

MAXIMUM CARDIAC PERFORMANCE OF RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) AT TEMPERATURES APPROACHING THEIR UPPER LETHAL LIMIT

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Summary

Numerous studies have examined the effect of temperature on *in vivo* and *in situ* cardiovascular function in trout. However, little information exists on cardiac function at temperatures near the trout's upper lethal limit. This study measured routine and maximum *in situ* cardiac performance in rainbow trout (*Oncorhynchus mykiss*) following acclimation to 15, 18 and 22 °C, under conditions of tonic (30 nmol l⁻¹), intermediate (60 nmol l⁻¹) and maximal (200 nmol l⁻¹) adrenergic stimulation. Heart rate increased significantly with both temperature and adrenaline concentration. The Q₁₀ values for heart rate ranged from 1.28 at 30 nmol l⁻¹ adrenaline to 1.36 at 200 nmol l⁻¹ adrenaline. In contrast to heart rate, maximum stroke volume declined by approximately 20% (from 1.0 to 0.8 ml kg⁻¹) as temperature increased from 15 to 22 °C. This decrease was not alleviated by maximally stimulating the heart with 200 nmol l⁻¹ adrenaline. Because of the equal and opposite effects of increasing temperature on heart rate and

stroke volume, maximum cardiac output did not increase between 15 and 22 °C. Maximum power output decreased (by approximately 10–15%) at all adrenaline concentrations as temperature increased. This reduction reflected a poorer pressure-generating ability at temperatures above 15 °C. These results, in combination with earlier work, suggest (1) that peak cardiac performance occurs around the trout's preferred temperature and well below its upper lethal limit; (2) that the diminished cardiac function concomitant with acclimation to high temperatures was associated with inotropic failure; (3) that Q₁₀ values for cardiac rate functions, other than heart rate *per se*, have a limited predictive value at temperatures above the trout's preferred temperature; and (4) that heart rate is a poor indicator of cardiac function at temperatures above 15 °C.

Key words: heart, stroke volume, heart rate, cardiac output, temperature, adrenaline, rainbow trout, *Oncorhynchus mykiss*.

Introduction

Much of our knowledge on fish cardiovascular responses to temperature change is limited to information on heart rate. There are two reasons for this. First, heart rate is the easiest cardiac variable to measure. Second, in mammals at least, heart rate is a reliable predictor of cardiac performance. If heart rate were equally reliable as a predictor of integrated cardiac function in fish, this large data base would have a tremendous predictive potential for fish cardiac performance. Unfortunately, there are many indications that heart rate alone is a poor predictor of integrated cardiac function in fish. Among the concerns are the following: (1) stroke volume can change by as much as, and even more than, heart rate when cardiac output increases (Farrell, 1991; Farrell and Jones, 1992); (2) the relative contributions of stroke volume and heart rate to changes in cardiac output vary between species and as a function of temperature (see Kolok and Farrell, 1994); (3) maximum stroke volume can decrease at high heart rates (Farrell *et al.* 1989); and (4) maximum isometric tension developed by cardiac muscle decreases at high contraction frequencies (i.e. a negative staircase effect) (Ask *et al.* 1981;

Ask, 1983; Driedzic and Gesser, 1985, 1988). In view of these observations, it would be unwise to predict changes in cardiac performance from temperature-induced changes in heart rate alone. Indeed, a temperature-induced increase in heart rate does not necessarily produce a proportional increase in cardiac output (Brett, 1971; Yamamitsu and Itazawa, 1990; Kolok and Farrell, 1994).

The present study, which reports the first measurements of maximum cardiac performance in rainbow trout at temperatures near their upper lethal limit (23–25 °C; Black, 1953), extends our knowledge of how numerous variables (heart rate, stroke volume, cardiac output, power output) affect cardiac function in fish. The results support our contention that heart rate is a poor indicator of integrated cardiac performance in fish. In addition, this novel information should prove valuable in predicting the effects of increased environmental temperature on fish performance. Our approach was to use an *in situ* perfused heart to measure routine and maximum cardiac performance at 15, 18 and 22 °C after the fish had acclimated to these temperatures. The *in situ* perfused rainbow trout heart

is an appropriate model for investigating the relationship between high environmental temperature and cardiac performance since it is capable of performing at work levels equal to maximum *in vivo* levels (Farrell *et al.* 1989).

Materials and methods

Experimental animals

Rainbow trout [*Oncorhynchus mykiss* (Walbaum)] (weighing 403–727 g) were obtained from a local supplier (West Creek Trout Farms, Aldergrove, BC, Canada) and maintained in a 2000 l fibreglass tank receiving dechlorinated Vancouver tapwater. Throughout the experiment only one stock of fish was used. All fish were initially maintained at 15 °C, before subsequent exposure to 18 and then 22 °C. Fish were acclimated at each temperature for at least 2 weeks prior to use. Water temperature was regulated to within 1 °C of the desired test temperature by a Min-O-Cool cooling unit (Frigid Units, Blissfield, MI, USA) and two countercurrent heat exchangers of local construction. Photoperiod was 12 h:12 h L:D. Fish were fed commercially prepared trout pellets daily.

Perfused heart preparations

Fish were anaesthetized in a buffered solution of tricaine methane sulphonate (0.1 g l⁻¹ MS 222, with 0.1 g l⁻¹ sodium bicarbonate) and transferred to an operating table where their gills were irrigated with aerated buffered anaesthetic at 4 °C (0.05 g l⁻¹ MS 222 in 0.1 g l⁻¹ sodium bicarbonate). Fish were injected with 0.6 ml of heparinized (100 i.u. ml⁻¹) saline *via* the caudal vessels, and an *in situ* heart preparation was obtained, as detailed in Farrell *et al.* (1986) and modified by Farrell *et al.* (1989). Briefly, an input cannula was secured into the sinus venosus through a hepatic vein and perfusion with saline containing 30 nmol l⁻¹ adrenaline was begun immediately. Silk thread was used to secure the input cannula and to occlude any remaining hepatic veins. The output cannula was inserted into the ventral aorta at a point confluent with the bulbus arteriosus and tied firmly in place. Finally, silk ligatures were tied around each ductus Cuvier to occlude these veins and to crush the cardiac branches of the vagus nerve. This procedure left the pericardium intact, while isolating the heart in terms of saline input and output.

