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# MYOCARDIAL OXYGEN CONSUMPTION IN THE SEA RAVEN, *HEMITRIPTERUS AMERICANUS*: THE EFFECTS OF VOLUME LOADING, PRESSURE LOADING AND PROGRESSIVE HYPOXIA

BY A. P. FARRELL*, S. WOOD, T. HART AND W. R. DRIEDZIC

*Biology Department, Mount Allison University, Sackville, New Brunswick, Canada, EOA 3CO.*

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## SUMMARY

1. Myocardial oxygen consumption ($\dot{V}_{O_2}$) was measured using an *in situ*, perfused heart preparation at 10 °C. $\dot{V}_{O_2}$ increased in a linear fashion with power output when cardiac output ($\dot{V}b$) was elevated (volume loading). The increased $\dot{V}_{O_2}$ was possible through improved $O_2$ delivery (increased $\dot{V}b$), but $\triangle P_{O_2}$ (input $P_{O_2}$ − output $P_{O_2}$) was reduced. The mechanical efficiency of the heart was improved.

2. $\dot{V}_{O_2}$ also increased in a linear fashion with power output when output pressure was increased with $\dot{V}b$ constant (pressure loading). The increased $\dot{V}_{O_2}$ was supported by increased $O_2$ removal from the perfusate since oxygen delivery ($\dot{V}b$ and input $P_{O_2}$) was constant. Once more, improved mechanical efficiency was observed.

3. $\dot{V}_{O_2}$ decreased as $O_2$ delivery was reduced with progressive hypoxia. Even so, power output was maintained at a perfusate input $P_{O_2}$ of 81 Torr. Five of 11 hearts survived a 30-Torr $P_{O_2}$ exposure, but with a 29 % decrease in power output and a 5-fold reduction in $\dot{V}_{O_2}$. The increase in the apparent aerobic efficiency which enabled this is discussed.

## INTRODUCTION

Considerable information exists on oxygen uptake ($\dot{V}_{O_2}$) of the mammalian heart, which has a well-developed coronary circulation. In contrast information on $\dot{V}_{O_2}$ of the teleost heart, where coronaries are not always present, is limited. Driedzic, Scott & Farrell (1983) measured myocardial $\dot{V}_{O_2}$ to be about 0·28 $\mu l\,s^{-1}\,kg^{-1}$ fish weight in isolated, perfused sea raven hearts under conditions of low afterload and a reduced cardiac output ($\dot{V}b$). Energy metabolism in the perfused hearts was highly aerobic and $\dot{V}_{O_2}$ increased with power output of the heart.

In view of the modest amount of information concerning myocardial $\dot{V}_{O_2}$, a comprehensive study was initiated using an *in situ*, sea raven heart preparation. The suitability of the preparation for physiological studies is well established (Farrell,

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* Present address: Bioscience Department, Simon Fraser University, Burnaby, B.C., Canada, V5A 1S6.

MacLeod & Driedzic, 1982; Farrell, MacLeod, Driedzic & Wood, 1983), in that the *in situ* heart can generate an *in vivo* work load and the power output of the heart can be varied by simple changes in preload and afterload. The sea raven heart lacks a coronary circulation and so it derives its $O_2$ supply from venous blood being pumped through the heart. The present work measured myocardial $\dot{V}_{O_2}$ under different power output regimes and different levels of $O_2$ delivery.

## MATERIALS AND METHODS

### *Animals*

Sea ravens, *Hemitripterus americanus* Gmelin, were caught by otter trawl in Passamaquoddy Bay off St Andrews, New Brunswick. The fish were held in aerated, recirculating sea water tanks (9–10 °C) prior to use. A total of 35 fish was used for the study, weighing 0·77–2.05 kg ($\bar{x} = 1{\cdot}25$ kg).

### *Perfused heart preparation*

The *in situ* heart preparation is described in full by Farrell *et al.* (1982, 1983). In essence, the intact heart received a physiological perfusate at a constant input pressure head *via* a cannula placed in the hepatic vein. All other veins entering the sinus venosus were ligated. Cardiac output was delivered against an output pressure head *via* a cannula placed in the ventral aorta. Cardiac output was varied by adjusting the height of the input reservoir (preload). Afterload was varied by adjusting the height of the output pressure head. The nerve supply to the heart was severed and so the intrinsic rhythm of the sino-atrial pacemaker set the heart rate. During the preparation time of 10–15 min, the heart received venous blood or perfusate. The fish was fully immersed in a saline bath which acted as a reference for pressure measurements. The perfusate composition (in mmol $l^{-1}$) was NaCl, 150; $MgSO_4.7H_2O$, 2; KCl, 5; $CaCl_2$, 2·3; $Na_2HPO_4$, 2·3; $NaH_2PO_4$, 0·2; dextrose, 16·7; and 10 g $l^{-1}$ polyvinylpyrrolidone (PVP, $M_r = 40\,000$). Control perfusate was gassed with 0·5 % $CO_2$ balance air and, after equilibration, the pH was adjusted to pH 7·9 with the addition of $NaHCO_3$ (approximately 10·7 mmol $l^{-1}$). The perfusate reservoirs, delivery lines, and the saline bath containing the preparation were all water-jacketed to maintain the temperature at 10 °C.

### *Protocols*

*Control conditions*

Following the cannulation procedures, $\dot{V}b$ and mean output pressure were set at approximately 11 ml $min^{-1}$ $kg^{-1}$ fish weight and 40 cm$H_2O$, respectively. The heart performed under these conditions for 10–20 min. If $\dot{V}b$ did not stabilize during this period, the preparation was discarded. Representative traces of the control cardiovascular variables ($\dot{V}b$, heart rate, output pressure and input pressure) were collected at the end of this stabilization period. Input perfusate samples were taken from the reservoir to provide three consistent $P_{O_2}$ values. Likewise, the perfusate leaving the ventral aorta was sampled *via* a three-way tap to obtain two consistent $P_{O_2}$ values. $\Delta P_{O_2}$ was the difference between the input and output $P_{O_2}$.

