The Journal of Experimental Biology 201, 2701–2710 (1998)
Printed in Great Britain © The Company of Biologists Limited 1998
JEB1468

EFFECTS OF TEMPERATURE, ADRENALINE AND RYANODINE ON POWER PRODUCTION IN RAINBOW TROUT *ONCORHYNCHUS MYKISS* VENTRICULAR TRABECULAE

H. A. SHIELS[1,*], E. D. STEVENS[2] AND A. P. FARRELL[1]

[1]*Department of Biological Sciences, Simon Fraser University, Burnaby, British Columbia, Canada V5A 1S6* and [2]*Department of Zoology, University of Guelph, Guelph, Ontario, Canada N1G 2W1*

*e-mail: hollys@sfu.ca

Accepted 10 July; published on WWW 10 September 1998

Summary

This study is the first to examine the contractility of teleost ventricular muscle in an oscillating muscle preparation. The experiments were designed to test the relative importance of Ca^{2+} released from the sarcoplasmic reticulum (SR) and Ca^{2+} influx across the sarcolemma (SL) to cardiac performance in rainbow trout *Oncorhynchus mykiss*. Adrenaline and ryanodine were used to modulate Ca^{2+} flux through the SL and SR, respectively. Experiments were conducted at two temperatures (12 °C and 22 °C) (1) to investigate the effect of an acute temperature change (from 12 °C to 22 °C) on power production, and (2) to test the effects of acute temperature change on the relative contributions of the SR and SL Ca^{2+} flux to power production. Concordant with isometric studies, the results showed that trans-sarcolemmal influx was the major source of Ca^{2+} (approximately 90 %) for cardiac power production at all temperatures. This SL Ca^{2+} influx was increased with adrenergic stimulation. The power curves generated in this study suggest an optimum frequency for power production of approximately 1.0 Hz at 12 °C, which corresponds well to typical *in vivo* heart rates for rainbow trout at that temperature. Further, this study indicated that temperature-induced changes in power output cannot be predicted from temperature-induced changes in isometric tension because the temperature-sensitivity of work and power proved to be greater than that of isometric tension. This finding is important because many previous studies have assessed cardiac contractility using only isometric tension.

Key words: *Oncorhynchus mykiss*, rainbow trout, work loop, ventricle, temperature, adrenaline, ryanodine, isometric tension, work, power.

Introduction

The intracellular Ca^{2+} stored in the sarcoplasmic reticulum (SR) is the major source of Ca^{2+} for myofilament activation in mammalian cardiac muscle. However, despite this central role, there exists a graded dependence on SR Ca^{2+} release among different species, during development and regionally within the heart. For example, adult rabbit ventricle is less dependent on SR Ca^{2+} release than that of adult rat (Bers, 1991); within a species, neonate hearts are less dependent on SR Ca^{2+} than adult hearts, and the atrium is more SR-dependent than the ventricle (Bers, 1991). In fish, however, most studies examining isometric tension at physiologically relevant pacing frequencies have supported the view that SR Ca^{2+} release is a relatively unimportant source of Ca^{2+} for contraction (rainbow trout *Oncorhynchus mykiss*, Driedzic and Gesser, 1988; El-Sayed and Gesser, 1989; Hove-Madsen and Gesser, 1989; Hove-Madsen, 1992; Møller-Nielsen and Gesser, 1992; Keen *et al.* 1994; Shiels and Farrell, 1997; carp *Carassius carassius*, Vornanen, 1994, 1996). It is not known for fish whether this conclusion holds true for cardiac muscle that is both shortening and developing tension, as it does *in vivo*. Unlike the isometric muscle preparation, the work loop technique (Josephson, 1985) imposes cyclic length changes, and the muscle is activated to contract while it shortens, so that it does work. Power is calculated as the product of net work per cycle and cycle frequency. This technique is now routinely used with skeletal muscle (Stevens, 1996) and has also been successfully applied to cardiac tissue in mammals (Layland *et al.* 1995*a*,*b*) and amphibians (Syme, 1993; Syme and Josephson, 1995) but not to fish. Since measures of work or power should have more functional significance than measures of isometric tension, the purpose of the present experiments was to measure the relative Ca^{2+} contribution from the SR and the SL to power production in trout ventricular tissue using work loops.

A major tool used to investigate the relative roles of SR and SL in cardiac muscle is the neutral plant alkaloid ryanodine, which binds specifically and irreversibly to the SR Ca^{2+}-release channel (Rousseau *et al.* 1987). At nanomolar concentrations, ryanodine opens the SR Ca^{2+}-release channel

to a subconducting state, whereas at higher concentrations (>10 μmol l^{-1}) it completely blocks the channel, inhibiting Ca^{2+} release from the SR (Sitsapesan *et al.* 1991). Thus, in adult mammalian cardiac muscle, which relies primarily on SR Ca^{2+} release for activation of the myofilaments, ryanodine application causes a reduction in tension production varying from 20 to 90 %, depending on species. Although ryanodine generally has a small effect on fish ventricular strips (Driedzic and Gesser, 1988; Hove-Madsen and Gesser, 1989; Vornanen, 1996) and trout *in situ* perfused hearts (Keen *et al.* 1994), it is important to reconsider these responses in the light of the temperature-dependence of the SR Ca^{2+}-release channel. Mammalian studies have demonstrated an increase in the open probability of the SR Ca^{2+}-release channel with acute decreases in temperature (Bers, 1987, 1989; Sitsapesan *et al.* 1991). Thus, the unresponsiveness of fish hearts to ryanodine may reflect the fact that most studies are conducted at low temperatures (5–15 °C) where there may be cold-induced opening of the SR Ca^{2+}-release channel, rendering the SR ineffective in sequestering Ca^{2+} (Tibbits *et al.* 1991, 1992). Consistent with this suggestion, ryanodine reduced rest potentiation by more than 50 % in rainbow trout ventricle at 15 °C and 25 °C (Hove-Madsen, 1992) and reduced isometric tension at physiologically relevant contraction frequencies at temperatures exceeding 20 °C in both skipjack tuna *Katsuwonus pelamis* (25 °C) (Keen *et al.* 1992) and rainbow trout (22 °C) (Shiels and Farrell, 1997). In view of this temperature-dependence, the present experiments on the relative contributions of SR and SL Ca^{2+} to power production were conducted at both 12 °C and 22 °C. Furthermore, because previous studies have demonstrated the effects of ryanodine to be dependent on pacing frequency (Møller-Nielsen and Gesser, 1992; Keen *et al.* 1994; Shiels and Farrell, 1997) and on the level of adrenergic modulation (Shiels and Farrell, 1997), we studied power generation at different cycle frequencies and at two adrenaline concentrations.

Materials and methods

Fish origin and maintenance

Four-year-old rainbow trout *Oncorhynchus mykiss* (Walbaum) (approximately 2.5 kg body mass) of both sexes were obtained from Alma Aquaculture Research Station, Alma, Ontario, Canada, and held in aerated, dechlorinated tap water. Fish were acclimated to 12 °C for a minimum of 2 weeks prior to experimentation. They did not feed because they had spawned within the past month, resulting in a negative energy balance. We do not believe that this compromised cardiac performance as the salmonid life style involves a major migration under a negative energy balance.