Once the surgery had been completed (15–20 min), the fish was immersed in a temperature-controlled saline bath at 15, 18 or 22 °C. The input cannula was attached to a constant-pressure reservoir and the output cannula was connected to a constant pressure head. Output pressure was initially set at 5 kPa to simulate resting *in vivo* ventral aortic blood pressure (Stevens and Randall, 1967), and filling (input) pressure was adjusted to give a cardiac output of 20 ml min⁻¹ kg⁻¹ body mass for the 15 and 18 °C groups. Cardiac output was set at 25 ml min⁻¹ kg⁻¹ body mass for the 22 °C group to account for temperature effects on *in vivo* resting cardiac output (Farrell and Jones, 1992). At all temperatures, the heart maintained this initial control level of performance for a period of 20 min to allow for recovery from surgery and for equilibration to the organ bath.

The saline in the organ bath and the perfusion reservoirs was maintained at the desired acclimation temperature by a Lauda cooling unit (Brinkmann Instruments, Rexdale, Ontario, Canada). The saline (pH 7.8 at 15 °C) contained (in mmol l⁻¹): NaCl, 124; KCl, 3.1; MgSO₄·7H₂O, 0.93; CaCl₂·2H₂O, 2.52; glucose, 5.6; Tes salt, 6.4; and Tes acid, 3.6 (Keen *et al.* 1994). The Tes buffer system was selected to simulate the buffering capacity of trout plasma and the normal change in blood pH with temperature ($\Delta pK_a/dT=0.016$ pH units °C⁻¹). The saline was equilibrated with 100 % O₂ for at least 30 min prior to experimentation. The coronary artery, which supplies the outer compact myocardium of the ventricle, was not perfused and so oxygenated saline was used to ensure that a sufficient amount of oxygen diffused from the ventricular lumen to the compact myocardium. The oxygen gradient from the lumen to the myocardium of our perfused heart was at least 20 times greater than that *in vivo*. The control saline contained 30 nmol l⁻¹ adrenaline bitartrate because Graham and Farrell (1989) have established that tonic adrenergic stimulation with 10 nmol l⁻¹ adrenaline is essential for long-term viability of perfused hearts at 5 °C. In addition, Keen *et al.* (1994) showed that trout acclimated to high temperatures (18 °C) have a decreased cardiac sensitivity to adrenaline.

Experimental protocols

The maximum pumping ability of the heart was assessed by measuring the following: (1) the ability of the heart to maintain stroke volume when exposed to increases in output pressure (i.e. homeometric regulation); (2) maximum cardiac output; (3) maximum power output; and (4) output pressure at maximum power output. Homeometric regulation was investigated by increasing diastolic output pressure from 4 to 8 kPa in increments of 1 kPa, or until cardiac output fell by 40 %. During homeometric regulation, the input pressure was maintained at control levels. Output pressure was not increased further to ensure that the heart was not damaged prior to the measurement of maximum cardiac output and maximum power output. In fish swimming maximally, or exposed to high adrenaline doses, diastolic ventral aortic pressure is unlikely to exceed 8 kPa (Kiceniuk and Jones, 1977; Gamperl *et al.* 1994a). Maximum cardiac output was determined under control conditions by increasing filling pressure in 8–12 steps (in increments of 0.005–0.01 kPa) until cardiac output reached a maximum value. Once maximum cardiac output had been attained, output diastolic pressure was raised in steps of 0.5–1 kPa until the maximum power output was reached. The output pressure at this point was noted. Each step in filling and output pressure was maintained for approximately 1–2 min. The heart was returned to the control work load for a recovery period of 15 min after the determination of homeometric regulation and following the determination of maximum power output. This allowed the heart to recover fully between tests and/or to equilibrate to new adrenaline concentrations. This series of experimental procedures required approximately 1 h to complete.

All cardiovascular measurements were repeated at two additional adrenaline concentrations (60 and 200 nmol l⁻¹) to

cover the range for circulating catecholamine levels observed in stressed rainbow trout (Milligan *et al.* 1989; Gamperl *et al.* 1994b; Randall and Perry, 1992). In addition, preliminary experiments at 15 °C (Fig. 1) showed that 200 nmol l⁻¹ adrenaline achieved maximum adrenergic stimulation of the *in situ* preparation and that 60 nmol l⁻¹ adrenaline was near the EC₅₀ for maximum stimulation.