*Volume loading*

These experiments ($N = 9$ fish) examined the effect of changing power output of the heart while changing $O_2$ delivery to the heart. Control $\dot{V}b$ was altered by changing preload; power output and $O_2$ delivery therefore changed in proportion to $\dot{V}b$ since the output pressure head and the perfusate $P_{O_2}$ were unchanged. Three changes in $\dot{V}b$ were examined: control $\dot{V}b$ plus 40 %, control $\dot{V}b$ plus 60 % and control $\dot{V}b$ less 40 %. A 3-min stabilization period was allowed at each new level prior to sampling the output perfusate and the cardiovascular variables. Control conditions were restored for at least 3 min between each challenge and cardiovascular and perfusate samples were taken at the end of each control period.

*Pressure loading*

These experiments ($N = 15$ fish) examined the effect of changing power output of the heart while maintaining oxygen delivery ($\dot{V}b$ and $P_{O_2}$ constant). Mean output pressure was varied to alter power output, and preload was left unchanged. At each new level, a 3-min stabilization period preceded the sampling of the cardiovascular variables and the output perfusate. Output pressures of approximately 35, 40, 50 and 55 $cmH_2O$ were used. While these pressures span the physiological range for ventral aortic pressures, the net effect on power was small in comparison with the $\dot{V}b$ changes. Control conditions were restored for at least 3 min between each challenge, and samples were taken at the end of this control period.

*Progressive hypoxia*

These experiments ($N = 11$ fish) examined the effects of a stepwise reduction in the perfusate $P_{O_2}$ with constant preload and afterload. In addition to the control (air saturated) perfusate four other $P_{O_2}$ levels were examined: 105, 80, 55 and 30 Torr. The $P_{O_2}$ of the perfusate was constant. At each new level of hypoxia a stabilization period of 8–10 min was allowed prior to simultaneous sampling of the cardiovascular variables, the input $P_{O_2}$ and the output $P_{O_2}$. If the cardiovascular variables began to decline abnormally fast at any level of hypoxia, the heart was assumed to be dying and the experiment was stopped. In the hearts that survived the 30 Torr $P_{O_2}$ exposure, preload was increased to evoke the maximal increase in $\dot{V}b$.

*Instrumentation*

Cardiac output was measured in the outflow line with a flowthrough electromagnetic flow probe and its associated BL 610 Biotronix flowmeter. Input and output pressures were monitored *via* saline-filled cannulae with a Micron pressure transducer (Narco Life Sciences, Houston, Texas). The flow and pressure signals were suitably amplified and displayed on a chart recorder (Biotronix BL 882, Kensington, Maryland). The $P_{O_2}$ of the perfusate was measured at 10 °C with an IL 113 acid-base analyser with associated $P_{O_2}$ electrode and water jacket. The electrode was calibrated with water-saturated gases (100 % $N_2$ and 12 % $O_2$) prior to each experiment and the calibration was rechecked with air prior to each sample. The hypoxic gas mixtures

Table 1. *Cardiac variables for control conditions in the different experiments*

| | Heart rate (beats $min^{-1}$) | $\dot{V}_b$ (ml $min^{-1}$ $kg^{-1}$) | Mean pressure ($cmH_2O$) | Power (mW $g^{-1}$) | $\Delta P_{O_2}$ (Torr) | $\dot{V}_{O_2}$ ($\mu l$ $s^{-1}$ $g^{-1}$) | Efficiency (%) | Fish weight (kg) | Ventricular weight (g) |
|---|---|---|---|---|---|---|---|---|---|
| Volume loading ($N = 9$) | 40·8 ± 1·4 | 10·6 ± 0·6 | 38·6 ± 0·5 | 1·08 ± 0·09 | 23·2 ± 1·4 | 0·316 ± 0·031 | 16·2 ± 1·0 | 1·13 ± 0·07 | 0·86 ± 0·06 |
| Pressure loading (winter) ($N = 9$) | 38·8 ± 1·8 | 11·6 ± 0·6 | 40·5 ± 0·3 | 1·21 ± 0·09 | 23·6 ± 1·0 | 0·365 ± 0·024 | 16·5 ± 0·8 | 1·20 ± 0·09 | 0·78 ± 0·09 |
| Presure loading (summer) ($N = 9$) | 47·8 ± 2·8 | 10·9 ± 0·4 | 40·7 ± 0·2 | 1·06 ± 0·12 | 31·3 ± 1·9 | 0·420 ± 0·027 | 12·4 ± 0·7 | 1·34 ± 0·11 | 0·95 ± 0·14 |
| Hypoxia ($N = 11$) | 39·2 ± 1·9 | 11·4 ± 0·3 | 43·8 ± 0·4 | 0·95 ± 0·05 | 24·1 ± 0·6 | 0·282 ± 0·017 | 16·9 ± 0·5 | 1·34 ± 0·15 | 1·14 ± 0·15 |