Tissue preparation

Fish were killed with a blow to the head, and the heart was removed and placed in ice-cooled saline. Ventricular trabeculae were dissected from the lumen of the ventricle. Mean trabecular length was 3.0±0.12 mm and mean trabecular wet mass was 6.0±1.0 mg (mean ± S.E.M., N=24). One end of the muscle was attached to the movable arm of an ergometer (Cambridge Technology) and the other end was tied to the fixed arm of an isometric force transducer (Harvard Apparatus) using 6-0 surgical silk. The muscle was lowered into an oxygenated, water-jacketed organ bath superfused with physiological saline containing (in mmol l^{-1}): Na^+, 136.8; K^+, 3.08; Ca^{2+}, 2.52; Mg^{2+}, 0.93; Cl^-, 129.7; SO_4^{2-}, 0.93; HCO_3^-, 2.7; Tes acid 3.49; and Tes base 6.37. Sodium pyruvate (10 mmol l^{-1}) was used as an exogenous substrate to fuel oxidative phosphorylation (Layland *et al.* 1995*a*,*b*; Franklin and Johnston, 1997; Altringham and Block, 1997). In a recent study, Xu *et al.* (1995) found that the SR Ca^{2+}-ATPase in SR vesicles isolated from rabbit hearts preferred ATP derived from glycolysis than from oxidative phosphorylation. We do not feel, however, that our use of pyruvate, which bypasses the glycolytic machinery, compromised SR function in our experiments. This is because the lack of glycolytic-derived ATP only influenced Ca^{2+}-pumping ability under mitochondrial-derived ATP-limiting conditions (Xu *et al.* 1995) whereas, in our study, an ample supply of ATP should be ensured through oxidative phosphorylation. Extracellular pH was 7.58. Experimental temperature was held at either 12 ° or 22 °C. Since the fish were acclimated to 12 °C, the experimental temperature of 22 °C represented an acute temperature change, employed to examine the temperature-dependency of the SR Ca^{2+}-release channel. Previous fish studies (Keen *et al.* 1994; Shiels and Farrell, 1997) have shown that an acute temperature change is more effective than temperature acclimation in invoking SR involvement during force development. A temperature of 22 °C was selected to be as high as possible to induce SR Ca^{2+} involvement, while keeping within the upper tolerance limits for this species (23–25 °C; Black, 1953).

Oscillatory measurements

The muscle was stimulated using platinum plate electrodes at a stimulus voltage (approximately 5 V) adjusted to 1.5 times that required to produce maximal isometric twitch force. Each stimulus train consisted of 16 pulses at a pulse interval of 2 ms and with a pulse width of 1 ms. Stimulus train duration was 31 ms. A train of true bipolar pulses rather than a single long direct current pulse was used to prevent oxidation at the stimulating electrodes. Muscle length was adjusted so that work per cycle at 0.2 Hz was optimized; this length was designated as L_0. This initial length optimization procedure took up to 2 h as it was necessary to leave the muscle at each new length for approximately 15 min to stabilize; during this time, the muscle was continuously stimulated at 0.2 Hz (i.e. 12 contractions min^{-1}).

To measure work and power, a sinusoidal signal was used to cycle muscle length about L_0. The amplitude of the length change cycle (i.e. strain or excursion amplitude) was approximately 0.1 mm for all trials. Strain (excursion amplitude expressed as a fraction of muscle length) was approximately 0.03 (3 %) and was chosen on the basis of

preliminary tests with different strain amplitudes. Trout trabeculae seemed to be stiffer than frog and rat heart, and produced maximum power at lower strains. The strain used in our study (3 %) was less than that used by Layland *et al.* (1995*a*,*b*) for rat heart muscle (10 %) and within the range of strains used by Syme and Josephson (1995) for frog heart (1.5 %, 3 % and 9 %). The cycle frequency refers to the frequency of the imposed length change and was varied from 0.2 Hz to 2.0 Hz in increments of 0.2 Hz. The phase of stimulation refers to the timing of the start of the stimulus train compared with the start of the imposed length change. Phase of stimulation is presented as a percentage of the length change cycle. Phases of stimulation that produced maximal power were different at different cycle frequencies; the tissue had to be stimulated earlier in the length cycle when cycled faster. For a frequency of 0.2 Hz, the phase of stimulation was 35 %, for 0.4 Hz it was 23 %, for 0.6 Hz it was 16 %, for 0.8 Hz it was 11 %, for 1.0 Hz it was 7 %, for 1.2 Hz it was 5 %, for 1.4 Hz it was 3 %, for 1.6 Hz it was 2 %, for 1.8 Hz it was 1 % and for 2.0 Hz it was 0 %. The muscle was stimulated continuously throughout the experiment and was oscillated continuously except at the end of each cycle frequency, where it was subjected to two isometric contractions before cycle frequency was increased to the next level (see below). To ensure that the work loops were stable, they were measured at the end of each cycle frequency, before the muscle was contracted isometrically.

Displacement refers to the change in muscle length and was measured with the ergometer. Force was measured with the isometric transducer. Force and displacement were recorded using a Nicolet digital storage oscilloscope. Work is the product of force and displacement and was calculated by integrating the force–displacement curve. Positive work is the work done by the muscle during shortening, and negative work is the work done on the muscle to lengthen it. Net work is the difference between positive and negative work.

Experimental protocols

The muscle was stabilized at L_0 for 30 min under basal (0.2 Hz) stimulation before being subjected to one of two experimental protocols. For both protocols, the first force–frequency experiment served as a control for subsequent measurements. Work and tension production at 0.2 Hz were compared before and after the first force–frequency trial; if measurements were appreciably different, the preparation was discarded. For the first force–frequency experiment, saline containing 10 nmol l^{-1} adrenaline was added to the organ bath, and measurements of both work and isometric tension were made at a series of cycle frequencies starting at 0.2 Hz and ending at 2.0 Hz. After each change in cycle frequency, the muscle was allowed to stabilize (for 1–2 min) before making new tension and work measurements. Subsequently, for protocol 1, the control saline was replaced with one containing 10 μmol l^{-1} ryanodine and 10 nmol l^{-1} adrenaline. The muscle was incubated for either 30 min at 22 °C or 45 min at 12 °C before work and isometric tension were re-measured at each cycle frequency. The saline was then replaced with one containing 10 μmol l^{-1} ryanodine and 10 μmol l^{-1} adrenaline, and work and tension were re-measured at each cycle frequency after the preparation had stabilized (approximately 10 min). The purpose of protocol 1 was to observe the effects of ryanodine under tonic adrenergic stimulation and also to test whether these effects could be overcome by increasing Ca^{2+} influx across the SL *via* maximal adrenergic stimulation.

