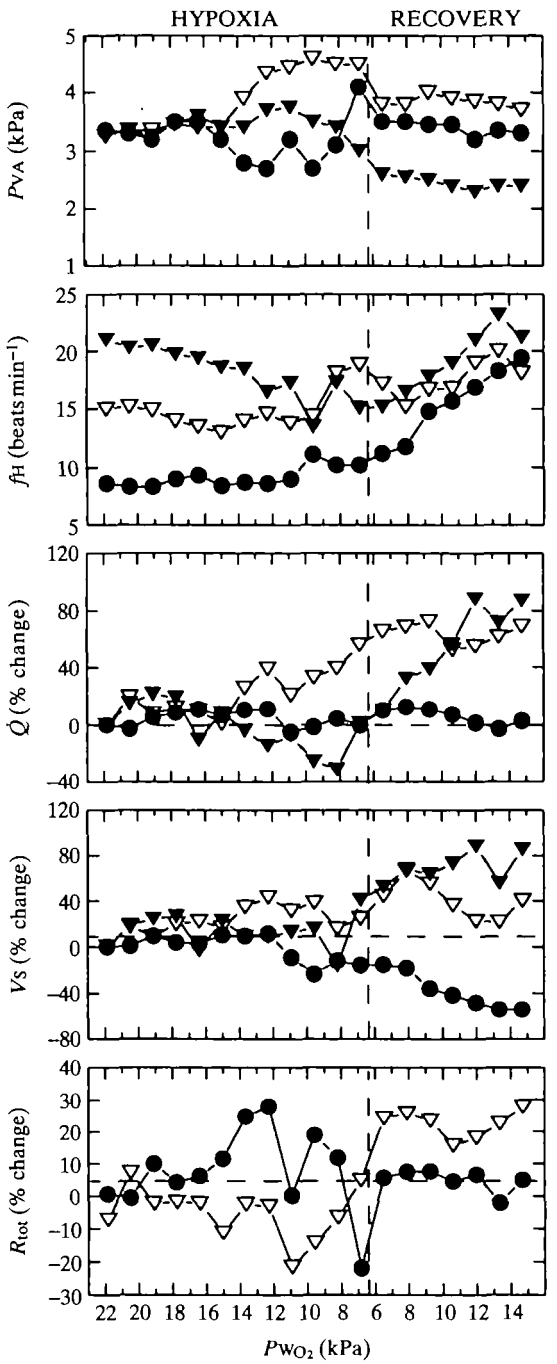

Fig. 6. Cardiovascular responses of *Pagothenia borchgrevinki* to hypoxia. Measured variables as for Fig. 1, N=3 animals, each animal represented by a different symbol. Each point represents a single measurement, so errors (and hence significance) could not be calculated. The dashed vertical line indicates the beginning of the recovery period. The dashed horizontal line indicates starting (zero) values.

reflect a change in either the gill circulation or the systemic circulation or some combination of the two.

Comparison of haemoglobin-free antarctic fish and temperate teleost species

Studies of *C. aceratus* have been used to exemplify cardiovascular adaptations to the antarctic environment. We can now compare red-blooded and haemoglobin-free antarctic fishes to ascertain whether there are any common adaptations. As in *C. aceratus*, P_{VA} in *P. bernacchii* (3.09 kPa) and *P. borchgrevinki* (3.60 kPa) is low compared to that of temperate species of teleosts, even though the nototheniids have haematocrits that are not exceptionally low by comparison. Furthermore, since $\dot{Q}$ is not unusually low in either species of nototheniid, a low vascular resistance is a common feature in all antarctic species examined thus far, whether or not they have red blood cells. *C. aceratus* has a lower P_{VA} (2.13–2.46 kPa) than the red-blooded nototheniids and, since $\dot{Q}$ is higher in *C. aceratus*, its vascular resistance (35 Pa min kg ml^{-1}, using a P_{VA} of 2.13 kPa and $\dot{Q}$ of 61 ml min^{-1} kg^{-1}) is 5–6 times lower. Interestingly, the total vascular resistance of the antarctic fishes is more similar to that of hagfishes than to that of temperate species of teleosts (Axelsson *et al.* 1990).

