

# Can Temperature-Dependent Changes in Myocardial Contractility Explain Why Fish Only Increase Heart Rate When Exposed to Acute Warming?

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**Summary Statement:** Measurements of myocardial function provide novel insights as to why fish may only increase heart rate when acutely warmed, and why this parameter may be constrained at cooler temperatures.

## ABBREVIATIONS:

A/D	analog to digital
ANOVA	analysis of variance
BPM	beats per minute
CCAC	Canadian Council on Animal Care
CT <sub>Max</sub>	critical thermal maximum
CVP	central venous pressure
D/A	digital to analog
f <sub>H</sub>	heart rate
HSD	honest significant difference
PO <sub>2</sub>	oxygen partial pressure
Q	cardiac output
RVM	relative ventricular mass
SEM	standard error of the mean
T <sub>crit</sub>	upper critical temperature
V <sub>s</sub>	stroke volume

## ABSTRACT

Fish increase heart rate ( $f_H$ ), not stroke volume ( $V_S$ ), when acutely warmed as a way to increase cardiac output ( $Q$ ). To assess whether aspects of myocardial function may have some basis in determining temperature-dependent cardiac performance, we measured work and power (shortening, lengthening and net) in isolated segments of steelhead trout (*Oncorhynchus mykiss*) ventricular muscle at the fish's acclimation temperature (14°C), and at 22°C, when subjected to increased rates of contraction (30–105 min<sup>-1</sup>, emulating increased  $f_H$ ) and strain amplitude (8–14%, mimicking increased  $V_S$ ). At 22°C, shortening power (indicative of  $Q$ ) increased in proportion to  $f_H$ , and the work required to re-lengthen (stretch) the myocardium (fill the heart) was largely independent of  $f_H$ . In contrast, the increase in shortening power was less than proportional when strain was augmented, and lengthening work approximately doubled when strain was increased. Thus, the derived relationships between  $f_H$ , strain, and myocardial shortening power and lengthening work, suggest that: increasing  $f_H$  would be preferable as a mechanism to increase  $Q$  at high temperatures; or in fact, may be an unavoidable response given constraints on muscle mechanics as temperatures rise. Interestingly, at 14°C, lengthening work increased substantially at higher  $f_H$ s, and the duration of lengthening (i.e., diastole) became severely constrained when  $f_H$  was increased. These data suggest that myocardial contraction / twitch kinetics greatly constrain maximal  $f_H$  at cool temperatures, and may underlie observations that fish elevate  $V_S$  to an equal or greater extent than  $f_H$  to meet demands for increased  $Q$  at lower temperatures.

## INTRODUCTION

Temperature has been described as the 'ecological master factor' for fish (Brett, 1965; Farrell et al., 2007) as it effects numerous physiological processes including the functional capacity of the cardiovascular system (Eliasson and Antilla, 2017). When exposed to an acute rise in temperature, fish increase cardiac output ( $Q$ ) in an attempt to meet their increased demands for oxygen (metabolic rate), a response that is mediated almost exclusively by increases in heart rate ( $f_H$ ). However, increases in  $f_H$  cannot carry on indefinitely, and at a certain point  $f_H$  plateaus before becoming arrhythmic and dropping back to resting levels just prior to the fish's upper critical temperature ( $T_{crit}$ ) (Farrell et al., 1996; Farrell, 2009; Gollock et al., 2006; Leeuwis et al., 2021; Steinhausen et al., 2008; Verhille et al., 2013). Research indicates

that this cardiac collapse is likely due to a combination of factors, including a loss of ventricular excitability due to disruptions in cardiomyocyte ionic currents (Haverinen and Vornanen, 2006 and 2020), a decrease in the efficiency of mitochondrial oxidative phosphorylation (Christen et al., 2018; Gerber et al., 2020 and 2021; Iftikar and Hickey, 2013; Penney and Gamperl 2014), and potentially, a loss of nervous function (Andreassen et al., 2020). However, why  $f_H$  alone is responsible for temperature-dependent increases in  $Q$  is not known, especially when experiments where the capacity to increase  $f_H$  is limited by pharmacological agents (e.g., zatebradine) have shown that increases in  $Q$  can be mediated through stroke volume ( $V_S$ ) as temperatures rise (Gamperl et al., 2011; Keen and Gamperl, 2012). This data suggests that there are either physiological constraints which prevent increases in  $V_S$  as temperatures and  $f_H$  increase, that fish preferentially increase  $f_H$  over  $V_S$ , or that increases in  $f_H$  are inescapable due to some aspect of temperatures' direct effect on the heart.