The purpose of protocol 2 was to observe maximal adrenergic stimulation of Ca^{2+} influx across the SL and to determine whether ryanodine effects could be detected at high adrenaline levels. To do this, after the first force–frequency experiment, the control saline solution was replaced with one containing 10 μmol l^{-1} adrenaline, and work and tension were measured at each cycle frequency after the preparation had stabilized (approximately 10 min). The saline was then replaced with one containing 10 μmol l^{-1} ryanodine and 10 μmol l^{-1} adrenaline. After 30 or 45 min of incubation (depending on test temperature as indicated above), work and tension were re-measured at each cycle frequency.

Because adrenaline is present at nanomolar concentrations in the circulation of resting fish (Milligan *et al.* 1989) and adrenaline has been shown to preserve cardiac tonus *in situ* (Graham and Farrell, 1989; Davie and Farrell, 1991) and *in vitro* (Shiels and Farrell, 1997), we utilized a tonic level (10 nmol l^{-1}) of adrenergic stimulation in all experiments. Further, as circulating levels of adrenaline increase drastically in response to stress (McDonald and Milligan, 1992), we employed a maximal level of adrenergic stimulation (10 μmol l^{-1}) to observe its effect on the relative importance of SR and SL Ca^{2+} flux to power generation.

In a separate experiment, the effects of an incremental acute temperature change were studied by making measurements on the same preparation at 12, 14, 16, 18, 20 and 22 °C. This muscle was stimulated with 10 μmol l^{-1} adrenaline to maximize performance and stimulated at 0.4, 0.6, 0.8, 1.0, 1.2 and 1.4 Hz. The muscle was left at each temperature for 20 min to stabilize before making work and tension measurements.

Chemicals, calculations and statistical analyses

All chemicals were purchased from either Sigma (St Louis, MO, USA) or BHD (Toronto, Ontario, Canada), with the exception of ryanodine which was purchased from Calbiochem (San Diego, CA, USA). Force (tension) is expressed as mN mm^{-2}. Mean cross-sectional area was calculated using wet muscle mass, trabecular length and an assumed muscle density of 1.06 g cm^{-3} (Layland *et al.* 1995*a*). For each fish (N=6), the results for each frequency within the physiological range (indicated on the figures and in the Results) were averaged. Significant increases after adrenaline stimulation and significant decreases after ryanodine incubation were assessed for the results averaged over the physiologically relevant frequency range for each temperature using one-way Student's t-tests (P<0.05). Significant differences are indicated in the text and in Table 1.

Results

Control (10 nmol l^{-1} adrenaline) trials: the effects of cycle frequency

Results for peak isometric tension during contraction are presented in Figs 1A,B, 2A,B. A negative force–frequency relationship, typical of teleost cardiac muscle (Driedzic and Gesser, 1988; Hove-Madsen, 1992; Matikainen and Vornanen, 1992), was obtained when cycle frequency was increased from 0.2 Hz until contractions became irregular (between 1.6 and 2.0 Hz, depending on the test temperature). Peak tension decreased significantly by approximately 75 % over the entire frequency range at both temperatures (the entire range of test frequencies is not shown in Figs 1–3, since only test frequencies near the physiologically relevant frequency range for each temperature are shown). The rates of contraction and relaxation increased (i.e. became faster) at higher frequencies at both 12 °C (Fig. 3) and 22 °C (data not shown).

The results for work per contraction cycle are presented in Figs 1C,D, 2C,D. At 12 °C, work decreased significantly with increasing cycle frequency (Fig. 1C,D). However, at 22 °C, work was significantly (P=0.04) lower than at 12 °C and did not change significantly with cycle frequency (Fig. 2C,D). At 22 °C, seven out of twelve preparations produced zero or

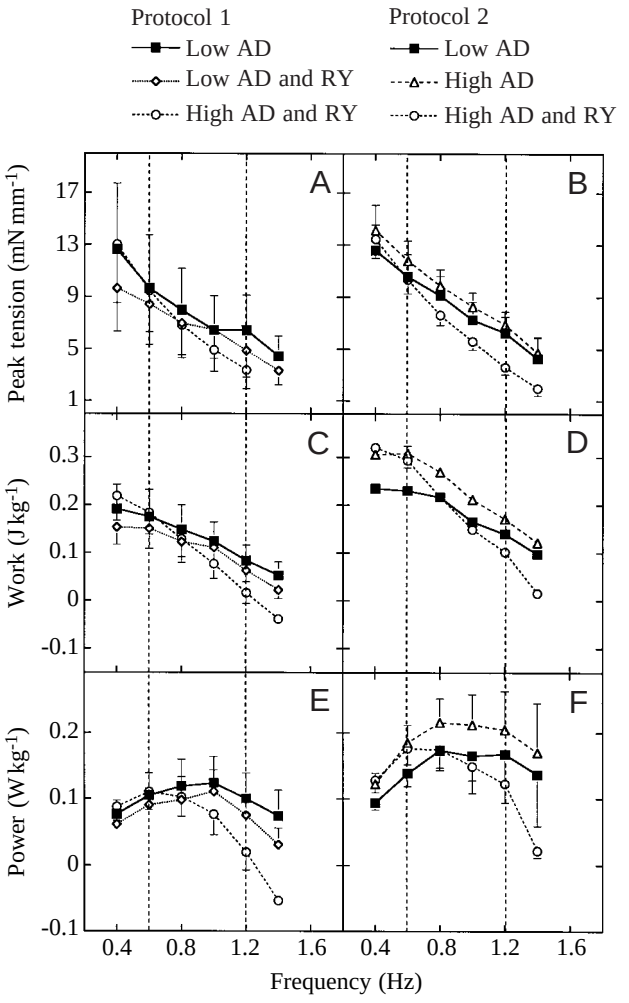

Fig. 1. The effects of ryanodine (RY) and adrenaline (AD) on the tension, work and power developed by trabecular muscle from trout ventricle tested at 12 °C. For each treatment, the muscle was paced at a series of frequencies starting at 0.2 Hz, and frequency was increased until contractions became irregular. Only frequencies near the physiological range are given on the figure. The physiologically relevant contraction frequencies for 12 °C (from 0.6–1.2 Hz) are within the vertical broken lines, which demarcate typical minimum and maximum *in vivo* heart rates for rainbow trout at 12 °C obtained from the literature (Kiceniuk and Jones, 1977; Wood *et al.* 1979; Gamperl *et al.* 1995; Farrell *et al.* 1996). The left and right panels are from protocols 1 and 2, respectively, and are independent trials using trabeculae from different fish (see Materials and methods). All points are means ± S.E.M., N=6.