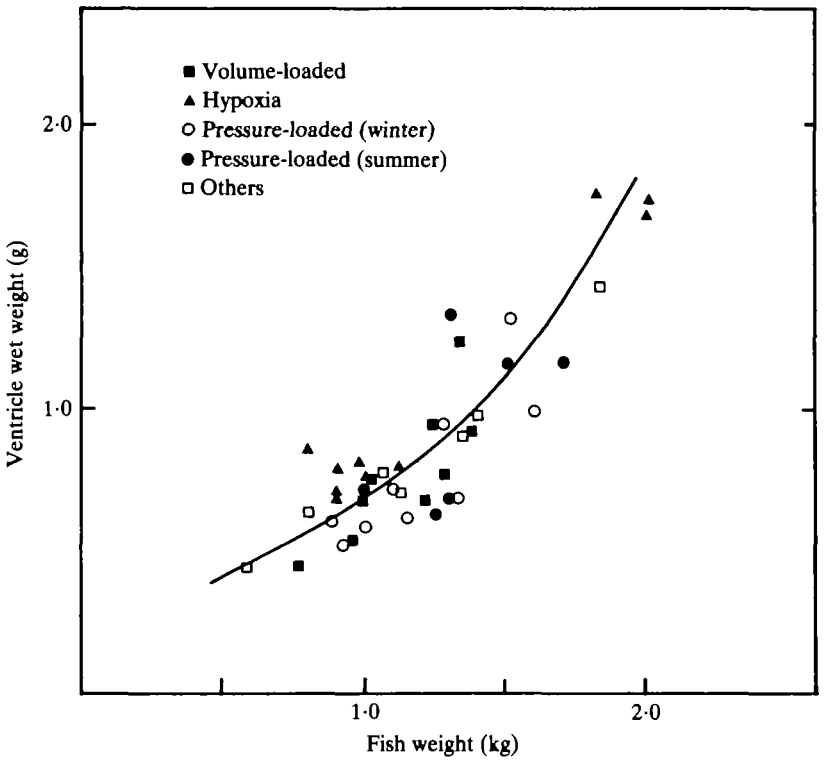


Fig. 1. The relationship between ventricular wet weight and fish body weight. The experiment in which each heart was used is indicated. The line was fitted by eye.

were obtained using a multiple flow controller (Matheson model 8249, East Rutherford, New Jersey).

### *Calculations*

Mean values for input and output pressures and $\dot{V}b$ were obtained from area determinations on the pulsatile traces. The pressures were referenced to the saline level in the chamber and appropriate corrections were made for the pressure drops across the input and output cannulae. All pressures are expressed in $cmH_2O$ (1 $cmH_2O$ = 0·098 kPa). Heart rate was determined from the periodicity of the flow trace. Cardiac output ($ml\,min^{-1}$) = heart rate × stroke volume. Power output of the heart (mW) = (mean output pressure − mean input pressure) × $\dot{V}b$ × (980/60) × $10^{-4}$. Oxygen uptake of the heart ($\mu l\,O_2\,s^{-1}$) = ($\dot{V}b/60$) × ($\alpha/760$) × ($\Delta P_{O_2}$) × $10^3$, where $\alpha$ = 0·038 ml $O_2\,ml^{-1}\,Torr^{-1}$ partial pressure (Altman & Dittmer, 1971). Mechanical efficiency of the heart (%) = [100] [power (mW) × 0·0498]/[$\dot{V}_{O_2}$ ($\mu l\,O_2\,s^{-1}$)]. Cardiac output was normalized per kg fish weight, and power output and $\dot{V}_{O_2}$ were normalized per g ventricular wet weight. The fish was weighed prior to the experiment and the ventricle was weighed following each experiment (Fig. 1). Each fish acted as its own control and mean values ± S.E. are given where appropriate. Statistical differences ($P < 0·05$) were determined with either a Wilcoxon signed-rank test or a Student's *t*-test.

## RESULTS

The control values for the cardiovascular performance and $O_2$ consumption show good agreement between the three experimental protocols (Table 1). All the experiments were performed during winter months (November–February), except six pressure loading and three volume loading experiments which were performed in early summer (May–June). The summer pressure loading experiments were treated separately in Table 1 since they had a significantly higher intrinsic heart rate, a higher $\dot{V}_{O_2}$ and lower efficiency compared to the winter experiments. There were also small variations in ventricular weight expressed as a percentage of total body weight (Fig. 1).