With regards to whether there is a reason why fish preferentially increase  $f_H$  vs.  $V_S$  (i.e., if there is a physiological or mechanical advantage to the heart), the research to date has not provided such evidence. For example, Syme et al. (2013) showed that measures of work (shortening, lengthening and net) from cod (*Gadus morhua*) trabeculae at 20°C responded similarly under conditions of falling oxygen ( $PO_2$  values) whether tested at low  $f_H$  (35 beats  $min^{-1}$ ) and high  $V_S$  (8% strain) vs. high  $f_H$  (70 beats  $min^{-1}$ ) and low  $V_S$  (2.2% strain). However, the strain trajectory was not adjusted to match changes in twitch kinetics in that study. Further, Syme et al. (2013) also showed that lengthening work increased greatly when cod myocardium was paced at high rates (from 75 – 115 bpm, the normal maximum  $f_H$  in this species is 75 bpm). These latter data suggest that lengthening work (that required to stretch the myocardium) may limit cardiac filling at high  $f_H$ . The Frank-Starling mechanism is also intimately involved in the regulation of  $V_S$  in fishes, and describes how elevated venous return increases end-diastolic volume, and this results in stretching of the myocardium and an increase in contractility, and therefore,  $V_S$  (Farrell and Smith, 2017). However, we have very limited knowledge of the relationship between central venous pressure (CVP) and increased temperature, and thus, of the possible role that CVP plays with regard to the lack of an increase in  $V_S$  as fish are acutely warmed. This is in part because the contribution of venous pressure (preload) to cardiac filling in fishes varies between species (Ho et al., 2002). Further, the effects of temperature on cardiac preload in fish have only been studied in one species, and over a very limited temperature range (Sandblom and Axelsson, 2006). These authors reported no change in CVP in trout

(*Oncorhynchus mykiss*) at temperatures from 10 to 16°C, and this may constrain  $V_s$ , in particular as the period of cardiac relaxation (diastolic filling) also decreases as the heart beats faster.

As highlighted in a recent special issue in this journal, whether organisms have the capacity to compensate for climate change-related impacts requires a mechanistic understanding of the effects of environmental drivers, and how their interactions influence physiological homeostasis (Franklin and Hoppeler, 2021). Thus, we examined how myocardial work and power at relatively cool (14°C) and high (22°C) temperatures are affected by  $f_H$  (contraction rate) and strain amplitude (related to  $V_s$ ). Because fish increase  $f_H$  preferentially over  $V_s$  when warmed, we hypothesized that increasing  $f_H$  would enhance power to a greater extent than strain (i.e.,  $V_s$ ) at 22°C, and perhaps be advantageous from the perspective of diastolic filling. Importantly, in these experiments, the period of muscle shortening during the strain cycle was adjusted to match the period over which force was generated by the muscle during contraction, with the remainder of the cycle being diastolic lengthening. This refinement in experimental paradigm resulted in the myocardium generating force while shortening, then relaxing at the onset of lengthening, at all temperature / strain /  $f_H$  combinations, as would occur during the cardiac cycle *in vivo*. This is opposed to a symmetrical strain cycle which is commonly used in work-loop studies, but which does not accurately mimic the function of a beating heart, particularly at high and low  $f_H$ .

The results of this study show/suggest that: 1) myocardial work (i.e., stroke work) and power rise substantially with increases in both strain amplitude (i.e.,  $V_s$ ) and  $f_H$ , but that increases in  $V_s$  may be less effective than elevating  $f_H$  with regard to increasing cardiac power output when fish are acutely warmed to temperatures approaching their  $CT_{Max}$ . 2) The work required to lengthen the muscle (i.e., diastolic filling work) was very sensitive to changes in strain amplitude (and thus  $V_s$ ), but was almost independent of  $f_H$  at 22°C, and this may assist the heart to fill at high temperatures and  $f_H$ . 3) In contrast, there was a substantial increase in lengthening work at 14°C with increased  $f_H$ , and this combined with constraints on the duration of lengthening at high  $f_H$ , may limit maximal  $f_H$  at cooler temperatures. Collectively, these data provide considerable, and novel, insights into the temperature dependence of cardiac function, and mechanistic explanations (hypotheses) for why tachycardia is the predominant / sole mechanism by which fish increase  $Q$  when acutely exposed to elevated temperatures.