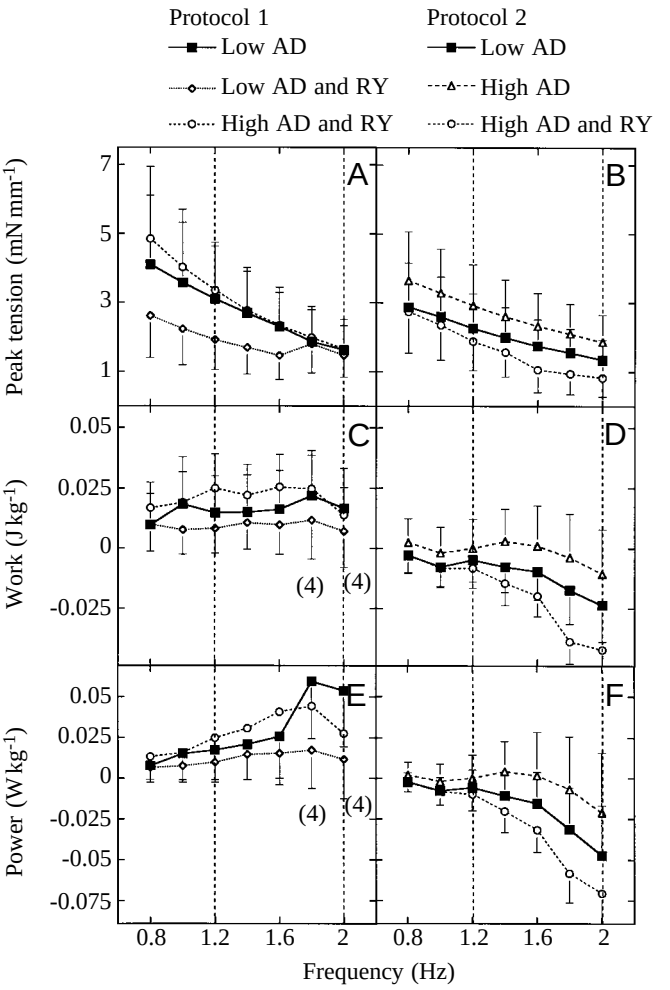

Fig. 2. The effects of ryanodine (RY) and adrenaline (AD) on the tension, work and power developed by trabecular muscle from trout ventricle tested at 22 °C. The physiologically relevant contraction frequencies for 22 °C (from 1.2 to 2.0 Hz) occur within the vertical lines, which demarcate typical minimum and maximum *in vivo* heart rates for rainbow trout at 12 °C obtained from the literature (Kiceniuk and Jones, 1977; Wood *et al.* 1979; Gamperl *et al.* 1995; Farrell *et al.* 1996). Other details as in Fig. 1. N=6 except where indicated in parentheses.

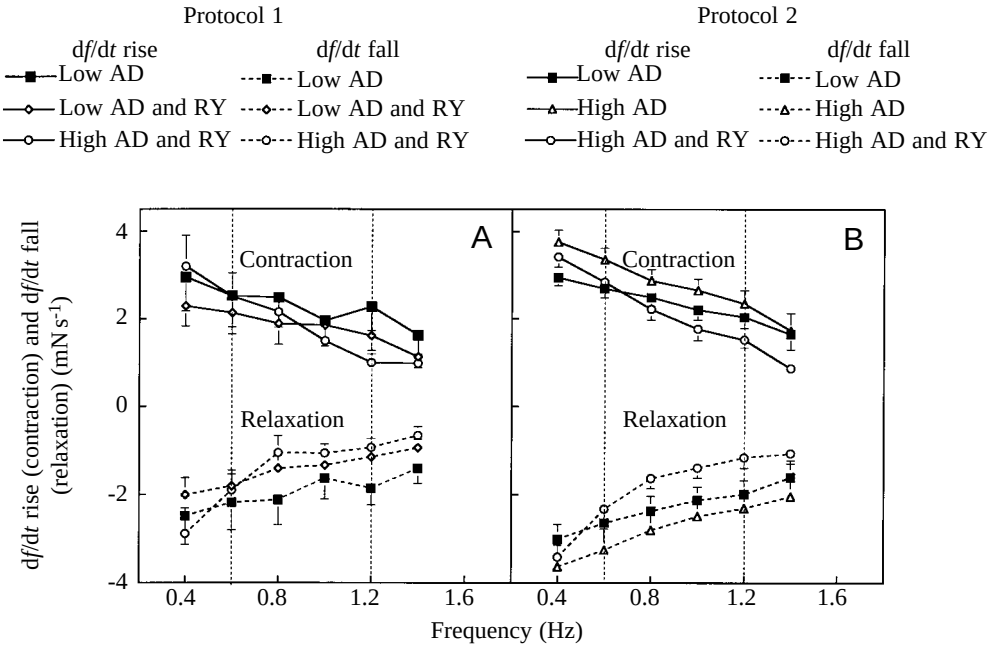

Fig. 3. The effects of ryanodine (RY) and adrenaline (AD) on the rate of development (d*f*/d*t* rise) and the rate of relaxation (d*f*/d*t* fall) of tension in trabecular muscle from trout ventricle tested at 12 °C. *N*=6 for all points. Other details are as in Fig. 1.

negative net work over the physiological range of frequencies.

The results for power output (the product of work per cycle and cycle frequency) are presented in Figs 1E,F, 2E,F. At 12 °C, power was independent of cycle frequency over the physiological range of heart rates for rainbow trout at this temperature. Outside the range of *in vivo* heart rates, power decreased significantly with increasing cycle frequency (high frequencies are not shown in figure). The apex of the power curve indicates an optimum frequency for power production of approximately 1.0 Hz at 12 °C for rainbow trout ventricle. Power output at 22 °C was significantly (P=0.03) lower than that observed at 12 °C because the majority of preparations produced either zero or negative net work at 22 °C (Fig. 2C,D). Power output was independent of cycle frequency over the physiological range of pacing frequencies.

High adrenaline concentration

The effects of 10 μmol l^{-1} adrenaline on tension, work and power were revealed in protocol 2 (see right-hand panels in Figs 1–3). At 12 °C, 10 μmol l^{-1} adrenaline significantly increased work by 21 %, and power by 20 %, compared with the first force–frequency trial (Table 1). Peak tension increased by approximately 10 % with 10 μmol l^{-1} adrenaline stimulation, but this difference was not significant (P=0.08). High adrenaline concentration significantly increased the rate of tension development (19 %) and the rate of relaxation (19 %) (Table 1). At 22 °C, 10 μmol l^{-1} adrenaline did not significantly affect any of the variables over the physiological range in protocol 2 (Table 1).

Ryanodine and low adrenaline concentration

Protocol 1 examined the effect of blocking SR Ca^{2+} release using ryanodine in the presence of 10 nmol l^{-1} adrenaline (see left-hand panels in Figs 1–3). At 12 °C, there were no significant effects of ryanodine. Thus, in an oscillating trout ventricular muscle preparation at 12 °C, under tonic (10 nmol l^{-1}) adrenergic modulation, SR Ca^{2+} does not significantly contribute to the total free Ca^{2+} available for interaction with the myofilaments. At 22 °C, ryanodine significantly reduced tension (36 %) and rates of contraction (38 %) and relaxation (50 %) (Table 1), indicating greater SR involvement during tension development at 22 °C.