It is possible to estimate whether the difference in R_{tot} between the red-blooded and haemoglobin-free notothenioids can be attributed entirely to differences in blood viscosity and blood flow rates. To do this, the component of vascular resistance due to viscosity must be separated from that due to vascular geometry. Wells *et al.* (1990) measured the viscosity of blood from *P. bernacchii* as 0.68 Pa s at −1.8 °C at a shear rate of 225 s^{-1} and a haematocrit of 15.4 %. This was 80 % higher than the blood viscosity of two haemoglobin-free channichthyids measured under similar conditions. Such a difference in blood viscosity clearly does not account for the five- to sixfold difference in R_{tot}. Thus, differences in vascular geometry must account for the majority of the higher R_{tot} of red-blooded compared to haemoglobin-free antarctic fish.

The low vascular resistance due to vascular geometry in red-blooded antarctic fish more than compensates for the high blood viscosity at 0 °C and keeps cardiac work at a level comparable to that found in temperate species of teleosts. The resting cardiac power output is similar for *P. bernacchii* (0.82 mW g^{-1} ventricle mass), *P. borchgrevinki* (1.05 mW g^{-1} ventricle mass), *C. aceratus* (0.72 mW g^{-1} ventricle mass), *Oncorhynchus mykiss* (1.7 mW g^{-1} ventricle mass) and *Hemitripterus americanus* (1.0 mW g^{-1} ventricle mass) (Farrell *et al.* 1988; Farrell, 1991). In *Chionodraco hamatus,* the peak power output of the heart *in vitro* exceeds 3 mW g^{-1} ventricle mass, but stroke work dimishes at afterloads exceeding 3.0 kPa, for although there is cardiomegaly, the myocardium is of the spongy type alone (Tota *et al.* 1991*a*,*b*). To what degree red-blooded antarctic fish can maximally increase myocardial power output is not clear. The maximum increase in myocardial power output observed was less than 100 % in *P. bernacchii* and approximately 100 % in *P. borchgrevinki*. In contrast, the rainbow trout and sea raven can increase myocardial power output by three- to fourfold (Farrell, 1991).

Perhaps red-blooded antarctic fishes have a much lower scope for cardiac output than temperate species. In this regard it is interesting to note that recovery from exercise and hypoxia are accompanied by large reductions in R_{tot}, which tend to keep blood pressure and cardiac work low, even though cardiac output and haematocrit (in *P. borchgrevinki*) are elevated.

While cardiac power output is not unusually high in antarctic fishes, they have a relatively large heart. The relative ventricle mass in *C. aceratus* is 0.3 % (Holeton, 1970; Harrison *et al.* 1991) and in *Chionodraco hamatus* it is 0.39 % (Tota *et al.* 1991*a*): this is three times larger than in a rainbow trout at 10°C (0.1 %) and is comparable to the largest values in teleosts (yellowfin tuna, 0.3 %; skipjack tuna, 0.4 %) (Brill and Bushnell, 1991; A. P. Farrell, P. S. Davie, C. E. Franklin, J. A. Johansen and R. W. Brill, in preparation). The relative ventricle mass of *P. bernacchii* (0.1 %) is as large as that in rainbow trout and is larger than that in temperate species of benthic teleosts (0.035–0.07 %; Santer, 1985; Farrell, 1991). There are at least two explanations for the large heart. One relates to the observation that antarctic fish are characterized by relatively large stroke volumes (3–6 ml kg^{-1} in *C. aceratus*; 2.19 ml kg^{-1} in *P. borchgrevinki*; 1.56 ml kg^{-1} in *P. bernacchii*) compared with temperate species, in which the maximum stroke volume is more often 1 ml kg^{-1} or less (Farrell, 1991). Assuming that Laplace's law can be applied to the fish heart, a disproportionately thicker ventricle will be needed to generate the same wall tension with the greater radius (internal volume) associated with a higher stroke volume.

Another explanation for the larger ventricle in antarctic fish may be related to muscle hypertrophy which could compensate for the effect of temperature on muscle contractility. Low temperatures reduce cardiac contractility in fish. Hearts from rainbow trout acclimated to 5°C generate only half the maximum mass-specific power output of hearts from fish acclimated to 15°C. However, cold-acclimated rainbow trout compensate for this by having a ventricle that is 50 % larger and, as a result, total cardiac power output is only 34 % lower than in the warm-acclimated fish (Graham and Farrell, 1989).