Instrumentation and analysis

An in-line electromagnetic flow probe (Zepeda instruments,

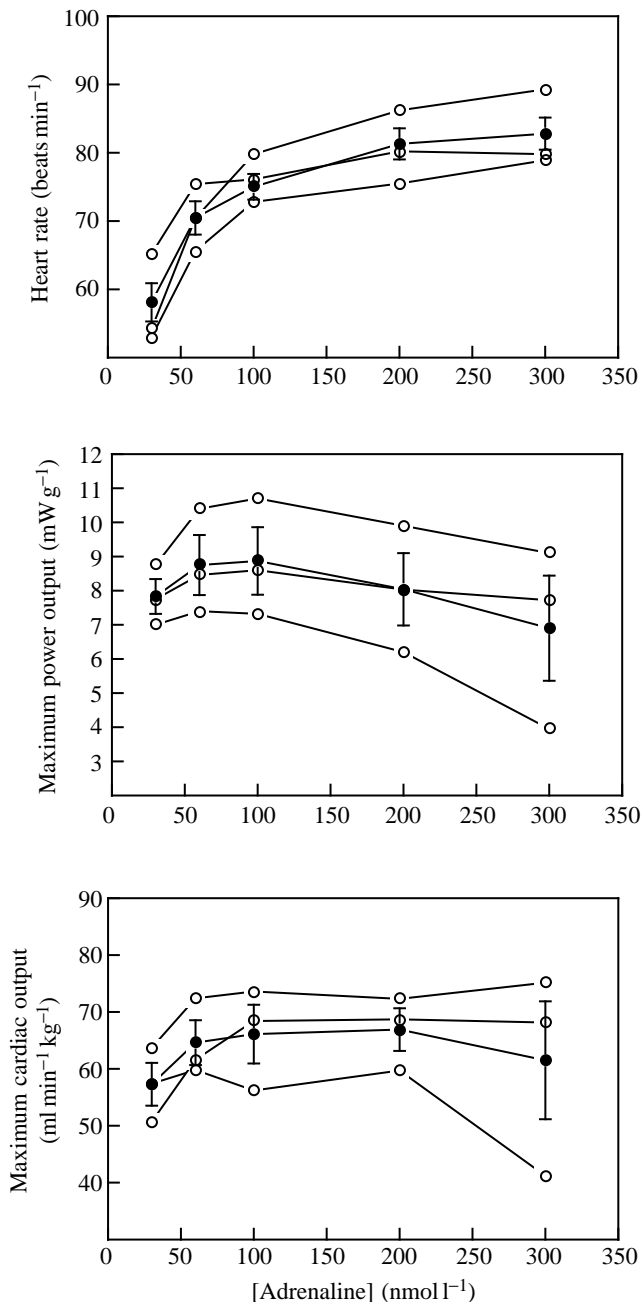


Fig. 1. Relationship between adrenaline concentration and cardiovascular variables for *in situ* perfused trout hearts at 15 °C. Open circles represent individual hearts and filled circles represent group means ($N=3$). Vertical bars represent ± 1 S.E.M.

Seattle, Washington, USA) was used to record mean cardiac output, and pressure transducers (Narco Life Sciences, Houston, TX, USA) were used to measure input and output pressures through saline-filled side-arms. Prior to experimentation, pressure changes due to cannula resistance were calculated at known flow rates. These values were then used to adjust input and output pressure to the levels experienced by the sinus venosus and bulbus arteriosus, respectively. Calibration of the pressure transducers was performed daily against a static water column. Pressure and flow signals were amplified and displayed on a four-channel chart recorder (Gould, Cleveland, OH, USA) in conjunction with a microcomputer running Labtech Notebook (Laboratory Technologies Corporation, Wilmington, MA). Data were collected at 5 Hz, and block averages were calculated every 15 s. Heart rate was measured by counting the number of systolic peaks recorded during a 10 s period.

Stroke volume and power output were calculated as follows:

$$V_s = (\dot{Q}/f_H)/M_b, \quad (1)$$

$$p = [\dot{Q} \times (P_o - P_i) \times \alpha]/M_v, \quad (2)$$

where \dot{Q} (ml min⁻¹) is cardiac output, P_o is mean output pressure (kPa), P_i is mean filling pressure (kPa), V_s is stroke volume (ml kg⁻¹ body mass), f_H is heart rate (beats min⁻¹), M_b is body mass (kg), p is power output (mW g⁻¹ ventricle mass), M_v is ventricular mass (g) and α is 0.00162 mW min ml⁻¹ kPa⁻¹.

Within each temperature group, paired *t*-tests were used to identify statistical differences between cardiovascular variables recorded at 30, 60 and 200 nmol l⁻¹. The effect of temperature within a particular adrenaline concentration was assessed using a one-way analysis of variance (ANOVA). A covariant analysis of variance (ANCOVA) was applied to the stroke volume–heart rate relationship to isolate the interactive effects of temperature and adrenaline. A general linear model (Zar, 1974) was used to examine whether temperature and adrenaline affected the relationship between filling pressure and stroke volume (i.e. the Starling curve) (Proc GLM, SAS Institute). For all statistical analyses, the fiducial limit of significance was chosen as 5%. Values throughout the text are expressed as means \pm S.E.M.

Results

Homeometric regulation

In our experience, cardiac failure is not normally a problem once the surgery has been completed. This fact is illustrated by the success of all seven of the preparations attempted at 15 °C. However, at temperatures above 15 °C, increases in output pressure either during the initial elevation to control conditions (5 kPa final pressure) or during the first homeometric regulation test caused cardiac failure (indicated by sustained cardiac arrhythmia) in some hearts. At 18 °C, two of the 13 attempts failed, and at 22 °C the failure rate reached 40% (six out of 15 preparations). In these failing preparations, increasing the adrenaline concentration to 60 nmol l⁻¹ occasionally

restored the normal beat frequency, but only temporarily. These observations suggest that above 15 °C there was a particularly strong negative effect of temperature on the pressure-generating ability of certain hearts. As a result, it should be remembered that the mean values we report for cardiac variables do not take into account the fact that a proportion of heart preparations failed at 18 °C and 22 °C.

At all temperatures, an increase in diastolic output pressure significantly decreased resting stroke volume (Fig. 2). There was also a significant effect of temperature on the relationship between resting stroke volume and temperature. At 15 °C, stroke volume was maintained above 90 % of the resting value even at an output pressure of 8 kPa. In contrast, at 18 °C stroke volume was reduced to less than 80 % of the resting value at an output pressure of only 7 kPa (Fig. 2). There was no significant difference between the curves for 15 °C and 22 °C.

Adrenergic stimulation had no marked effect on the general shape of the homeometric relationships (Fig. 2). However, adrenaline consistently displaced the relationship downwards because adrenergically mediated increases in heart rate meant that the set point for resting stroke volume was lower (see below).

Heart rate

Heart rate increased significantly with both temperature and adrenaline concentration (Fig. 3A). Increasing the temperature from 15 to 22 °C increased heart rate by 13.9 beats min^{-1} (from 69.9 to 83.8 beats min^{-1}) with 30 nmol l^{-1} adrenaline, and by 20.8 beats min^{-1} (from 81.5 to 102.3 beats min^{-1}) with 200 nmol l^{-1} adrenaline. The Q_{10} values for 30 nmol l^{-1} and 200 nmol l^{-1} adrenaline were calculated as 1.28 and 1.36, respectively.

Increasing the adrenaline concentration from 30 to 200 nmol l^{-1} significantly increased heart rate at all temperatures. This increase was 18.5 beats min^{-1} (22 %) at 22 °C and approximately 10 beats min^{-1} at 15 °C (17 %) and 18 °C (13 %).

Stroke volume

In almost all cases, the filling pressures at the routine work loads were subambient and there were no significant effects of temperature on the filling pressure required to generate routine cardiac output (Table 1). Increasing the filling pressure generated a typical Starling curve for stroke volume at all temperatures. However, acclimation temperature significantly altered the shape of the Starling curve (Fig. 4). Acclimation to higher temperatures (18 and 22 °C) caused a significant downward shift in the upper arm of the Starling curve (Fig. 4) and significantly decreased maximum stroke volume (Figs 3B, 4). Maximum stroke volumes were approximately 1 ml kg^{-1} at 15 °C and 0.8 ml kg^{-1} at 22 °C. Adrenaline had no significant effect on maximum stroke volume regardless of the acclimation temperature (Fig. 3B).