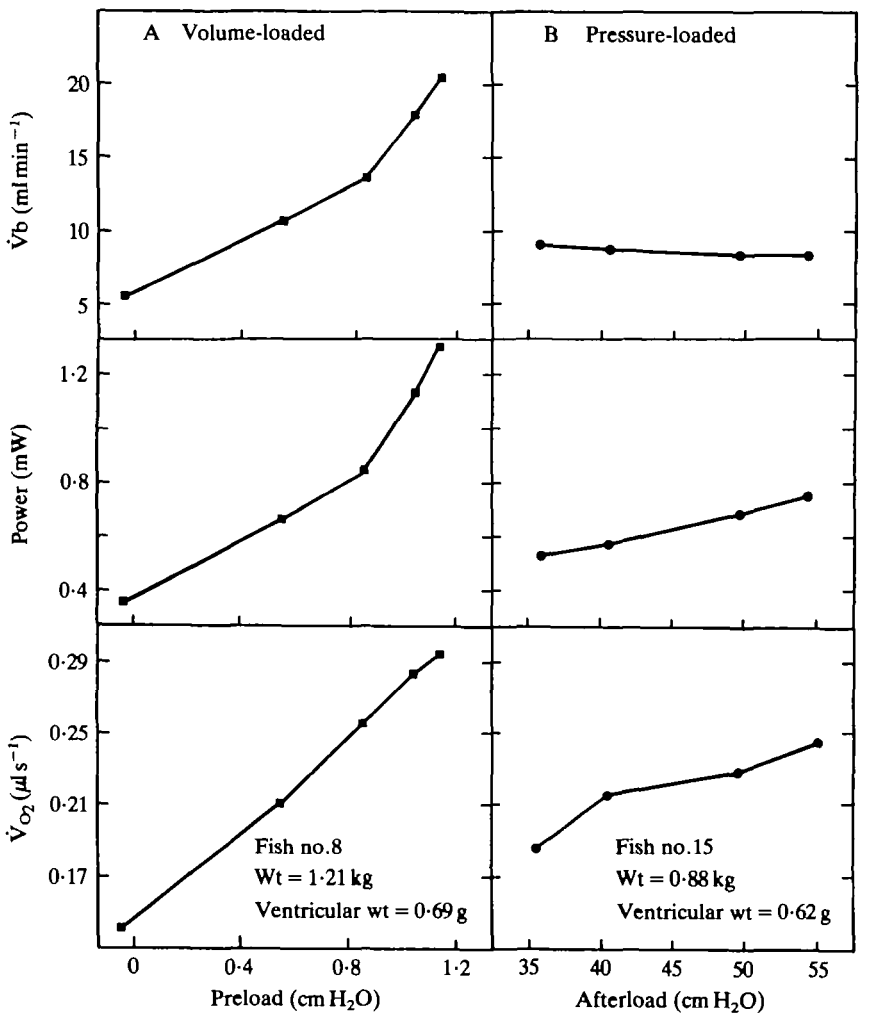


Fig. 2. Cardiac variables [power, oxygen uptake ($\dot{V}_{O_2}$) and cardiac output ($\dot{V}b$)] for individual fish during volume loading (A) and pressure loading (B). Preload was varied in volume-loaded hearts to increase $\dot{V}b$ and power. In pressure-loaded hearts, afterload was varied over a physiological range to increase power with a minimal effect on $\dot{V}b$. Note that physiological changes in $\dot{V}b$ had a far greater effect on myocardial power than physiological changes in afterload.

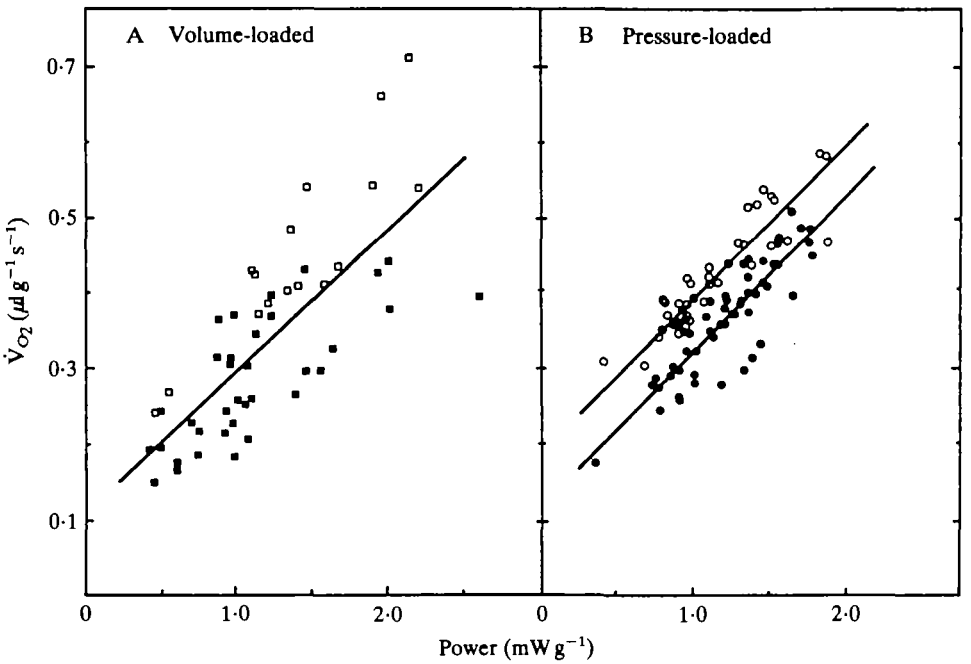


Fig. 3. The relationship between myocardial power output and myocardial oxygen consumption, $\dot{V}_{O_2}$, for volume-loaded (A) and pressure-loaded (B) hearts. Winter experiments (solid symbols) are distinguished from summer experiments (open symbols). For values of linear correlations, see text.

Therefore, the control power output per g ventricle wet weight varied amongst preparations because $\dot{V}b$ was set according to body weight .

Using these data, the average $\dot{V}_{O_2}$ is 0·25 $\mu$l s$^{-1}$ for a 1-kg sea raven generating a myocardial power output of 0·85 mW at 10 °C.

### *Volume loading*

An increase in preload produced increases in $\dot{V}b$, $\dot{V}_{O_2}$ and power output (Fig. 2A). All individual fish showed a significant linear correlation between $\dot{V}_{O_2}$ and power output ($r > 93$ % in all nine fish). $\dot{V}_{O_2}$ was linearly related to power output (Fig. 3A), where $\dot{V}_{O_2} = 0{\cdot}188 \times \text{power} + 0{\cdot}110$ ($r = 75{\cdot}9$ %, 50 df, $P < 0{\cdot}05$).

Mechanical efficiency of the heart increased with volume loading. For example, a three-fold increase in power output (0·8 to 2·4 mW g$^{-1}$) improved efficiency from 15·3 % to 21·3 % (Table 2).

For the $\dot{V}b$ range used in these experiments, $\Delta P_{O_2}$ decreased with increases in $\dot{V}b$ (Fig. 4A). Thus, when power output was increased by increasing $\dot{V}b$, there was an increase in $\dot{V}_{O_2}$, an increase in mechanical efficiency, an increase in oxygen delivery, and a decrease in oxygen removal from the perfusate.

### *Pressure loading*

Changes in afterload altered power output without major changes in $\dot{V}b$ (Fig. 2B). Overall, a linear relationship existed between the $\dot{V}_{O_2}$ and power output (Fig. 3B).