## METHODS

### ***Animal husbandry***

All procedures were approved by the Memorial University of Newfoundland and Labrador's (MUN's) and the University of Calgary's animal care committees, and followed CCAC guidelines. Adult steelhead trout (*Oncorhynchus mykiss*) were sourced from Nova Fish Farms (Nova Scotia, Canada) and initially housed in a circular, 3000 litre (L), tank supplied with aerated 14°C seawater for approximately two months in the Dr. Joe Brown Aquatic Research Building at the Ocean Sciences Centre (MUN; St. John's, Newfoundland, Canada). A photoperiod of 12 h light : 12 h dark was maintained throughout, and the fish were fed a commercial pelleted trout diet three times a week at 1.5% of body mass per feeding. Eighty fish were then removed from the 3000 L stock tank, and transferred to a 1200 L tank for a 4-week acclimation period prior to experimentation; conditions in this tank mirrored those described for the stock tank except that the ration was changed to 1% body mass per day.

### ***Preparation of isolated myocardium***

Prior to an experiment, fish (583 ± 39 g; SEM) were netted from the tank and euthanized by cerebral percussion, and the ventricle was excised, cut in half along the sagittal plane and then rinsed in ice-cold physiological saline for marine teleosts (Petersen and Gamperl, 2010). Trabeculae from the spongy ventricular myocardium were isolated on a chilled (4°C) stage using a dissecting microscope. Trabecular preparations were selected so that the majority of fibers ran parallel to the long axis of the preparation, and there was minimal branching along the muscle's length. Preparations (N=10, each from a different fish) averaged 1.89 ± 0.35 mg wet mass and had a resting length of 4.87 ± 0.37 mm. A segment of 6–0 silk suture was tied around each end of the preparation and used to attach the muscle strip to stainless steel pins on the arm of a servomotor (Model 300C-LR, Aurora Scientific: Aurora, ON, Canada) and a force transducer (Model 404A, Aurora Scientific). The muscle segments were bathed in physiological saline that was bubbled with air, and the temperature was maintained at 14 ± 0.2°C with Peltier thermoelectric modules and a temperature controller (Model TC-24-12, TE Technology; Traverse City, MI). Platinum plate stimulating electrodes, used to activate the muscle, were positioned on both sides of the preparation and connected to an amplifier circuit powered by a wet cell battery that followed a stimulator (Isostim A320, World Precision Instruments; Sarasota, FL) that was in turn controlled by a computer. A 3 ms, supra-maximal,

square voltage pulse was used to stimulate the muscle strips. Custom software written using Lab-View (National Instruments: Austin, TX) controlled a 12-bit analog/digital converter card (PCI MIO 16E 4, National Instruments) that operated the stimulator and servomotor (5 kHz D/A output) and collected force, muscle length (servomotor arm position) and stimulus signals (1 kHz A/D input). Once attached to the apparatus, the length of each preparation was increased systematically until net power output, measured using the method described below at 30 cycles  $\text{min}^{-1}$ , approached maximal and mimicked the operation of a beating heart on the ascending limb of the force-length relationship.

### ***Measuring work and power***

We evaluated the effects of temperature, contraction rate (i.e.,  $f_H$ ) and strain amplitude (i.e., changes in muscle length, simulating that seen with changes in  $V_S$ ) on work and power output of the ventricular muscle using the work-loop method (e.g., see Carnevale et al., 2021; Johnson and Johnston, 1991; Syme, 1993; Syme and Stevens, 1989). The length of the muscle strips was cycled in a sinusoidal trajectory at a rate of 30, 45, 60, 75, 90 and 105 times per minute (analogous to  $f_H$  in beats per minute, BPM). The order in which the series of contraction rates was tested (low-to-high vs. high-to-low) was alternated between each muscle strip. Strain amplitude was set to 8%, 11% and 14% peak-to-peak (where strain is the change in length relative to the resting length, in percent). Eight percent strain is the muscle strain measured at resting stroke volume ( $V_S = 0.4 \text{ ml g}^{-1}$  ventricle) in a beating steelhead trout heart (Carnevale, 2019), similar to the value of 9% measured in resting cod hearts (Syme et al., 2013), and 11% and 14% strain bracket that estimated at maximum  $V_S$  in trout ( $1.3 \text{ ml g}^{-1}$  ventricle,  $\sim 12\%$  strain assuming that the ventricle is a sphere) (Carnevale, 2019). The largest strain of 14% also ensured that all strips were operating at, or near, maximum strain due to the inherent variability between fish and the varying orientation of trabeculae *in vivo* (Sanchez-Quintana et al., 1995). Experiments were performed at 14°C and 22°C, however, a contraction rate of 105 BPM was not used at 14°C as the strips became refractory and could not follow this contraction rate at this temperature. The 14°C temperature was always tested first, followed by 22°C, because higher temperatures tend to be more stressful on muscle. However, to assess the stability of the preparations over the time-course of the experiment, work was measured at a reference strain of 11% and 30 BPM at the beginning of the experiment at 14°C, again at 22°C

about mid-way through the experiment, and again at the end of the experiment when preparations were returned to 14°C.