Maximum cardiac output

Although there was some variability in cardiac output

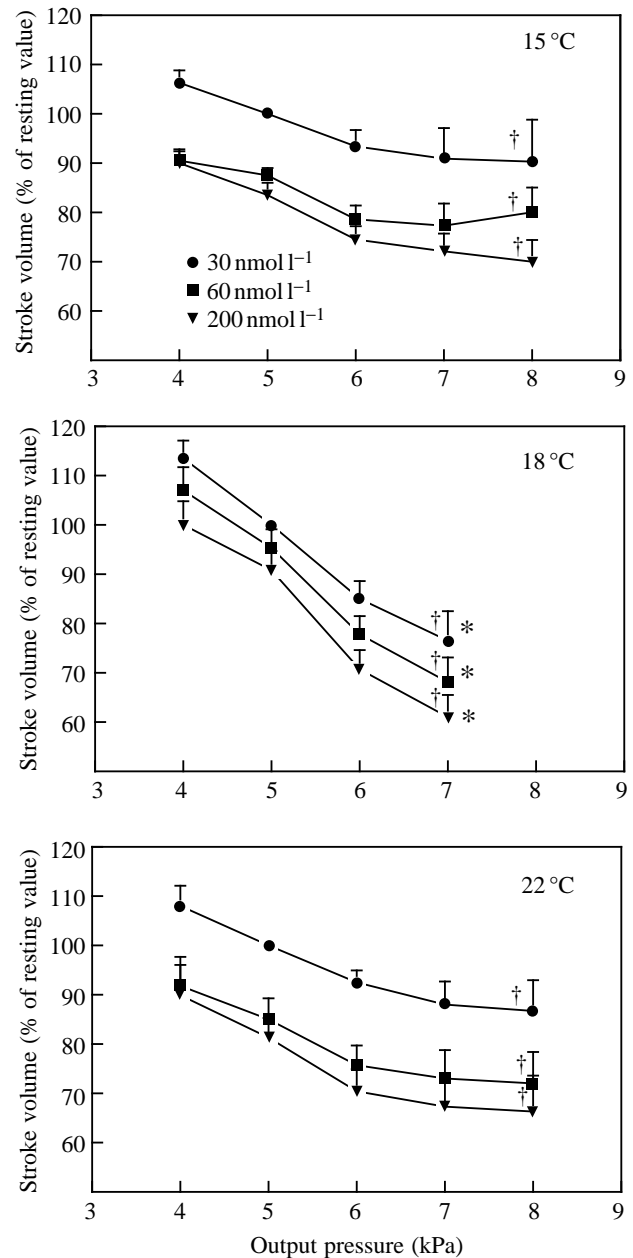


Fig. 2. Relationship between stroke volume and output pressure for *in situ* hearts exposed to various temperatures (15 °C, $N=7$; 18 °C, $N=11$; 22 °C, $N=8$) and adrenaline concentrations. Measurements for hearts at 18 °C and 8 kPa of output pressure are not shown because further increases in output pressure greatly reduced cardiac output (see Materials and methods). A dagger denotes a significant ($P<0.05$) decrease in the stroke volume at the highest output pressure tested when compared with the resting value. An asterisk indicates a significant difference from the value for stroke volume of the 15 °C fish tested at the highest output pressure. Vertical bars represent ± 1 S.E.M.

between temperature groups, there were no significant differences between values. Furthermore, it is clear that maximum cardiac output with 60 and 200 nmol l^{-1} adrenaline was unchanged by acclimation temperature (Fig. 3C). This indicates that the temperature-induced increases in heart rate