Antarctic fish are also characterized by low heart rates. Heart rates in *P. bernacchii* (10.5 beats min^{-1}) and *P. borchgrevinki* (11.3 beats min^{-1}) are lower than those reported for *C. aceratus* (14–17 beats min^{-1}). Steady heart rates as low as 3–6 beats min^{-1} were observed in some *P. bernacchii*. The low heart rate in the red-blooded nototheniids was clearly a result of a high inhibitory cholinergic tone. In fact, the cholinergic tone found in *P. bernacchii* (80.4 %) is the highest so far observed in a teleost fish. The low heart rate was not related to a low intrinsic heart rate. The isolated perfused heart of the icefish *C. hamatus* beat at 26 beats min^{-1} (Tota *et al.* 1991*a*), suggesting that the channichthyids too may have a high inhibitory cholinergic tone. Factors other than catecholamines and acetylcholine may affect the heart, but the two major influences on the heart are the adrenergic and cholinergic systems (Farrell, 1991). Once their effects are blocked, it is likely that the intrinsic heart rate is revealed.

The intrinsic heart rate in *P. bernacchii* has a Q_{10} value of 1.96. It is readily

apparent that the intrinsic heart rate at 5 °C (32 beats min^{-1}) is very similar to those reported for rainbow trout at 5 °C (36 beats min^{-1}, Graham and Farrell, 1989) and for sea raven at 3 °C (24 beats min^{-1}, Graham and Farrell, 1985). Thus, the intrinsic pacemaker frequency in antarctic fish is probably not very different from that of temperate species of teleosts. The excitatory adrenergic component of the control of heart rate in *P. bernacchii* is similar to that found in other teleosts (Axelsson *et al.* 1988). The low level of excitatory adrenergic tone in *P. borchgrevinki*, while unusual, does not preclude either adrenergic inotropic effects or adrenergic chronotropy under other conditions.

The response to hypoxia

Several other aspects of cardiovascular control in addition to those discussed above were revealed by the present study. Perhaps the most unusual observation was the lack of bradycardia associated with hypoxia for *P. bernacchii*. Temperate and tropical teleosts typically respond to aquatic hypoxia with a reflex decrease in f_H (Randall, 1970; Daxboeck and Holeton, 1978; Smith and Jones, 1978; Bushnell and Brill, 1991; Farrell, 1991; Satchell, 1991), which may improve oxygen extraction by the working myocardium and oxygen transfer across the gill lamellae. The absence of hypoxic bradycardia in *P. bernacchii* may reflect the fact that f_H is already under a large inhibitory cholinergic tone, rather than the absence of the afferent arm of the reflex. Some fish, particularly those with the highest initial heart rates, showed periods of phasic increases and decreases in heart rate (Fig. 2). These phasic decreases in heart rate could be viewed as a form of hypoxic bradycardia followed by periods of escape from the vagal tone. The observation that atropinized fish showed a decrease in f_H at the lowest values of $P_{W_{O_2}}$ (which continued into the recovery period) suggests a direct effect of hypoxia on the heart.

Of the three *P. borchgrevinki* that were exposed to hypoxia, only the fish with the highest heart rate showed a marked reduction in f_H (41 %). Observations in *C. aceratus* are not clear-cut with respect to hypoxic bradycardia. In *C. aceratus*, Holeton (1972) reported an atropine-sensitive bradycardia associated with both hypoxia and hyperoxia, heart rate decreasing by 45–85 %. In *Pseudochaenichthys georgianus*, $\dot{Q}$, heart rate and ventral aortic blood pressure are reduced by up to 50 % during hypoxia (Hemmingsen and Douglas, 1977). In contrast, Hemmingsen *et al.* (1972) and Hemmingsen and Douglas (1977) reported a slight decrease in heart rate and a modest increase in $\dot{Q}$ in *C. aceratus*, while Hemmingsen and Douglas (1972) reported no change in heart rate during hypoxia.

Adrenaline, through an increase in vascular resistance, usually produces a pressor response that is often associated with a reflex bradycardia. This is presumably a barostatic reflex (Wood and Shelton, 1980*a*,*b*; Farrell, 1991). It is noteworthy that such a response was absent in *P. bernacchii*. Instead, a decrease in stroke volume reduced the pressor response. Since f_H increased immediately after the injection of adrenaline, it seems likely that there was a decrease rather than the expected increase in inhibitory cholinergic tone to the heart associated with the

pressor response. This is further suggested by the lack of tachycardia in response to adrenaline after pretreatment with atropine. This was not the case in the two *P. borchgrevinki* examined. Heart rate was either unchanged or decreased under these conditions. The physiological basis for this interspecific difference is worth further study.