Table 2. *Regression equations for $\dot{V}_{O_2}$ and power output: a comparison of normalized data and absolute values*

| | | Gradient | Intercept | $r^2$ | df | Mechanical efficiency 0·8 mW | 2·4 mW |
|---|---|---|---|---|---|---|---|
| Volume-loaded | normalized* | 0·188 | 0·110 | 57·6 | 50 | 15·3 | 21·3 |
| | absolute | 0·174 | 0·101 | 74·1 | 50 | 16·6 | 23·1 |
| Winter pressure-loaded | normalized | 0·205 | 0·120 | 73·0 | 50 | 14·0 | 19·2 |
| | absolute | 0·251 | 0·049 | 65·3 | 50 | 16·0 | 18·4 |
| Summer pressure-loaded | normalized | 0·204 | 0·192 | 84·3 | 38 | 11·2 | 17·5 |
| | absolute | 0·333 | 0·058 | 61·7 | 38 | 12·3 | 13·9 |

* Normalized to g ventricular wet weight. Average ventricular wet weights are presented in Table 1.

Since the winter experiments ($N = 9$) differed the summer experiments ($N = 6$), two linear regressions are presented: winter $\dot{V}_{O_2} = 0{\cdot}205 \times \text{power} + 0{\cdot}120$ ($r = 85{\cdot}4\,\%$, 50 df, $P < 0{\cdot}05$) and summer $\dot{V}_{O_2} = 0{\cdot}204 \times \text{power} + 0{\cdot}192$ ($r = 91{\cdot}8\,\%$, 38 df, $P < 0{\cdot}05$). Thus for the summer experiments, $\dot{V}_{O_2}$ was slightly higher for a given power output, i.e. efficiency was lower, but both data sets had the same gradient.

Mechanical efficiency increased with pressure loading. For example, a three-fold increase in power output (0·8 to 2·4 mW g$^{-1}$) improved efficiency from 10·0 % to 19·2 % and from 11·2 % to 17·5 % in winter and summer experiments, respectively (Table 2).

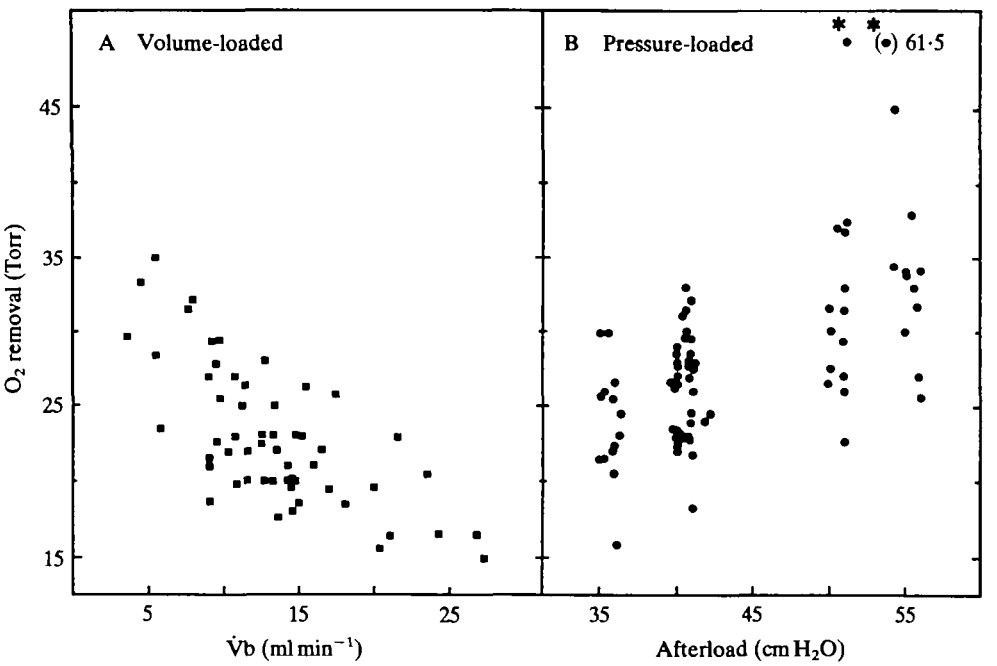


Fig. 4. Scatter diagrams to represent the relationship between $O_2$ removal from the perfusate (input $P_{O_2}$ − output $P_{O_2}$) and cardiac output ($\dot{V}b$) in volume-loaded hearts (A) and afterload in pressure-loaded hearts (B). * Denotes that $\dot{V}b$ was not maintained during pressure loading.

$\Delta P_{O_2}$ increased with pressure loading in order to meet the added $O_2$ demand, provided $\dot{V}b$ (i.e. $O_2$ delivery) was constant (Fig. 4B). Whenever $\dot{V}b$ decreased during pressure loading, $P_{O_2}$ increased markedly. This was particularly evident in the three experiments where a mean output pressure could not be increased to 55 $cmH_2O$ and $\dot{V}b$ decreased as output pressure was raised beyond 50 $cmH_2O$ (Fig. 4B). Here the reduction in $O_2$ delivery was not completely offset by the increase in $O_2$ extraction. Control conditions could be restored subsequently, indicating that the heart may not have been damaged by this challenge.

Thus, when power output is increased with a constant $O_2$ delivery ($\dot{V}b$ and input $P_{O_2}$ constant), an increase in $\dot{V}_{O_2}$ is achieved through improved $O_2$ removal from the perfusate.

### *Progressive hypoxia*

These experiments lasted up to 80 min. At each level of hypoxia the cardiovascular variables were stable for many minutes and so the data summarized in Fig. 5 apply to relatively steady-state conditions.