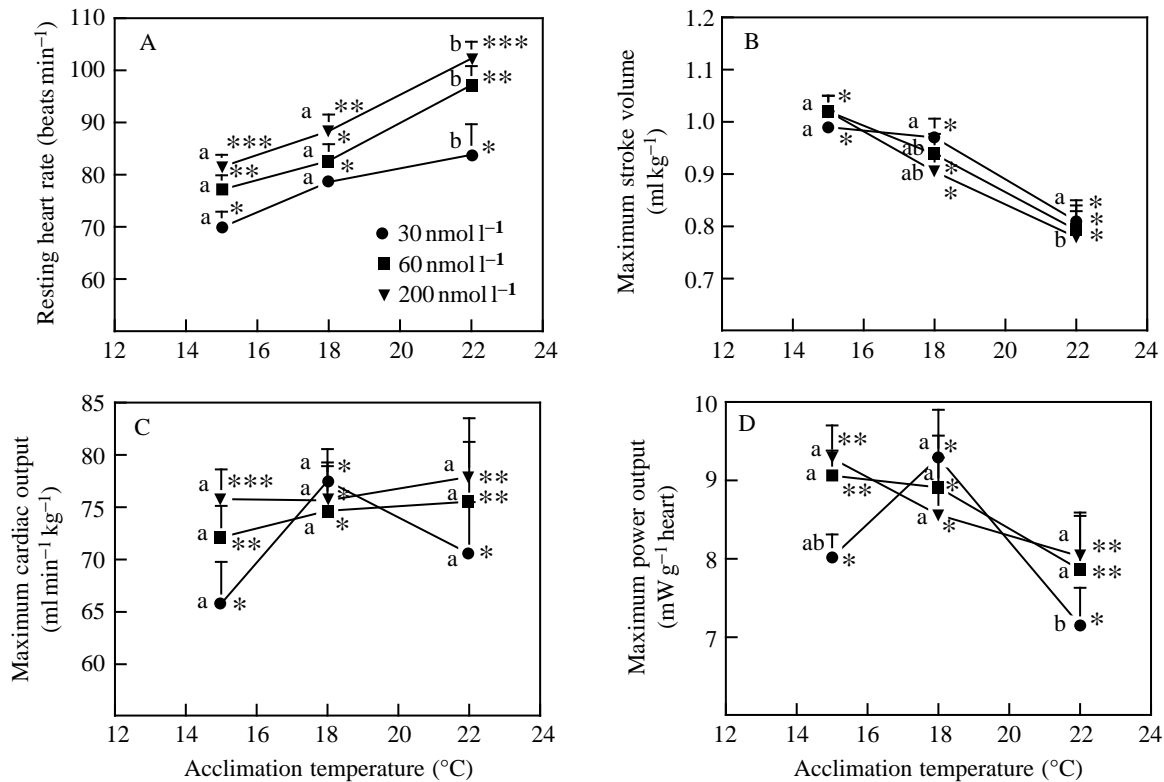


Fig. 3. Relationship between acclimation temperature and (A) resting heart rate, (B) maximum stroke volume, (C) maximum cardiac output and (D) maximum power output for *in situ* perfused trout hearts at 15 ($N=7$), 18 ($N=11$) and 22 °C ($N=8$). Vertical bars represent 1 s.e.m. Dissimilar letters indicate values that are significantly different ($P<0.05$) between acclimation temperatures within each adrenaline concentration. Within each temperature, means with an unequal number of asterisks indicate significant differences between adrenaline concentrations ($P<0.05$).

were offset by equal and opposite changes in maximum stroke volume. This point is illustrated in Fig. 5, where the 29% increase in heart rate between 15 and 22 °C was associated with a 23% reduction in stroke volume.

Within the 15 and 22 °C temperature groups, there were statistically significant effects of adrenaline (Fig. 3C). Increasing the adrenaline concentration from 30 to 200 nmol l⁻¹ increased maximum cardiac output by 10 ml min⁻¹ kg⁻¹ (15%) at 15 °C and by 7.5 ml min⁻¹ kg⁻¹

(10%) at 22 °C. There was no significant effect of adrenaline on cardiac output at 18 °C.

Maximum power output

While the homeometric regulation test and the failure of a high proportion of hearts hinted at poorer inotropic performance under resting conditions at 22 °C, a reduced maximum power output was a clear indicator of inotropic failure at this acclimation temperature. Maximum power

Table 1. Morphometric and cardiovascular variables for rainbow trout (*Oncorhynchus mykiss*) acclimated to 15, 18 and 22 °C

Test temperature (°C)	Body mass (g)	Heart mass (g)	RVM (%)	Resting P_i (kPa)			P_o at maximum power (kPa)		
				30 nmol l ⁻¹	60 nmol l ⁻¹	200 nmol l ⁻¹	30 nmol l ⁻¹	60 nmol l ⁻¹	200 nmol l ⁻¹
15 ($N=7$)	493.1±29.7 ^a	0.40±0.1 ^a	0.081±0.01	-0.08±0.02	-0.08±0.02	-0.08±0.03	7.14±0.28 ^a	7.38±0.23 ^a	7.20±0.33 ^a
18 ($N=11$)	515.1±28.9 ^{a,b}	0.40±0.0 ^a	0.078±0.00	-0.07±0.04	0.00±0.03	-0.01±0.04	6.84±0.15 ^{a,b}	6.80±0.16 ^{a,b}	6.14±0.19 ^b
22 ($N=9$)	606.5±41.7 ^b	0.53±0.0 ^b	0.088±0.00	-0.07±0.03	-0.06±0.02	-0.07±0.03	6.26±0.21 ^b	6.60±0.27 ^b	6.13±0.24 ^b

Resting input pressure, and output pressure at maximum power output, were recorded at three different adrenaline concentrations (30, 60 and 200 nmol l⁻¹) using an *in situ* heart preparation.

Values are expressed as means ± 1 s.e.m.

RVM, relative ventricular mass.

Dissimilar letters indicate significantly different values ($P<0.05$) within a column.

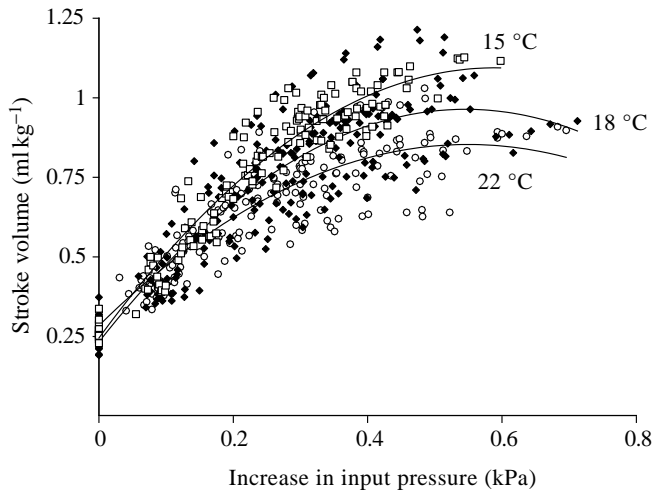


Fig. 4. Starling curves for *in situ* perfused trout hearts at acclimation temperatures of 15 ($N=7$, \square), 18 ($N=11$, \blacklozenge) and 22 °C ($N=9$, \circ). Within each temperature, each point represents data for an individual heart at adrenaline concentrations of 30, 60 and 200 nmol l^{-1} . Best-fitting equations for each acclimation temperature were: 15 °C, $y = -2.439x^2 + 2.878x + 0.245$ ($r^2=0.942$); 18 °C, $y = -2.454x^2 + 2.678x + 0.233$ ($r^2=0.829$); 22 °C, $y = -1.887x^2 + 2.072x + 0.284$ ($r^2=0.824$). Analysis of covariance showed that the stroke volume–input pressure relationships at all temperatures were significantly different from each other ($P < 0.05$).

output was significantly lower at 22 °C than at 15 °C, irrespective of the adrenaline concentration (Fig. 3D). This reduction in maximum power output occurred primarily because the maximum pressure-generating ability of the heart was significantly lower (Table 1), since maximum cardiac output was unaffected (Fig. 3C).