Ryanodine and high adrenaline concentration

The combined effects of adrenaline and ryanodine were examined in both protocols 1 and 2; however, the order of drug application differed between protocols. In protocol 1, when a high (10 μmol l^{-1}) level of adrenaline was applied to muscle pre-treated with ryanodine there were no significant effects at 12 °C. However, at 22 °C, work, power and the contraction and relaxation rates were increased significantly by application of 10 μmol l^{-1} adrenaline after ryanodine pre-treatment (Fig. 2A,C,E; Table 1). We have no explanation for this discrepancy between temperatures. It is important to note that, despite this adrenergically stimulated increase in work and power, work and power at 22 °C still remained less than 50 % of those observed at 12 °C (Table 1). When ryanodine was applied during maximal adrenergic stimulation in protocol 2, all variables decreased significantly at 12 °C and 22 °C (with the exception of work and power at 22 °C, where P=0.06).

Effect of temperature

The most notable effect of temperature was a substantial reduction in cardiac performance at 22 °C (i.e. significantly lower tension, work and power). To elucidate better the effects of acute temperature change, we conducted a single experiment with one important methodological difference

Table 1. *Effects of adrenaline and ryanodine on tension, work, power and rates of contraction and relaxation of trout ventricular trabeculae at 12 °C and 22 °C*

	Tension ($mN\,mm^{-2}$)	Work ($J\,kg^{-1}$)	Power ($W\,kg^{-1}$)	d*f*/d*t* rise ($mN\,s^{-1}$)	d*f*/d*t* fall ($mN\,s^{-1}$)
12 °C, protocol 1					
Low AD (control)	7.35±1.50	0.132±0.04	0.117±0.03	2.16±0.29	–1.76±0.19
Low AD and RY	5.63±1.27	0.104±0.03	0.080±0.02	1.76±0.19	–1.37±0.19
High AD and RY	6.33±1.23	0.107±0.03	0.081±0.02	1.86±0.29	–1.27±0.19
12 °C, protocol 2					
Low AD (control)	8.40±0.80	0.189±0.03	0.159±0.03	2.35±0.1	–2.25±0.1
High AD	9.32±0.70	0.242±0.04†	0.203±0.03†	2.94±0.19†	–2.74±0.19†
High AD and RY	7.66±0.67*	0.208±0.03*	0.159±0.02*	2.16±0.29*	–1.66±0.19*
22 °C, protocol 1					
Low AD (control)	2.38±0.55	0.014±0.01	0.025±0.02	1.27±0.19	–0.98±0.1
Low AD and RY	1.51±0.32*	0.008±0.01	0.013±0.01	0.78±0.1*	–0.49±0.1*
High AD and RY	2.41±0.43	0.021±0.01†	0.033±0.02†	1.37±0.1†	–1.08±0.1†
22 °C, protocol 2					
Low AD (control)	1.78±0.34	–0.013±0.01	–0.022±0.01	1.27±0.1	–1.08±0.1
High AD	2.37±0.41	–0.002±0.01	–0.004±0.02	1.66±0.1	–1.47±0.1
High AD and RY	1.46±0.31*	–0.021±0.01	–0.038±0.01	0.88±0.1*	–0.59±0.1*

Tension, work, power and rates of contraction (d*f*/d*t* rise) and relaxation (d*f*/d*t* fall) averaged over physiologically relevant frequencies for the two independent protocols at 12 °C and 22 °C. Each value is the average of results from all the relevant frequencies (for 12 °C, average of results from 0.6, 0.8, 1.0 and 1.2 Hz; for 22 °C, average of results from 1.2, 1.4, 1.6, 1.8 and 2.0 Hz) from N=6 fish at each frequency.

* denotes a significant decrease with ryanodine treatment (RY); † denotes a significant increase with a high concentration (10 $\mu mol\,l^{-1}$) of adrenaline (AD) using one-way Student's *t*-tests.

Low AD, 10 $nmol\,l^{-1}$.

Values are means ± S.E.M.

from the previous series of experiments; the acute temperature change was more gradual (from 12 °C to 22 °C in increments of 2 °C). The consequence of gradual temperature increases on work loops (Fig. 4) and peak isometric tension (vertical line at L_0 in Fig. 4) at three cycle frequencies was examined. The results show that tension was not especially sensitive to temperature at any cycle frequency, but was highest at 14 °C (Fig. 5A). As expected, however, work (Fig. 5B) and power (Fig. 5C) decreased several-fold with an acute increase in temperature above 14 °C, primarily as a result of faster relaxation rates (see Fig. 4). At 1.0 Hz and 1.4 Hz, power and work appeared to be maximal at 14 °C, providing a clearer prediction of optimal temperature than isometric tension. Power was greatest at the highest cycle frequency (1.4 Hz), unlike work and tension, which were greatest at the lowest cycle frequency (0.4 Hz).

Discussion

Relative Ca^{2+} contribution from the SR and SL

The present experiments used ryanodine to estimate the relative importance of SR and SL Ca^{2+} flux to power generation in trout ventricle at 12 °C and 22 °C. Our results suggest that, at both temperatures, the majority of the Ca^{2+} needed for activation of the myofilaments crosses the SL, with SR Ca^{2+} release playing a more modest role. These results are in agreement with studies examining the role of the SR in contributing Ca^{2+} to tension generation in trout cardiac muscle (see review by Tibbits *et al.* 1991; see also Hove-Madsen, 1992; Keen *et al.* 1994; Shiels and Farrell, 1997). At 12 °C, ryanodine application tends to shift the apex of the power curve to the left (Fig. 1E,F), suggesting that ryanodine affects the optimum frequency for power production such that, without SR Ca^{2+} release, power production is reduced at a given frequency. However, these results were not resolved statistically (Table 1). Further studies might be useful in clarifying these relationships. It was anticipated that SR Ca^{2+} involvement would be greater at 22 °C owing to the temperature-dependency of the SR Ca^{2+}-release channel. This would be true only from the perspective of a percentage change in tension, work and power after ryanodine treatment, measured in a muscle barely performing work. Indeed, work production was low at 22 °C, which resulted in low power production. Myocardial power output calculated from *in vivo* measurements from rainbow trout (11 °C) is 1.53 $W\,kg^{-1}$ (Jones and Randall, 1978). Our values for power output from the isolated muscle are lower by a factor of 10 (0.159 $W\,kg^{-1}$). This is probably a result of the low strains (3 %) applied to the muscle. Studies with isolated frog ventricular muscle have shown that, at cycle frequencies comparable with *in vivo* heart rates, lower strains result in lower work production. At 9 % strain, power output (2.3 $W\,kg^{-1}$; Syme and Josephson, 1995)