Power output and $\dot{V}b$ were not significantly different from their control levels at an input $P_{O_2}$ of 81 Torr, but $\dot{V}_{O_2}$ was significantly reduced (Fig. 5). Maintenance of power output in association with a decrease in $\dot{V}_{O_2}$ resulted in an increase in the apparent aerobic efficiency of the heart. Below an input $P_{O_2}$ of 81 Torr, power output and $\dot{V}b$ were reduced significantly, even though preload and afterload were unchanged. One heart died during the transition from 81 Torr to 55 Torr and five hearts died during the transition from 55 Torr to 30 Torr.

$\dot{V}_{O_2}$ was reduced almost three-fold at 55 Torr and over five-fold at 30 Torr, yet the decreases in power output were small by comparison (81 % and 72 % of control, respectively). At these extremes of hypoxia there was an increase in the apparent aerobic efficiency of the heart.

The intrinsic heart rate ($39{\cdot}8 \pm 1{\cdot}9$ beats $min^{-1}$) did not change significantly with progressive hypoxia. Heart rate was reduced by 3–5 beats $min^{-1}$ in three of the four hearts that survived at 30 Torr. One additional fish showed an atypical, progressive decrease in heart rate (33·3 beats $min^{-1}$ at 165 Torr to 18·8 beats $min^{-1}$ at 30 Torr, even though $\dot{V}b$, power output, $\dot{V}_{O_2}$ and efficiency were not atypical compared to the other 10 fish.

At a $P_{O_2}$ of 30 Torr, only small increases in $\dot{V}b$ were possible when preload was raised to evoke a maximal increase in $\dot{V}b$. Cardiac output increased by 8 %, 22 %, 46 % and 55 % with no significant change in heart rate in the four fish examined. Such increases in $\dot{V}b$ were terminal in that $\dot{V}b$ decreased rapidly in three of the hearts after only 20–180 s, and the control $\dot{V}b$ could not be restored subsequently. One heart maintained a 22 % increase in $\dot{V}b$ for 10 min without dying.

## DISCUSSION

This is the first comprehensive study of myocardial $\dot{V}_{O_2}$ in a teleost. Based on the present observations, myocardial $\dot{V}_{O_2}$ is 0·25 $\mu l\, O_2\, s^{-1}$ for a 1-kg fish with a 0·8-g ventricle generating a power output of 0·85 mW. Driedzic *et al.* (1983), using isolated sea raven hearts, reported a similar $\dot{V}_{O_2}$ value, but the power output was only 0·2 mW