Discussion

The present study, which is the first to measure the maximum performance of a perfused salmonid heart at temperatures near the upper lethal temperature, clearly shows

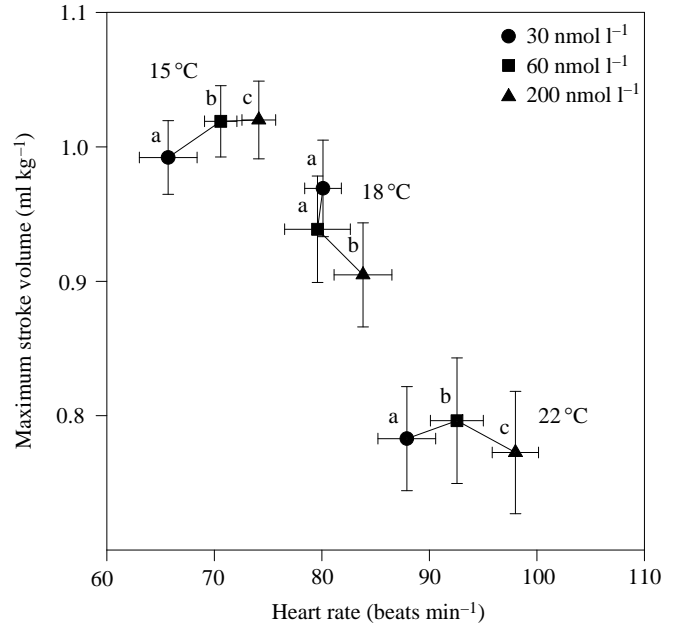


Fig. 5. The relationship between maximum stroke volume and heart rate for *in situ* perfused trout hearts at various temperatures and adrenaline concentrations. Values are expressed as means \pm 1 S.E.M. Dissimilar letters within temperature groups indicate significant differences ($P < 0.05$) between adrenaline concentrations. Means for each adrenaline concentration were significantly different between temperatures ($P < 0.05$).

that maximum cardiac output reaches a plateau at approximately 15 °C and that temperatures above 18 °C are associated with a reduced pressure-generating ability. This conclusion is consistent with *in vivo* measurements made on another salmonid, the sockeye salmon (*Oncorhynchus nerka*). Davis (1968) and Brett (1971) found that cardiac output in swimming sockeye salmon was essentially unchanged between 15 and 22 °C. In addition, they estimated that cardiac work during activity had a peak at 15 °C because ventral aortic blood pressure was lower at 22 °C than at 15 °C. If it is assumed that maximal prolonged swimming activity elicits a maximal

Table 2. A comparison of maximum cardiac performance variables at various temperatures using *in situ* heart preparations with intact pericardia and tonic (5–30 nmol l^{-1} adrenaline) or maximal levels of adrenergic stimulation (values in parentheses)

Temperature (°C)	Heart rate (beats min^{-1})	Stroke volume (ml kg^{-1})	Cardiac output ($\text{ml min}^{-1} \text{kg}^{-1}$)	Power output (mW g^{-1})	Reference
8	52	0.96	50	6.1	Keen and Farrell (1994)
10	50	0.97	53	5.9	Farrell <i>et al.</i> (1988)
10	66	0.69	46	6.5	Milligan and Farrell (1991)
10	62 (73)	0.89 (0.86)	56 (63)	5.2 (6.9)	Farrell <i>et al.</i> (1991)
10 (TR)	66 (75)	1.05 (1.05)	67 (75)	6.7 (8.7)	Farrell <i>et al.</i> (1991)
15	70 (82)	0.99 (1.02)	66 (76)	8.00 (9.3)	Present study
18	78	0.79	62	8.81	Keen and Farrell (1994)
18	79 (88)	0.97 (0.91)	78 (76)	9.3 (8.6)	Present study
22	84 (102)	0.81 (0.77)	71 (78)	7.2 (8.0)	Present study

TR indicates that this group of fish was exercise-trained for 1 month.

cardiac response in sockeye salmon, then the sockeye salmon's maximal *in vivo* cardiac performance peaks at its preferred temperature (12–14 °C; Brett, 1971) and well below its upper lethal limit (24 °C). We believe that a similar conclusion can be drawn for the rainbow trout, whose preferred temperature and upper lethal temperature are almost identical to those of the sockeye salmon (Black, 1953; Garside and Tait, 1958).

When using a perfused trout heart preparation without a coronary circulation, the exchange of gases and solutes between the myocardium and the perfusate will be affected. This is of particular concern in the present study for two reasons. First, the experimental conditions promoted cardiac failure and, second, any problems with diffusion may have been exacerbated in the hearts of the 22 °C fish because their ventricles were 30% larger than those of the 15 °C fish. Nonetheless, there are several important reasons why the absence of a coronary circulation was unlikely to bias the experiments towards the poorer cardiac performance observed at 22 °C. Foremost, there is good agreement between the cardiac performance measures for our perfused *in situ* hearts and reported *in vivo* values for swimming sockeye salmon (Davis, 1968; Brett, 1971). In both studies, heart rate increased at 22 °C while stroke volume, cardiac output, pressure generation and cardiac power output decreased. Second, by limiting fish size to less than 750 g and oxygenating the perfusate, we believe that any problem with oxygen diffusion into the myocardium was largely eliminated. The oxygen partial pressure gradient was at least 20 times that normally found in venous blood, and the thickness of the compact myocardium in our fish (<1 mm) was no more than that used in examining cardiac performance with electrically paced, isolated strips. Third, Davie and Farrell (1991) were unable to improve the performance of normoxic dogfish hearts by perfusing the coronary circulation with air-saturated saline. Although we may have eliminated the possibility of an oxygen limitation, there is an additional concern regarding solute transfer, particularly the removal of H⁺ and K⁺, which in themselves could reduce heart contractility. We know from previous studies that lactate and H⁺ are released into the lumen and can be measured in the perfusate leaving the trout heart (Farrell and Milligan, 1986). Therefore, transfers of solute from the trout myocardium are far from completely inhibited. If the larger hearts of the 22 °C group did lead to a poorer cardiac performance, we would predict a negative correlation between heart size and cardiac power output. However, no significant relationship exists between these two variables ($r^2=0.30$). In view of the above discussion, we feel confident in extrapolating our observations to the *in vivo* situation and in providing mechanistic explanations.