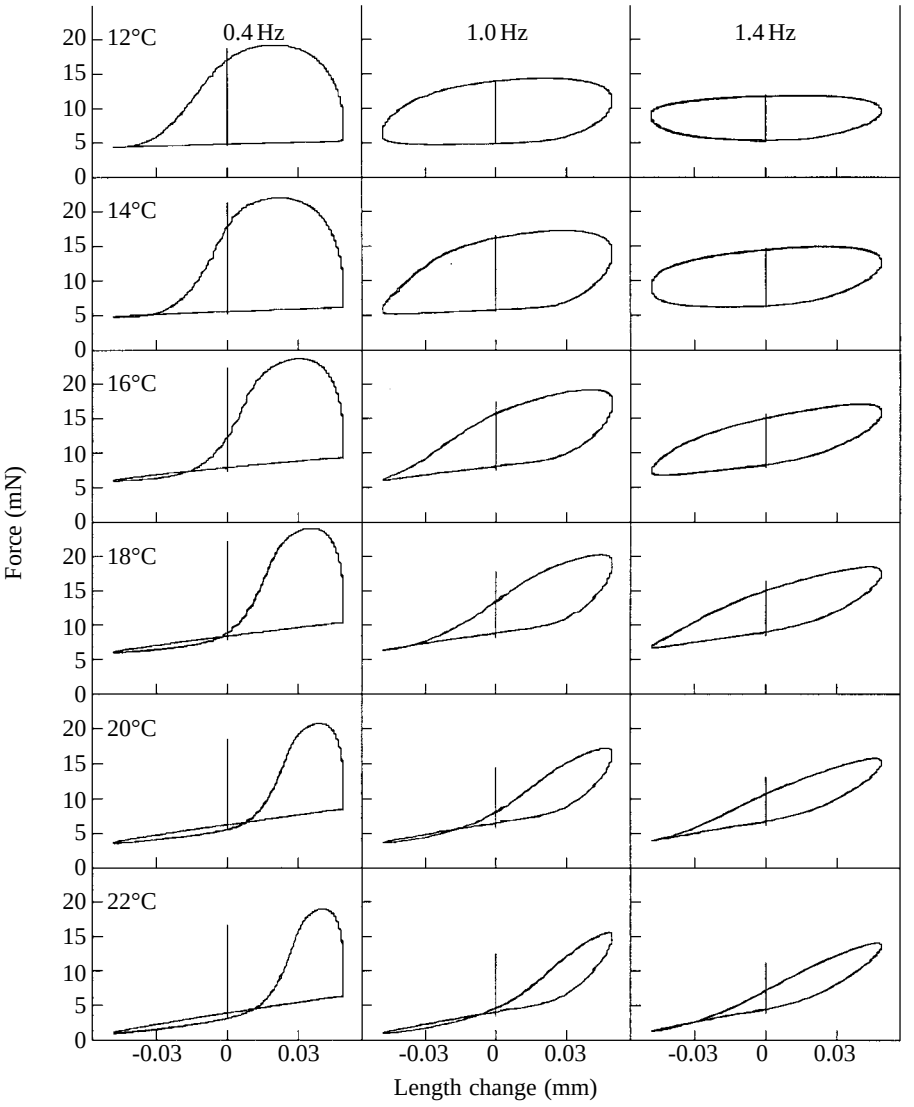

Fig. 4. Effect of temperature on work loops of trout ventricle trabecular strips under maximal ($10\,\mu mol\,l^{-1}$) adrenergic stimulation. Note the relative change in work at increasing temperatures and frequencies. Loops run counterclockwise. The area inside the loop is equal to the net work per cycle. Power is the product of work and cycle frequency. Positive work decreased markedly with an increase in temperature, whereas negative work (the area between the *x*-axis and the bottom of the loop) changed much less. The vertical line in the centre of each trace indicates isometric tension at that temperature. For this particular preparation, excursion amplitude was 0.097 mm, muscle length (i.e. L_0) was 3.8 mm and peak-to-peak force at 12 °C and 1.0 Hz was 9.758 mN.

is comparable with *in vivo* calculations of myocardial power output ($4.52\,W\,kg^{-1}$; Hillman *et al.* 1987); however, if strain is reduced to 3 % or 1.5 %, power output is reduced to approximately $1.2\,W\,kg^{-1}$ and $0.55\,W\,kg^{-1}$, respectively (Syme and Josephson, 1995).

Our study also examined how power production was influenced by adrenergic stimulation. In the trout heart, adrenaline is the predominant catecholamine involved in modulating cardiac contractility (Farrell and Jones, 1992). Adrenaline stimulates β-adrenergic receptors, causing the phosphorylation of the SL L-type Ca^{2+} channel *via* cyclic AMP and protein kinase A pathways (Tibbits *et al.* 1992). This phosphorylation increases the open probability of the L-type Ca^{2+} channel (Reuter, 1983), allowing for greater trans-sarcolemmal Ca^{2+} influx with each depolarization. Concordant with other studies (Ask, 1983; Farrell *et al.* 1986; Vornanen, 1989; Keen *et al.* 1992; Shiels and Farrell, 1997), we demonstrate a positive inotropic effect of adrenaline, which was statistically significant at 12 °C. The only other study to investigate the effects of adrenaline on an oscillatory cardiac muscle preparation (rat papillary muscle; Layland *et al.* 1997) found that the power curve was shifted significantly upwards and to the right with adrenergic stimulation. The increase in isometric tension production after adrenergic stimulation in the present study was smaller than that observed in a previous study where the muscle was contracting only isometrically (Shiels and Farrell, 1997). The reasons for this difference are unclear. Shiels and Farrell (1997) report a peak tension of approximately 1–2 $mN\,mm^{-2}$, which is lower than that found in the present study, approximately 7–8 $mN\,mm^{-2}$. It is possible that, by subjecting the muscle to a length change, we increased the Ca^{2+}-sensitivity of the myofilaments (Allen and Kentish, 1985) such that myofilament activation was higher in the working muscle, resulting in a higher basal peak tension and a smaller positive inotropic effect of adrenaline. Clearly, further studies are needed in this area.

Effects of temperature and cycle frequency on power production

By studying the effects of cycle frequency on power

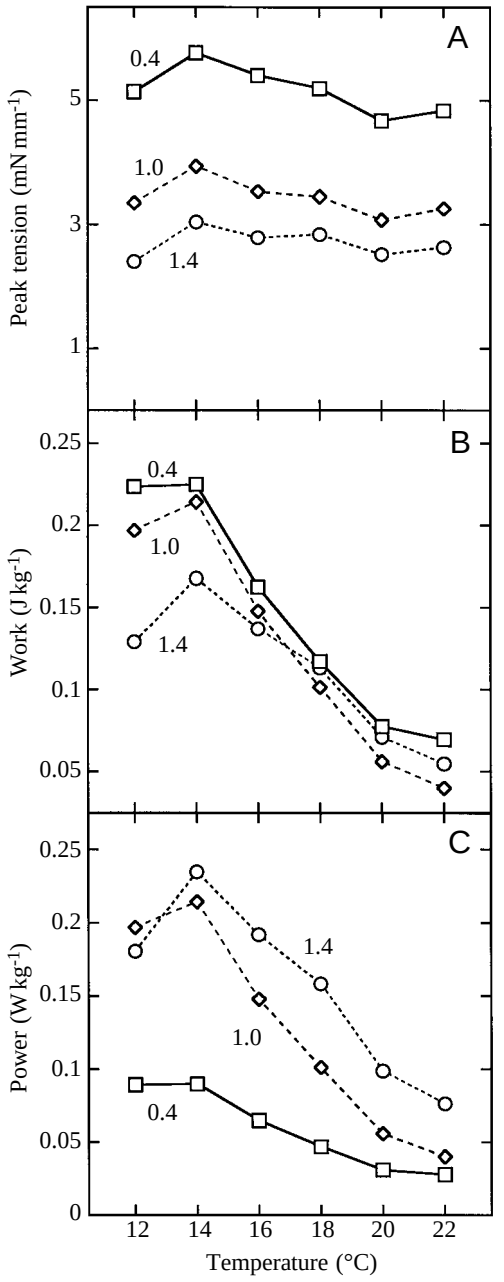

Fig. 5. The effects of temperature on tension, work and power production of ventricular trabeculae from a single trout under maximal (10 μmol l^{-1}) adrenergic stimulation. Results are given for three cycle frequencies: 0.4, 1.0 and 1.4 Hz.

generation, it is possible to evaluate whether there is an optimum frequency for power output. Further, if an optimum frequency exists *in vitro*, this can be compared with *in vivo* heart rates. The optimum frequency for power production at 12 °C is between 0.8 Hz and 1.2 Hz, as indicated by the apex of the power curves. This optimum frequency corresponds well with the 55 beats min^{-1} (approximately 1.0 Hz) *in vivo* heart rates measured in trout at 12 °C (Wood *et al.* 1979).