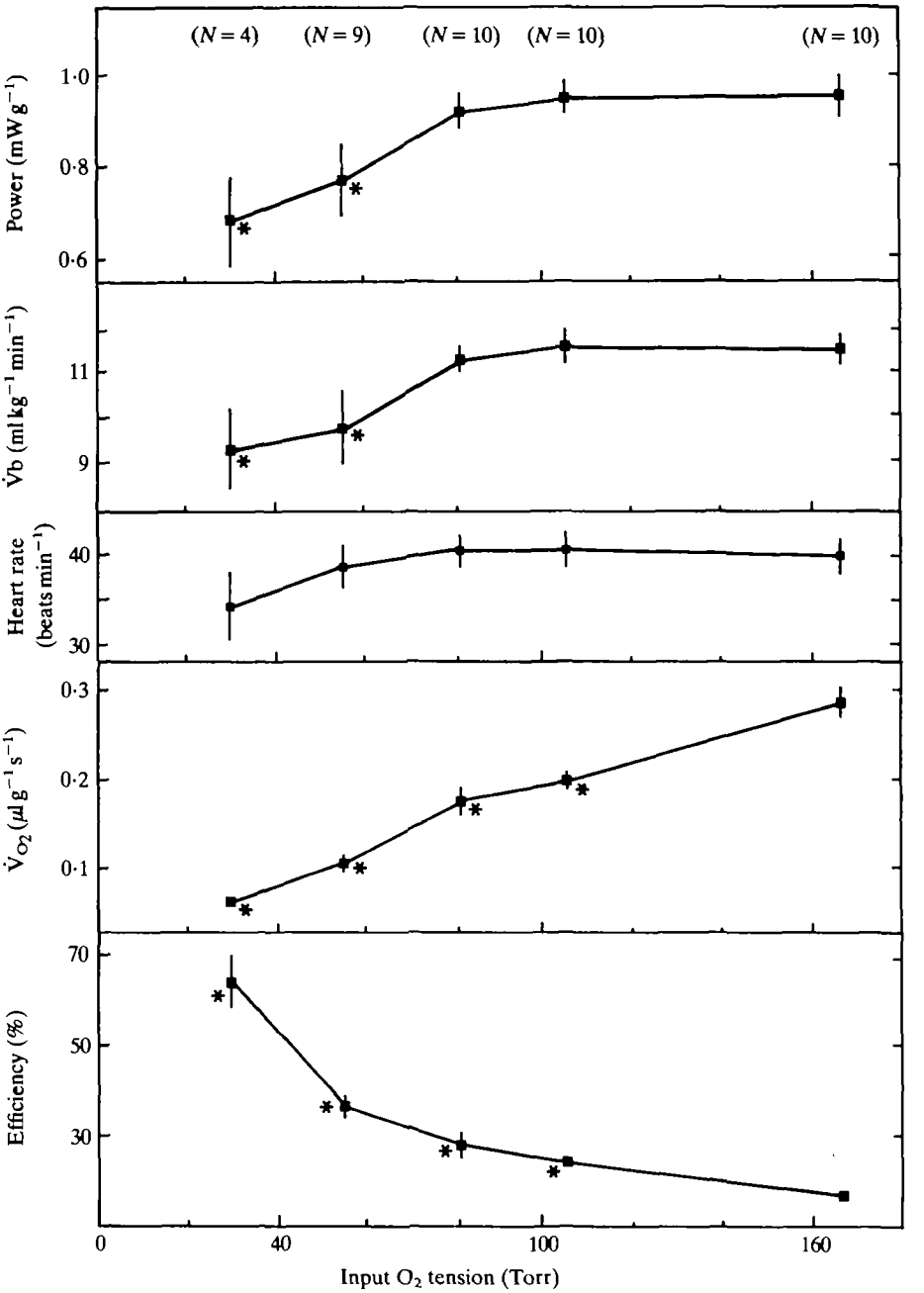


Fig. 5. The effect of four levels of progressive hypoxia on cardiac performance. Each point represents a mean value for $N$ preparations with the standard error indicated by the vertical bar. * Denotes a statistically significant difference from the normoxic control value.

and the mechanical efficiency was 4·2 %. The mechanical efficiency of the *in situ* heart was about 15 %, which is comparable to that of mammalian hearts (rat 11 %, Neely, Liebermeister, Battersby & Morgan, 1967; human 9–11 %, Gibbs & Chapman, 1979). The highest efficiency observed in an *in situ* sea raven heart with normoxic perfusate was 26 %, a value close to the 30 % observed in trained athletes after severe exercise (Gibbs & Chapman, 1979). It seems unlikely that the assumption that the trout heart is 40 % efficient (Jones, 1971) will be substantiated.

The present myocardial $\dot{V}_{O_2}$ measurement can be used to estimate what proportion of the $O_2$ contained in venous blood is consumed by the heart of the intact fish. Each heart stroke supplies about 10 $\mu$l $O_2$, assuming stroke volume is 0·34 ml $kg^{-1}$ and venous $O_2$ content is 3 vol%. Yet the myocardium of a 1-kg fish requires about 0·37 $\mu$l $O_2$ per heart beat, assuming heart rate is 40 beats $min^{-1}$. Thus, myocardial $\dot{V}_{O_2}$ removes less than 4 % of the $O_2$ available in venous blood. Myocardial $\dot{V}_{O_2}$ is also about 0·6 % of the standard $O_2$ uptake of the resting animal. This estimate is based on a standard $O_2$ uptake measurement of 64 $\mu$l $O_2$ $s^{-1}$ $kg^{-1}$ for the lingcod, a fish with a similar lifestyle to the sea raven (Farrell & Daxboeck, 1981), and is in the middle of the theoretical range (0·08 % to 4 %) proposed by Cameron (1975). The above calculations confirm theoretical predictions that the fish heart is efficient (Jones, 1971), has a low metabolic demand in terms of the whole animal (Cameron, 1975), and has a venous $O_2$ supply that is more than adequate for the myocardial demands of resting fish (Jones & Randall, 1978).

$\dot{V}_{O_2}$ is linearly correlated to power output of the heart and mechanical efficiency is improved as power output is increased. These findings are consistent with observations on mammalian hearts (Neely *et al.* 1967; Gibbs & Chapman, 1979; Suga *et al.* 1981, 1982) but do not support the assumption made for the trout heart (Jones, 1971) that efficiency is constant over a large range of $\dot{V}b$ values.

Using the normalized $\dot{V}_{O_2}$ *versus* power output regression equations, the present findings appear to indicate that pressure work and stroke work have the same metabolic cost in the fish heart. The increases in $\dot{V}_{O_2}$ were equivalent in both pressure-loaded and volume-loaded hearts and efficiency was improved by 5–6 % in both cases when power output was increased three-fold (Table 2). Observations on mammalian hearts demonstrate that an increase in pressure (pressure work) is more costly than an increase in flow (stroke work). Neely *et al.* (1967), for instance, demonstrated that if $\dot{V}b$ was increased three-fold without a significant change in systolic pressure, i.e. diastolic pressure was lowered, heart work increased two- to five-fold without a significant increase in $\dot{V}_{O_2}$. In contrast, $\dot{V}_{O_2}$ increased 65 % with a doubling of heart work when the heart generated a greater aortic pressure and stroke volume was constant. However, when $\dot{V}b$ was increased without regulating systolic pressure (diastolic pressure constant), $\dot{V}_{O_2}$ increased significantly (a two-fold increase for a six-fold increase in heart work). Thus one explanation for the apparently similar metabolic cost of volume and pressure loading in sea raven hearts is that the volume-loaded heart performed additional pressure work as systolic pressure rose with stroke volume (diastolic afterload was unchanged).

The present $\dot{V}_{O_2}$ and power output data were normalized because the animal weight varied substantially (see Neely *et al.* 1967). For the volume-loaded heart, absolute and normalized values give the same relationship between $\dot{V}_{O_2}$ and power output

(Table 2). However, with pressure loading, efficiency does not improve appreciably (1–2 %, Table 2) when absolute values are used, unlike the 5–6 % with normalized values. This difference is probably related to the cost of pressure development in fish of different sizes. Only relatively small changes in power output within individual fish were possible with pressure loading (Fig. 2B) and the maximum power output of the smaller fish did not necessarily overlap with the minimum power output of the larger fish. This separation between the absolute data for large and small fish and the different gradient for $\dot{V}_{O_2}$ *versus* power output, implies that pressure work is more costly in larger fish. This additional cost may be related to the fact that larger fish have relatively larger (thicker?) hearts (Fig. 1).

A seasonal difference in $\dot{V}_{O_2}$ was apparent. Summer fish had a higher $\dot{V}_{O_2}$ and a lower efficiency. Whether this is related to the higher intrinsic heart rate of summer fish observed here and in a separate study (M. S. Graham & A. P. Farrell, in preparation) is not known.