To illustrate the point that peak cardiac performance occurs at approximately 15 °C in rainbow trout, Table 2 summarizes the available data on maximum cardiac performance of perfused rainbow trout hearts at various temperatures. The data in Table 2 are comparable because they were collected in the same laboratory using the same type of heart preparation (i.e. an *in situ* heart with an intact pericardium) and an initial tonic

adrenergic stimulation (5–30 nmol l⁻¹ adrenaline). Table 2 clearly shows that the maximum stroke volume under conditions of tonic adrenergic stimulation occurs between 10 and 15 °C, whereas maximum cardiac output and maximum power output occur at 18 °C. Although these data suggest that maximum cardiac performance in rainbow trout occurs at 18 °C, it is unlikely that *in vivo* maximal cardiac performance is achieved without significant adrenergic stimulation (humoral and/or sympathetic). Under conditions of 'maximal' adrenergic stimulation, cardiac output remains constant between 10 and 22 °C, an effect which shifts the optimum temperature for maximum power output to 15 °C (Table 2; Fig. 3D). Because power output is the most appropriate index of integrated cardiac performance, it appears that maximum performance of rainbow trout hearts is achieved at 15 °C.

Temperature is generally regarded as having positive chronotropic and negative inotropic effects on the teleost myocardium (Lennard and Huddart, 1992; Matikainen and Vornanen, 1992). The present study supports this generalization with regard to both the chronotropic and inotropic effects of temperature. Matikainen and Vornanen (1992) nicely illustrated the simultaneous and opposing effects of temperature-related negative inotropy and positive chronotropy using isolated carp cardiac muscle. By deriving a maximum tissue pumping capacity term (the product of the spontaneous heart rate and the maximum isometric force; g mg⁻¹ tissue min⁻¹), they demonstrated a peak tissue pumping capacity at approximately 20 °C, a temperature well below the upper lethal temperature of carp (approximately 35 °C). The performance curve for isolated carp cardiac muscle strips had an inverted U shape as a function of temperature. Consequently, the decline in tissue pumping capacity of the carp heart at warm temperatures bears a striking resemblance to the decline in maximum power output in *in situ* rainbow trout hearts (see Table 2; see Fig. 6) and *in vivo* in sockeye salmon (Brett, 1971). In all three instances, there was a decrease in inotropic performance and/or decreased maximum stroke volume at higher temperatures.

Inotropic failure in our rainbow trout hearts at temperatures greater than 15 °C was demonstrated by lower values for maximum power output and maximum output pressure at 18 and 22 °C. In addition, the significance of this result is magnified when one considers that the failure of a number of preparations at these temperatures resulted in only the stronger hearts being tested (this bias may in fact explain why the homeometric regulation curves were similar for 15 and 22 °C hearts). The finding that rainbow trout hearts had a poorer pressure-generating ability at temperatures above 15 °C has indirect support from *in vivo* studies. For example, Davis (1968) reported reduced ventral and dorsal aortic blood pressures in swimming sockeye salmon at 22 °C compared with values at 15 °C, even though cardiac output was the same. Also, Wood *et al.* (1979) found a significant attenuation of the increase in dorsal aortic blood pressure in rainbow trout in response to adrenaline injections at 22 °C compared with 12 °C. Thus, in both of these studies, the heart performed less

pressure work at 22 °C. These *in vivo* observations could be related to increased temperature directly affecting the normal neural, hormonal and local control of vasomotor status in the systemic circulation (i.e. either a greater vasodilatory capacity or a weaker vasoconstrictory capacity). However, on the basis of the present observations, we can include another possibility. In response to a poorer cardiac pressure-generating ability at 22 °C, the vasomotor system may produce a vasodilatation to allow for the maintenance of cardiac output. Interestingly, Gamperl *et al.* (1994a) reported that adrenaline injection into rainbow trout resulted in significantly lower *in vivo* dorsal aortic pressures if the pericardium was opened. Opening the pericardium is known to cause poorer pressure generation in both the rainbow trout (Farrell *et al.* 1988) and the eel (*Anguilla dieffenbachii*) (Franklin and Davie, 1991), and reduces maximum power output in the rainbow trout heart by approximately 45 %.

Work on isolated cardiac muscle strips from teleost fish clearly shows that maximum tension decreases with increasing pacing frequencies, a negative staircase effect (Driedzic and Gesser, 1985; Vornanen, 1989; Bailey and Driedzic, 1990). It seems likely that this negative staircase effect would explain the negative inotropic effect of warm temperature in our hearts. Indeed, there is a decrease in force when the spontaneous beat frequency increases with temperature (Ask, 1983; Matikainen and Vornanen, 1992), and indications are that factors associated with either a shortening of the duration of the active state or a reduction in the intensity of the active state may become limiting at high beat frequencies (Vornanen, 1989; Driedzic and Gesser, 1994). The factors could include the inability of the contractile proteins to generate maximal force at shortened active states or impaired calcium delivery to and removal from the contractile proteins (Vornanen, 1989; Matikainen and Vornanen, 1992).

Nevertheless, alternative explanations for the reduction in maximum power output and maximum pressure-generating ability with increasing temperature should not be excluded at this time. For example, decreases in β -receptor number and/or affinity could have diminished the positive inotropic influence of adrenaline. In ventricular strips, Keen *et al.* (1993) showed that it takes approximately 10 times the adrenaline concentration at 18 °C to achieve the same level of tension generation measured at 8 °C, and that this effect was associated with fewer sarcolemmal β -adrenoreceptors. In addition, Ask *et al.* (1981), using atrial tissue, showed that the contractile force elicited by a maximally effective dose of adrenaline ($1.4 \mu\text{mol l}^{-1}$) at 14 °C was only 30 % of that produced at 2 °C. Although the observation that heart rate increased with increasing adrenaline concentration at all temperatures (Fig. 3A) is apparently inconsistent with diminished adrenergic influence at high temperatures, it must be remembered that positive chronotropy is mediated primarily through effects on the heart's pacemaker cells (Huang, 1973), whereas inotropic effects occur primarily because of adrenergic stimulation of the ventricle. Thus, there could be differential temperature effects on adrenergic sensitivity for these two regions of the heart.

It is clear from the present study that the application of Q_{10} values to maximum cardiac output has a limited value. At temperatures above the preferred temperature, Q_{10} values could be misleading because of the plateau in maximum cardiac output. Furthermore, because temperatures above 15 °C are associated with increased heart rates but constant cardiac output and falling power outputs, heart rate must be considered to be a very poor predictor of cardiac performance at these temperatures.