At 22 °C, work and, thus, power production were negligible at physiologically relevant contraction frequencies. This was unexpected and prompted us to examine more closely the effects of temperature on the kinetics of oscillatory contractions. The results from this experiment suggest that the rates of contraction and relaxation are elevated at 22 °C such that the muscle is fully relaxed during the shortening phase of the strain cycle, especially at frequencies exceeding 1.0 Hz (see Fig. 4). This causes a reduction in the amount of positive work (work done by the muscle during shortening), and net work production approaches zero. Therefore, the loss of work and, consequently, power output at 22 °C can be explained by a faster relaxation at warmer temperatures. This phenomenon can also be thought of in terms of total contraction duration. The duration of contraction determines how long tension is maintained during the shortening period of the cycle, which in turn influences the amount of work produced at a given cycle frequency (Layland *et al.* 1995*a*). At high temperatures, when contraction duration is reduced, work and power decline. Theoretically, maximum work would be achieved if the loop was a rectangle, with an instantaneous increase in tension at maximum muscle length and an instantaneous decrease in tension when the muscle relaxed at minimum muscle length. It should be noted that the effects of temperature on power would probably be less dramatic *in vivo*, because contraction frequency increases with temperature such that relaxation would only just be completed before the next contraction was initiated. Further, the fact that 22 °C is approaching the upper incipient lethal temperature for this species must also be considered, as it may cause contractility and maximum performance to decrease at high contraction frequencies. Indeed, we observed that some of the preparations could not maintain power production in the upper range of stimulation frequencies at 22 °C. This is in agreement with results from Farrell *et al.* (1996) which demonstrate that the maximum power output of the *in situ* trout heart decreases at temperatures above 18 °C. In general, the temperature study demonstrates that isometric tension is much less sensitive to temperature change (Fig. 5A) compared with work and power output (Fig. 5B,C). This an important result because it suggests that measuring temperature-induced changes in isometric tension may not be as reliable an indicator of respective changes in work or power output. Unfortunately, many studies assume that isometric tension production is a good index of contractility and overall cardiac performance, but the data presented here show that this assumption may not always be the case.

Interestingly, peak isometric tension did not change during the gradual temperature change. This suggests that, when fish cross a 10 °C thermocline, cardiac performance may be compromised to a greater degree than when temperature changes by 10 °C more gradually over the diurnal cycle, such as in animals trapped in streambeds. Furthermore, the discrepancy between the peak tension results from the two experimental designs (see Table 1) reiterates the point that peak isometric tension is not as reliable an indicator of contractility as are the more integrative measurements of work and power, whose responses were consistent between experiments.

In conclusion, we have examined the performance

capabilities of isolated trout ventricular trabeculae in an oscillating preparation. We believe that, by forcing the muscle to work against a cyclic length change, this preparation better approximates the *in vivo* condition of ventricular emptying and filling than does the traditional isometric tension preparation. Using this technique, we demonstrate that work and power are more sensitive to temperature change than is isometric tension. Further, although there may be some temperature-dependent and frequency-dependent interplay between SL and SR Ca^{2+} flux, power production, like tension production, depends primarily on extracellular Ca^{2+} influx across the SL.

We thank Rich Moccia at the Alma Aquaculture Research Station for the trout and Allison Holloway, Tom McDonald and Francoise Mercure for assistance in transporting them to Guelph. This research was supported through a National Science and Engineering Research Council of Canada grant awarded to A.P.F. and E.D.S. and by a Simon Fraser Graduate Fellowship awarded to H.A.S.

References

ALLEN, D. G. AND KENTISH, J. C. (1985). The cellular basis of the length–tension relation in cardiac muscle. *J. molec. cell. Cardiol.* **17**, 821–840.

ALTRINGHAM, J. D. AND BLOCK, B. A. (1997). Why do tuna maintain elevated slow muscle temperatures? Power output of muscle isolated from endothermic and ectothermic fish. *J. exp. Biol.* **200**, 2617–2697.

ASK, J. A. (1983). Comparative aspects of adrenergic receptors in the hearts of lower vertebrates. *Comp. Biochem. Physiol.* B **160**, 365–371.

BERS, D. M. (1987). Ryanodine and the Ca^{2+} content of the SR assessed by caffeine and rapid cooling contractures. *Am. J. Physiol.* **253**, C408–C415.

BERS, D. M. (1989). SR calcium loading in cardiac muscle preparations based on rapid cooling contractures. *Am. J. Physiol.* **256**, C109–C120.

BERS, D. M. (1991). *Excitation–contraction coupling and cardiac contractile force.* Boston: Kluwer Scientific Press.

BLACK, E. C. (1953). Upper lethal temperatures of some British Columbian freshwater fishes. *J. Fish. Res. Bd Can.* **10**, 196–210.

DAVIE, P. S. AND FARRELL, A. P. (1991). Cardiac performance of an isolated heart preparation from the dogfish (*Squalaus acanthias*): The effects of hypoxia and coronary artery perfusion. *Can. J. Zool.* **69**, 1822–1828.

DRIEDZIC, W. R. AND GESSER, H. (1988). Differences in force–frequency relationships and calcium dependency between elasmobranchs and teleost hearts. *J. exp. Biol.* **140**, 227–241.

EL-SAYED, M. F. AND GESSER, H. (1989). Sarcoplasmic reticulum, potassium and cardiac force in rainbow trout and plaice. *Am. J. Physiol.* **257**, R599–R604.

FARRELL, A. P., GAMPERL, A. K., HICKS, J. M. T., SHIELS, H. A. AND JAIN, K. E. (1996). Maximum cardiac performance of rainbow trout (*Oncorhynchus mykiss*) at temperatures approaching their upper lethal limit. *J. exp. Biol.* **199**, 663–672.

FARRELL, A. P. AND JONES, D. R. (1992). The heart. In *Fish Physiology*, vol. 12A (ed. W. S. Hoar, D. J. Randall and A. P. Farrell), pp. 1–88. San Diego: Academic Press.