The $O_2$ content of the air-equilibrated perfusate was 0·76 vol%, which is about four times lower that that of the venous blood supplying $O_2$ to the heart. Because of this, the $P_{O_2}$ of the perfusate decreases during its passage through the heart, whereas the blood $P_{O_2}$ probably does not change significantly. This raises the question whether $O_2$ delivery to the myocardium was limited by perfusion with aerated saline. If it is assumed that the *output* $P_{O_2}$ is indicative of the $O_2$ gradient driving diffusion, then $O_2$ delivery from aerated perfusate was probably not diffusion limited since the output $P_{O_2}$ ($> 130$ Torr) of the perfusate was always much greater than the venous $P_{O_2}$ in the intact sea raven (53 Torr, Farrell & Driedzic, 1980). Consequently, the cardiac performance *in situ* is probably directly comparable to the *in vivo* situation despite differences of $O_2$ content in the perfusion media. This conjecture is also supported by preliminary experiments where oxygenating the perfusate (99·5 % $O_2$) had no effect on cardiac performance or $\dot{V}_{O_2}$, and by the fact that the *in situ* heart performed physiological workloads and showed no deterioration after 2 h of experiments.

Unlike the situation with aerated perfusate, $O_2$ diffusion became limiting during perfusion with hypoxic saline. Power output was maintained with an input $P_{O_2}$ of 80 Torr, i.e., when the output $P_{O_2}$ of 60–65 Torr was slightly above the normal venous $P_{O_2}$ of 53 Torr. However, at an input $P_{O_2}$ below 55 Torr (output $P_{O_2}$ of 40–45 Torr) the decrease in $O_2$ removal as $\dot{V}b$ declined during hypoxia, which is the opposite of the situation with aerated perfusate (Fig. 3A), was a strong indication that $O_2$ diffusion became limiting. Perhaps the magnitude of the $O_2$ limitation is better highlighted by the death of some hearts and the poor and often terminal response to preload. Extrapolation of this conclusion to intact fish is restricted by the limited information on the cardiac and venous $P_{O_2}$ during hypoxia. Nevertheless it seems reasonable to assume that $O_2$ diffusion does become limiting in the intact sea raven at a $P_{O_2}$ perhaps a few Torr lower than that used in the present work: this would account for the negligible change in venous $P_{O_2}$ and the possible importance of facilitated $O_2$ diffusion by haemoglobin. In other species the limiting $P_{O_2}$ will undoubtedly vary because of myocardial myoglobin content, ventricular thickness and the presence of a coronary circulation. In intact lingcod, a water $P_{O_2}$ of 25–45 Torr reduces cardiac performance ($\dot{V}b$ and arterial pressure reduced by 31 % and 10 % respectively, Farrell 1982), but whether this response reflects an $O_2$ limitation is

unknown. In contrast, the trout heart, which is supplemented by arterial blood, maintains its performance during environmental hypoxia ($P_{O_2}$ of 40 Torr) when the venous and arterial $P_{O_2}$ levels are 10 and 22 Torr respectively (Holeton & Randall, 1967; Wood & Shelton, 1980).

Hypoxia had no major effect on pacemaker frequency. Any decreases in rate observed here were in unstable preparations exposed to stressful situations (e.g. excessive work loads). These decreases were irreversible. Consequently, the bradycardia observed in intact fish at extremes of environmental hypoxia (e.g. Smith & Jones, 1978; Daxboeck & Holeton, 1978; Wood & Shelton, 1980; Farrell, 1982) is probably entirely a central reflex.

Hearts receiving input perfusate with a $P_{O_2}$ of 80 Torr were able to sustain the same level of performance as control hearts. It has previously been shown that sea raven hearts perfused with air-equilibrated media generate essentially all of their ATP requirements *via* aerobic metabolism (Driedzic *et al.* 1983). Thus, a 1-kg fish, with a 0·8-g heart and an oxygen consumption rate of 0·25 $\mu l\, O_2\, s^{-1}$ would have an ATP turnover rate of approximately 80 nmol ATP $g^{-1}\, s^{-1}$. It may be calculated from the data of Turner & Driedzic (1980) that sea raven hearts subjected to anoxic conditions to stimulate glycolysis have a maximal anaerobic ATP production rate of approximately 20 nmol ATP $g^{-1}\, s^{-1}$. At an input $P_{O_2}$ of 80 Torr, ATP demand could be matched closely with the sum of ATP regeneration through aerobic and anaerobic metabolism. Larger decreases in external oxygen availability resulted in a decrease in performance, presumably due to the inability to increase further the rate of ATP production. Only 5 of 11 hearts withstood the 30 Torr exposure and their capacity to increase $\dot{V}b$ was reduced. Imposed increases in $\dot{V}b$ were also detrimental to the heart's survival. Only one heart sustained an increase in $\dot{V}b$ for longer that 2 min. The rapid collapse of the hearts at high work loads during hypoxia may reflect a problem not only with ATP production but also with the removal of anaerobic end products from the myocardium. Intracellular acidosis is detrimental to cardiac contractility (Gesser & Poupa, 1983; Farrell *et al.* 1983; Farrell, 1984) and it is recognised acidosis combined with anoxia impair contractility of ventricular strips considerably more than anoxia alone (Nielsen & Gesser 1983).

In summary, the present measurements of myocardial $\dot{V}_{O_2}$ under various power output regimes and levels of $O_2$ delivery indicate many similarities between the fish heart and the mammalian heart despite the anatomical differences and lack of a coronary circulation in the fish. Perhaps the most important difference is the relative tolerance of hypoxia by the fish myocardium. This aspect of myocardial metabolism would be worthy of further investigation.

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