Maximum stroke volume decreased with increasing temperature (Fig. 3B). Previous studies with *in situ* trout hearts have also reported that maximum stroke volume decreased with increasing temperature (Graham and Farrell, 1990; Keen and Farrell, 1994). Likewise, Yamamitsu and Itazawa (1990) showed that stroke volume decreased with increasing temperature in the isolated carp heart, although it is unlikely that they measured maximum performance. The data presented in Table 2 suggest that maximum stroke volume of the *in situ* rainbow trout heart (approximately 1 ml kg^{-1}) occurs at temperatures of 15 °C and below. In a heart preparation with a punctured pericardium, Graham and Farrell (1990) showed that stroke volume decreased from 1 ml kg^{-1} at 5 °C to 0.7 ml kg^{-1} at 15 °C. Because stroke volume in our *in situ* heart with an intact pericardium was still 1 ml kg^{-1} at 15 °C, it is possible that the pericardium plays an important role in maintaining maximum stroke volume at warm temperatures.

Heart rate clearly had an important influence on maximum stroke volume (Fig. 5). This agrees with numerous previous studies where temperature-induced decreases in stroke volume were associated with concomitant increases in heart rate (Graham and Farrell, 1989; Lennard and Huddart, 1992; Keen and Farrell, 1994). There are two possible explanations for this inverse relationship between heart rate and maximum stroke volume: either a limitation on cardiac filling or the negative staircase effect on cardiac contractility referred to above. To what degree these two factors influence stroke volume at higher heart rates can be resolved only by direct measurements of heart chamber volumes during the cardiac cycle. Using echocardiography, Franklin and Davie (1992) showed that ventricular end-systolic volume in rainbow trout is normally near zero. Therefore, if a negative staircase effect is involved in the reduced stroke volume at high heart rates, end-systolic volume would be found to increase. In contrast, a lower end-diastolic volume would account for the decrease in stroke volume if filling time was a problem, as suggested by Farrell *et al.* (1989) to explain a decrease in maximum stroke volume of 0.2 ml kg^{-1} when isolated trout hearts were paced at 60 beats min^{-1} . One piece of evidence which suggests that limitations on cardiac filling may contribute to the decrease in stroke volume at high heart rates comes from studies on *in situ* hearts with intact (present study) *versus* punctured pericardium (Graham and Farrell, 1990). Stroke volume decreased by 0.3 ml kg^{-1} (from 1 ml kg^{-1}) when intrinsic heart rate reached 60 beats min^{-1} in hearts with a punctured pericardium. In contrast, hearts with an intact pericardium were able to maintain maximum stroke volume until heart rate exceeded

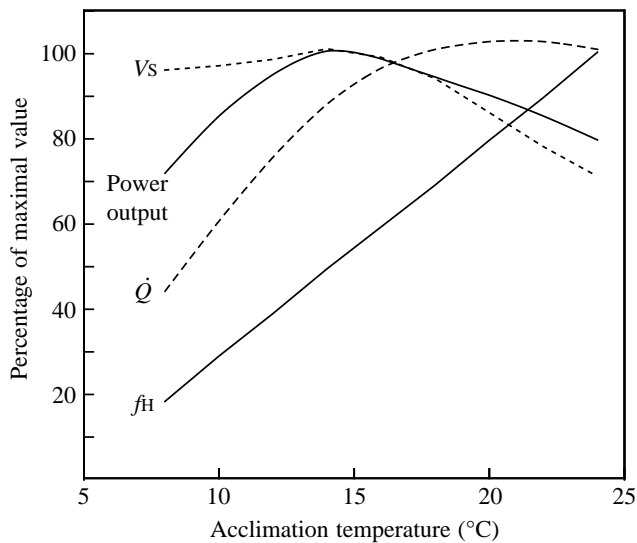


Fig. 6. Proposed relationship between acclimation temperature and the maximal level of cardiovascular variables for the rainbow trout. The 100% values for stroke volume (V_s), heart rate (f_H), power output and cardiac output (\dot{Q}) are 1 ml kg^{-1} , $120 \text{ beats min}^{-1}$, 9.5 mW g^{-1} and $80 \text{ ml min}^{-1} \text{ kg}^{-1}$, respectively.

$80 \text{ beats min}^{-1}$. A mechanistic explanation for the enhanced maintenance of maximum stroke volume in hearts with an intact pericardium is that the pericardium in rainbow trout permits *vis-a-fronte* filling of the heart (Farrell *et al.* 1988) and this type of cardiac filling is likely to be faster than *vis-a-tergo* filling (Farrell and Jones, 1992).

Fig. 6, while somewhat speculative at this time, summarizes our ideas on cardiac performance in rainbow trout as a function of acclimation temperature. We hope that it will provide a useful framework for further research in this area. Heart rate follows a Q_{10} relationship up to the upper lethal temperature, where it reaches its maximum level of approximately $120 \text{ beats min}^{-1}$. Maximum stroke volume (approximately 1 ml kg^{-1}) is maintained up to the preferred temperature, above which it decreases. For several degrees above the preferred temperature, the decrease in stroke volume is matched by the increase in heart rate. Consequently, maximum cardiac output (approximately $80 \text{ ml min}^{-1} \text{ kg}^{-1}$) has a broad plateau extending for several degrees higher than the preferred temperature. In contrast, the pressure-generating ability of the heart decreases at temperatures higher than the preferred temperature such that peak maximum power output (around 9.5 mW g^{-1}) occurs around the preferred temperature.

Mechanistic explanations for the decline in maximum performance above the preferred temperature require further work at both the organ and tissue level. However, the observation that maximum stroke volume was not maintained at high temperatures suggests that myocardial adaptations are quite limited above the preferred temperature. This is not the case at colder temperatures. For example, cold acclimation results in a larger cardiac mass (Graham and Farrell, 1989) and a greater number of sarcolemmal adrenoceptors that increase

the sensitivity of the trout heart to adrenaline (Keen *et al.* 1993). As a result of cold-acclimation, maximum stroke volume and power output tend to be higher than otherwise possible with the accompanying temperature-dependent decrease in heart rate.

Whether any of the above generalizations apply to other teleost species, such as sockeye salmon and carp, remains to be determined. However, it is clear for the rainbow trout (1) that maximum cardiac performance declines at temperatures above the preferred temperature; (2) that the usefulness of Q_{10} relationships for cardiac functions other than heart rate is highly dependent upon the section of the thermal regime of the fish under consideration; and (3) that heart rate is a poor indicator of integrated cardiac function at temperatures above the preferred temperature.

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