Importantly, at every combination of contraction rate and temperature, the period of muscle shortening during the strain cycle was adjusted to match the period over which force was generated by the muscle during contraction; the balance of the strain cycle comprised of muscle lengthening. As such, the strain trajectory was an asymmetrical sine pattern which resulted in the muscle generating force while shortening and then relaxing at the onset of lengthening (e.g., see Fig. 1), as would occur during the cardiac cycle *in vivo*. To calculate the relative proportion of the strain cycle that comprised muscle shortening, isometric contractions were recorded for each muscle strip at each combination of contraction rate and temperature, and the period over which the muscle generated force was measured as the time from stimulation until force fell to 10% of maximum. The proportion of the strain cycle that comprised shortening at each contraction rate and temperature was then set to match the period of force production, calculated as: the period of force production / total period of the cycle. The point that the stimulus was applied during the strain cycle was set to coincide with the point at which the muscle strip was at its maximum length, just prior to shortening. This resulted in elastic recoil of the myocardium initiating muscle shortening, but active force being produced primarily during muscle shortening, and avoided variability in stimulus timing as a complicating factor in data interpretation. This timing was expressed as a fraction of the total cycle period and calculated as:  $[\text{total period of one cycle} - \text{period of shortening}] / (2 \times \text{total period of one cycle})$ .

At each combination of strain, temperature and contraction rate, the muscle strip performed 30 consecutive cycles of work, with measurements taken from the last cycle in the series (i.e., where force and work had stabilized). Records of muscle force and length were subjected to a 10-point median filter before analysis to remove any small noise artifacts (Syme et al., 2013). Work was calculated as the sum of the products of length change and average force produced by the muscle over each collection interval. Shortening work was the sum over the shortening portion of the strain cycle, i.e., that associated with ejection of blood in a beating heart. Lengthening work, the work required to extend the ventricular muscle and associated with diastolic filling work of a beating heart, was the sum over the lengthening portion of the strain cycle. Net work was calculated as shortening work minus lengthening work. Values of work done per cycle are shown in the results as they are particularly useful for

interpreting the effects of strain amplitude and temperature on the work required to fill the heart (i.e., diastolic filling work) or eject blood (i.e., stroke work) during each cardiac cycle. However, myocardial power (calculated as the product of work per cycle and cycle frequency) is also presented as it is most useful for interpreting the effects of contraction rate and temperature on cardiac power output (which reflects cardiac output,  $Q$ ). Work is expressed as Joules  $\text{kg}^{-1}$  of muscle mass, and power as Watts  $\text{kg}^{-1}$  of muscle mass. The mass of the strips was measured at the conclusion of each experiment by removing the muscle preparation from the apparatus, trimming any obviously non-viable tissue, blotting it on filter paper to remove surface moisture, and weighing it on a microbalance (Mettler UMT2, Mettler Toledo; Columbus, OH).

### ***Statistical analyses***

Time dependent effects on shortening and lengthening work, measured at the beginning, mid-point and end of each experiment at a reference strain of 11% and 30 BPM contraction rate (i.e., to test for any loss of contractile performance over the experiment), were examined using one-way repeated measures ANOVAs followed by Dunnett's tests (Fig. 2). Split-plot mixed general linear models, and the R statistical package (Version 3.22), were used to examine the effects of the three controlled variables (contraction rate, strain and temperature) and one random variable (strip) on measures of work and power (Table 1, Fig. 3 and 4).

Ventricular trabeculae were not controlled for size, which contributed to inherent variability between strips, but was accounted for in the main model by including strip as a random factor.

Values of lengthening work done per unit strain amplitude at each temperature (Table 2, Fig. 5) were analyzed using a two-way repeated measures ANOVA (controlled variables contraction rate and strain). This was followed by one-way repeated measures ANOVAs and Tukey's HSD tests. Differences in the relationship between lengthening work and strain rate at the 3 different strain amplitudes (Fig. 7) were examined at each temperature (14 and 22°C) using linear regression analysis. This included testing whether the intercepts and slopes of the lines were significantly different at all temperatures. The duration of lengthening per cycle, as affected by temperature and contraction rate (Fig. 6), was examined using a one-way repeated measures ANOVA, followed by: 1) paired t-tests between temperatures; and 2) one-way ANOVAs followed by Tukey's HSD tests between contraction rates. Finally, specific (a priori) comparisons were made of lengthening work and shortening power measured at 14°C at 60



contractions  $\text{min}^{-1}$  and 8% strain (*in vivo* conditions), as compared to two particular strain/contraction rate combinations at 22°C, using repeated measures ANOVAs followed by Holm-Sidak tests (Table 3). These latter strain/rate combinations reflected possible *in vivo* conditions at warmer temperatures. The ANOVAs, Tukey's HSD tests and t-tests were performed using Prism 8 (GraphPad Software, San Diego, CA).  $P < 0.05$  was used as the level of statistical significance in all analyses, and all values in the text, tables and figures are means + standard errors (SEM).