FARRELL, A. P., MACLEOD, K. R. AND CHANCEY, B. (1986). Intrinsic mechanical properties of the perfused rainbow trout heart and the effects of catecholamines and extracellular calcium under control and acidotic conditions. *J. exp. Biol.* **125**, 319–345.

FRANKLIN, C. E. AND JOHNSTON, I. A. (1997). Muscle power output during escape responses in an Antarctic fish. *J. exp. Biol.* **200**, 703–712.

GAMPERL, A. K., AXELSSON, M. AND FARRELL, A. P. (1995). Effects of swimming and environmental hypoxia on coronary blood flow in rainbow trout. *Am. J. Physiol.* **269**, R1258–R1266.

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.

HILLMAN, S. S., WITHER, P. C., PALIOCA, W. B. AND RUBEN, J. A. (1987). Cardiovascular consequences of hypercalcemia during activity in two species of amphibian. *J. exp. Zool.* **242**, 303–308.

HOVE-MADSEN, L. (1992). The influence of temperature on ryanodine sensitivity and the force–frequency relationship in the myocardium of rainbow trout. *J. exp. Biol.* **167**, 47–60.

HOVE-MADSEN, L. AND GESSER, H. (1989). Force–frequency relationship in the myocardium of rainbow trout. *J. comp. Physiol.* **159**B, 61–69.

JONES, D. R. AND RANDALL, D. J. (1978). The respiratory and circulatory systems of fish during exercise. In *Fish Physiology: Locomotion*, vol. VII (ed. W. S. Hoar and D. J. Randall), pp. 425–501. San Diego: Academic Press.

JOSEPHSON, R. K. (1985). Mechanical power output from striated muscle during cyclic contraction. *J. exp. Biol.* **114**, 493–512.

KEEN, J. E., FARRELL, A. P., TIBBITS, G. F. AND BRILL, R. W. (1992). Cardiac physiology in tunas. II. Effect of ryanodine, calcium and adrenaline on force–frequency relationships in atrial strips from skipjack tuna, *Katsuwonus pelamis*. *Can. J. Zool.* **70**, 1211–1217.

KEEN, J. E., VIAZON, D.-M., FARRELL, A. P. AND TIBBITS, G. F. (1994). Effect of temperature and temperature acclimation on the ryanodine sensitivity of the trout myocardium. *J. comp. Physiol.* B **164**, 438–443.

KICENIUK, J. W. AND JONES, D. R. (1977). The oxygen transport system in trout (*Salmo gairdneri*) during sustained exercise. *J. exp. Biol.* **69**, 247–260.

LAYLAND, J., YOUNG, I. S. AND ALTRINGHAM, J. D. (1995*a*). The effect of cycle frequency on the power output of rat papillary muscle *in vitro*. *J. exp. Biol.* **198**, 1035–1043.

LAYLAND, J., YOUNG, I. S. AND ALTRINGHAM, J. D. (1995*b*). The length dependence of work production in rat papillary muscles *in vitro*. *J. exp. Biol.* **198**, 2491–2499.

LAYLAND, J., YOUNG, I. S. AND ALTRINGHAM, J. D. (1997). The effect of adrenaline on the work- and power-generating capacity of rat papillary muscle *in vitro*. *J. exp. Biol.* **200**, 503–509.

MATIKAINEN, N. AND VORNANEN, M. (1992). The effect of season and temperature acclimation on the function of crucian carp (*Carassius carassius*) heart. *J. exp. Biol.* **167**, 203–220.

MCDONALD, D. M. AND MILLIGAN, C. L. (1992). Chemical and physical properties of the blood. In *Fish Physiology*, vol. 12 (ed. W. S. Hoar, D. J. Randall and A. P. Farrell), pp. 55–133. New York: Academic Press.

MILLIGAN, C. L., GRAHAM, M. S. AND FARRELL, A. P. (1989). The response of trout red cells to adrenaline during seasonal acclimation and changes in temperature. *J. Fish Biol.* **35**, 229–236.

MØLLER-NIELSEN, T. AND GESSER, H. (1992). Sarcoplasmic reticulum and excitation–contraction coupling at 20 °C and 10 °C in rainbow trout myocardium. *J. comp. Physiol.* B **162**, 526–534.

REUTER, H. (1983). Calcium channel modulation by neurotransmitters, enzymes and drugs. *Nature* **301**, 569–574.

ROUSSEAU, E., SMITH, J. S. AND MEISSNER, G. (1987). Ryanodine modifies conductance and gating behaviour of single calcium release channels. *Am. J. Physiol.* **253**, C364–C368.

SHIELS, H. A. AND FARRELL, A. P. (1997). The effect of temperature and adrenaline on the relative importance of the sarcoplasmic reticulum in contributing calcium to force development in isolated ventricular trabeculae from rainbow trout. *J. exp. Biol.* **200**, 1607–1621.

SITSAPESAN, R., MONTGOMERY, R. A. P., MACLEOD, K. T. AND WILLIAMS, A. J. (1991). Sheep cardiac sarcoplasmic reticulum calcium release channels: modifications of conductance and gating by temperature. *J. Physiol., Lond.* **434**, 469–488.

STEVENS, E. D. (1996). The pattern of stimulation influences the amount of oscillatory work done by frog muscle. *J. Physiol., Lond.* **494**, 279–285.

SYME, D. A. (1993). Influence of extent of muscle shortening and heart rate on work from frog heart trabeculae. *Am. J. Physiol.* **265**, R310–R319.

SYME, D. A. AND JOSEPHSON, R. K. (1995). Influence of muscle length on work from trabecular muscle of frog atrium and ventricle. *J. exp. Biol.* **198**, 2221–2227.

TIBBITS, G. F., HOVE-MADSEN, L. AND BERS, D. M. (1991). Calcium transport and the regulation of contractility in teleosts, a comparison with higher vertebrates. *Can. J. Zool.* **69**, 2014–2919.

TIBBITS, G. F., MOYES, C. D. AND HOVE-MADSEN, L. (1992). Excitation–contraction coupling in the teleost heart. In *Fish Physiology*, vol. 12A (ed. W. S. Hoar, D. J. Randall and A. P. Farrell), pp. 267–296. New York: Academic Press.

VORNANEN, M. (1989). Regulation of contractility of the fish (*Carassius carassius* L.) heart ventricle. *Comp. Biochem. Physiol.* **2**C, 477–483.

VORNANEN, M. (1994). Seasonal and temperature-induced changes in myosin heavy chain composition of crucian carp hearts. *Am. J. Physiol.* **267**, R1567–R1573.

VORNANEN, M. (1996). Effects of extracellular calcium on the contractility of warm and cold acclimated crucian carp heart. *J. comp. Physiol.* B **165**, 507–517.

WOOD, C. M., PIEPRZAC, P. AND TROTT, J. N. (1979). The influence of temperature and anemia on the adrenergic and cholinergic mechanisms controlling heart rate in the rainbow trout. *Can. J. Zool.* **103**, 207–208.

XU, K. Y., ZWEIER, J. L. AND BECKER, L. C. (1995). Functional coupling between glycolysis and sarcoplasmic reticulum calcium transport. *Circulation Res.* **77**, 88–97.