Farrell (1991) concludes that temperate fishes generally regulate cardiac stroke volume to a greater degree than heart rate, with tunas presented as an exception to this generality. Based on the present study, red-blooded nototheniids may be another exception. Because heart rate is under such a large inhibitory cholinergic tone, *P. bernacchii* could increase f_H about threefold. We observed twofold increases in f_H during swimming in *P. borchgrevinki*. Such changes are greater than those observed in temperate teleosts. Furthermore, four observations support the idea that red-blooded nototheniids alter f_H to a greater degree than they alter stroke volume. In *P. bernacchiii*, the recovery from hypoxia was associated with tachycardia, with V_S only returning to, but not increasing beyond, resting values. In *P. borchgrevinki*, swimming was associated with a much larger increase in f_H than in V_S. In both *P. bernacchii* and *P. borchgrevinki*, $\dot{Q}$ was well regulated when f_H was elevated after atropine treatment. Lastly, in many of the manipulations, increases in f_H were accompanied by decreases in V_S. A question still to be resolved is whether these fish can increase stroke volume much beyond the relatively small (25 %) increases observed here.

It is unlikely that antarctic fish will be exposed to hypoxia in their well-oxygenated, cold and stenothermal environment. Nevertheless, they respond to experimentally induced hypoxia in a similar manner to temperate species. In *P. borchgrevinki* exposed to a $P_{W_{O_2}}$ of 8 kPa for 11–14 days there was a 93 % increase in haematocrit and the P_{50} of the blood was decreased by 33 %, a change associated with a 25 % reduction in erythrocyte ATP concentrations (Wells *et al.* 1989). In the present study, cardiac output increased and total vascular resistance declined on recovery from hypoxia as $P_{W_{O_2}}$ was restored to 20–21 kPa, results that are consistent with an improved oxygen delivery to tissues that previously may have either run down their oxygen stores or switched to anaerobic metabolism. There was a rise in P_{VA} at a $P_{W_{O_2}}$ of 6.7 kPa in both control and atropinized fish. *Gadus morhua* also showed a transient rise in P_{VA} in response to hypoxia, which was brought about by an increase in systemic resistance (Fritsche and Nilsson, 1989). A rise in P_{VA} might be expected to result in lamellar recruitment at the gill.

Comparison of Pagothenia bernacchii *and* Pagothenia borchgrevinki

Since *P. bernacchii* is a benthic species and *P. borchgrevinki* is a cryopelagic species, cardiovascular differences were expected. *P. bernacchii* would not swim in the swimming tunnel, a response typical of benthic fish. Although most of the work was performed on *P. bernacchii*, sufficient observations were made on *P. borchgrevinki* to note significant species differences. In keeping with the more active life style of *P. borchgrevinki*, $\dot{Q}$, P_{VA}, myocardial power output, haematocrit and the sizes of the atrium and ventricle were relatively greater than for

P. bernacchii. The difference in $\dot{P}_{VA}$ is not accounted for by the small difference in haematocrit (and thus blood viscosity). Instead, it is largely due to a lower total vascular resistance in *P. borchgrevinki*. Whether the branchial or systemic resistances are markedly different in the two species remains to be determined.

This work was support by grants from Natural Sciences and Engineering Research Council, Canada, by an Erskine Visiting Fellowship to A.P.F. and by University Grants Committee awards to W.D. and M.E.F. We thank Gavin Robinson for his help in setting up facilities and Professor Rufus Wells for drawing to our attention various papers in press.

References

ANDRIASHEV, A. P. (1987). A general review of the Antarctic bottom fish fauna. In *Proceedings of the Fifth Congress of European Ichthyologists, Stockholm 1985* (ed. S. O. Kullander and B. Fernholm), pp. 357–372. Stockholm: Swedish Museum of Natural History.

AXELSSON, M. (1988). The importance of nervous and humoral mechanisms in the control of cardiac performance in the Atlantic cod *Gadus morhua* at rest and during non-exhaustive exercise. *J. exp. Biol.* **137**, 287–303.

AXELSSON, M., EHRENSTRÖM, F. AND NILSSON, S. (1988). Cholinergic and adrenergic influence on the teleost heart *in vivo*. *Expl Biol.* **46**, 179–186.

AXELSSON, M., FARRELL, A. P. AND NILSSON, S. (1990). Effects of hypoxia and drugs on the cardiovascular dynamics of the Atlantic hagfish *Myxine glutinosa*. *J. exp. Biol.* **151**, 297–316.

AXELSSON, M. AND NILSSON, S. (1986). Blood pressure regulation during exercise in the Atlantic cod *Gadus morhua*. *J. exp. Biol.* **126**, 225–236.

BOULENGER, G. A. (1902). Pisces. In *Report on the Collections of Natural History made in the Antarctic Regions during the Voyage of the 'Southern Cross'*, pp. 174–189. London: British Museum (Natural History).

BRILL, R. W. AND BUSHNELL, P. G. (1991). Metabolic and cardiac scope of high energy demand teleosts – the tunas. *Can. J. Zool.* **69**, 2002–2009.

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

DAVISON, W., FORSTER, M. E., FRANKLIN, C. E. AND TAYLOR, H. H. (1988). Recovery from exhausting exercise in an Antarctic fish, *Pagothenia borchgrevinki*. *Polar Biol.* **8**, 167–171.

DAVISON, W., FRANKLIN, C. E. AND CAREY, P. W. (1990). Oxygen uptake in the Antarctic teleost *Pagothenia borchgrevinki*. Limitations imposed by X-cell gill disease. *Fish Physiol. Biochem.* **8**, 69–77.

DAXBOECK, C. AND HOLETON, G. (1978). Oxygen receptors in the rainbow trout, *Salmo gairdneri*. *Can. J. Zool.* **56**, 1254–1259.

EASTMAN, J. T. (1991). Evolution and diversification of Antarctic notothenioid fishes. *Am. Zool.* **31**, 93–109.

FARRELL, A. P. (1986). Cardiovascular responses in the sea raven, *Hemitripterus americanus*, elicited by vascular compression. *J. exp. Biol.* **122**, 65–80.

FARRELL, A. P. (1991). From hagfish to tuna – a perspective on cardiac function. *Physiol. Zool.* **64**, 1137–1164.

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.

FITCH, N. A., JOHNSTON, I. A. AND WOOD, R. E. (1984). Skeletal muscle capillary supply in a fish that lacks respiratory pigments. *Respir. Physiol.* **57**, 201–211.

FORSTER, M. E., FRANKLIN, C. E., TAYLOR, H. H. AND DAVISON, W. (1987). The aerobic scope of an Antarctic fish, *Pagothenia borchgrevinki* and its significance for metabolic cold adaptation. *Polar Biol.* **8**, 155–159.

FRANKLIN, C. E., DAVISON, W. AND CAREY, P. W. (1991). The stress response of an Antarctic teleost to an acute increase in temperature. *J. Therm. Biol.* **16**, 173–177.

FRITSCHE, R. AND NILSSON, S. (1989). Cardiovascular responses to hypoxia in the Atlantic cod, *Gadus morhua*. *Expl Biol.* **48**, 153–160.

GRAHAM, M. AND FARRELL, A. P. (1985). The seasonal intrinsic cardiac performance of a marine teleost. *J. exp. Biol.* **118**, 173–183.

GRAHAM, M. S. AND FARRELL, A. P. (1989). The effect of temperature acclimation and adrenaline on the performance of a perfused trout heart. *Physiol. Zool.* **62**, 38–61.

GRAHAM, M. S. AND FLETCHER, G. L. (1985). On the low blood viscosity of two cold water marine sculpins. A comparison with the winter flounder. *J. comp. Physiol.* B **155**, 455–459.

HARRISON, P., ZUMMO, G., FARINA, F., TOTA, B. AND JOHNSTON, I. A. (1991). Gross anatomy, myoarchitecture, and ultrastructure of the heart ventricle in the haemoglobinless icefish *Chaenocephalus aceratus*. *Can. J. Zool.* **69**, 1339–1347.

HEMMMINGSEN, E. A. AND DOUGLAS, E. L. (1970). Respiratory characteristics of the hemoglobin-free fish *Chaenocephalus aceratus*. *Comp. Biochem. Physiol.* **33**, 733–744.

HEMMMINGSEN, E. A. AND DOUGLAS, E. L. (1972). Respiratory and circulatory responses in a hemoglobin-free fish, *Chaenocephalus aceratus*, to changes in temperature and oxygen tension. *Comp. Biochem. Physiol.* **43**A, 1031–1043.

HEMMINGSEN, E. A. AND DOUGLAS, E. L. (1977). Respiratory and circulatory adaptations to the absence of hemoglobin in chaenichthyid fishes. In *Adaptations Within Antarctic Ecosystems – Proceedings of the Third* SCAR *Symposium on Antarctic Biology* (ed. G. A. Llano), pp. 479–487. Houston, Texas: Gulf Publishing Co.

HEMMINGSEN, E. A., DOUGLAS, E. L., JOHANSEN, K. AND MILLARD, R. W. (1972). Aortic blood flow and cardiac output in the hemoglobin-free fish *Chaenocephalus aceratus*. *Comp. Biochem. Physiol.* **43**A, 1045–1051.

HOLETON, G. (1970). Oxygen uptake and circulation by a hemoglobinless Antarctic fish (*Chaenocephalus aceratus* Lönnberg) compared with three red-blooded Antarctic fish. *Comp. Biochem. Physiol.* **34**, 457–471.

HOLETON, G. (1972). Gas exchange in fish with and without hemoglobin. *Respir. Physiol.* **14**, 142–150.

HOLM, S. (1979). A simple sequentially rejective multiple test procedure. *Scan. J. Stat.* **6**, 65–70.

LIN, Y.-C. AND HORVATH, S. M. (1972). Autonomic nervous control of cardiac frequency in the exercise-trained rat. *J. appl. Physiol.* **33**, 796–799.

MACDONALD, J. A., MONTGOMERY, J. C. AND WELLS, R. M. G. (1987). Comparative physiology of McMurdo Sound fishes. *Adv. mar. Biol.* **24**, 321–388.

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

RANDALL, D. J. (1970). The circulatory system. In *Fish Physiology*, vol. 4 (ed. W. S. Hoar and D. J. Randall), pp. 132–172. New York: Academic Press.

RANDALL, D. J. (1985). Shunts in fish gills. In *Cardiovascular Shunts, Alfred Benzon Symposium 21* (ed. K. Johansen and W. W. Burggren), pp. 71–87. Copenhagen: Munksgaard.

RICKENBACH, A. F. (1990). A comparison of blood viscosity between several Antarctic teleosts and a warm temperate teleost. MSc thesis, University of Auckland.

SANTER, R. M. (1985). Morphology and innervation of the fish heart. *Adv. Anat. Embryol. Cell Biol.* **89**, 1–102.

SATCHELL, G. H. (1991). *Physiology and Form of Fish Circulation*. Cambridge: Cambridge University Press. 235pp.

SMITH F. M. AND JONES, D. R. (1978). Localisation of receptors causing hypoxic bradycardia in trout (*Salmo gairdneri*). *Can. J. Zool.* **56**, 1260–1265.

TOTA, B., ACIERNO, R. AND AGNISOLA, C. (1991*a*). Mechanical performance of the isolated and perfused heart of the haemoglobinless Antarctic icefish *Chionodraco hamatus* (Lönnberg): effects of loading conditions and temperature. *Phil. Trans. R. Soc. Lond. B* **332**, 191–198.

TOTA, B., AGNISOLA, C., SCHIOPPA, M., ACIERNO, R., HARRISON, P. AND ZUMMO, G. (1991*b*). Structural and mechanical characteristics of the heart of the icefish *Chionodraco hamatus* (Lönnberg). In *The Biology of Antarctic Fishes* (ed. G. diPrisco, B. Maresca and B. Tota). Berlin: Springer-Verlag. 250pp.

WELLS, R. M. G., GRIGG, G. C., BEARD, L. A. AND SUMMERS, G. (1989). Hypoxic responses in a

fish from a stable environment: blood oxygen transport in the antarctic fish *Pagothenia borchgrevinki*. *J. exp. Biol.* **141**, 97–111.

WELLS, R. M. G., MACDONALD, J. A. AND DIPRISCO, G. (1990). Thin-blooded Antarctic fishes: a rheological comparison of the haemoglobin-free icefishes *Chionodraco kathleenae* and *Cryodraco antarcticus* with a red-blooded nototheniid, *Pagothenia bernacchii*. *J. Fish Biol.* **36**, 595–609.

WOOD, C. M. AND SHELTON, G. (1980*a*). Cardiovascular dynamics and adrenergic responses of the rainbow trout *in vivo*. *J. exp. Biol.* **87**, 267–270.

WOOD, C. M. AND SHELTON, G. (1980*b*). 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.