## RESULTS

The shortening work performed by myocardial strips (at a reference strain of 11% and 30 contractions  $\text{min}^{-1}$ ) at 14°C at the end of the experiment was not significantly different compared to the work measured at 22°C mid-way through the experiment, or at 14°C at the beginning of the experiment (Fig. 2A). Lengthening work was also not significantly different between the start and end measurements at 14°C, and was only about 4% higher ( $P < 0.05$ ) at 22°C (Fig. 2B). The preparations, were thus, very stable over the course of an experiment.

### *Effects of strain on work and power*

At both temperatures, changes in shortening work done per cycle, and changes in shortening power, were positively but less than proportionally related to changes in strain amplitude (Table 1); shortening work and power were about 33% greater at 11 vs. 8% strain, and about 63% greater at 14 vs. 8% strain (Figs. 3 and 4, A and B). Lengthening work and power also increased with strain amplitude (Table 1). However, the increase was proportionally greater than the increase in strain amplitude (Figs. 3 and 4, C and D). For example: at 14°C lengthening work/power were about 150% greater at 14 vs. 8% strain at 30 BPM and about 100% greater at 90 BPM; and at 22°C lengthening work and power were about 100% greater at 14 vs. 8% strain across all contraction rates. This disproportionate increase in lengthening work was further examined by plotting work per unit of strain amplitude (Fig. 5, Table 2), where more negative values indicate more work required for a given amount of lengthening. Clearly, there was a significant increase in the amount of work required to lengthen the myocardium per unit strain at larger strain amplitudes. Net work and power also had a positive relationship with strain amplitude (Figs. 3 and 4 E and F, Table 1), but the magnitude of the increase was

noticeably less at higher contraction rates at 14°C. This reflected the high levels of lengthening work under these conditions, particularly at high strains.

### ***Effects of contraction rate on work and power***

Shortening work done per cycle declined slightly with increased contraction rate at all strain amplitudes, although the extent of the decline was marginally greater at 14 vs. 22°C, particularly at higher contraction rates (Fig. 3A and B, Table 1). At 14°C, these changes in work per cycle resulted in an increase in shortening power that was approximately proportional to contraction rate up to ~75 BPM, but then approached a plateau at 90 BPM (Fig. 4A). Whereas at 22°C, power continued to increase in approximate proportion to contraction rate up to 105 BPM (Fig. 4B). Conversely, while there was almost no change in lengthening work per cycle as contraction rate increased at 22°C, a large increase in lengthening work was observed in muscle working at 14°C (~ 5% vs. 60%, respectively; Fig. 3C and D, Table 1). This is highlighted by the approximately 2-fold increase in lengthening work per unit strain with increasing contraction rate at 14°C, but almost no change at 22°C (Fig. 5, Table 2). Lengthening power also increased with contraction rate (Table 1, Fig. 4C and D), but the extent of the increase was greater than the increase in contraction rate, and highly dependent on temperature. For example, at 14°C lengthening power increased about 6-fold over the 3-fold range of contraction rates (30 to 90 BPM), while at 22°C it increased less than 4-fold.

In combination, these changes in shortening and lengthening work lead to a decrease in net work with increased contraction rate, with the decline considerably greater in muscle working at 14 vs. 22°C (Fig. 3D and E, Table 1). As a result, net power at 14°C initially increased with contraction rate, but then attained a maximum at ~75 BPM and subsequently declined. In contrast net power at 22°C continued to increase with contraction rate, with little evidence of approaching a maximum (Fig. 4E and F).

### ***Lengthening rate, duration and work***

In a beating heart, the period of muscle shortening is determined largely by the duration of the cardiac twitch, with diastolic lengthening comprising the remainder of the cycle; a situation that we mimicked in the present study (Fig. 1). This period of lengthening, and in turn the rate of lengthening, is thus constrained by both the duration of the twitch and  $f_H$  (which sets the total period of time available for shortening and lengthening). Therefore, we examined

how contraction rate ( $f_H$ ) affected the time available for muscle lengthening (equivalent to the duration of diastolic filling) (Fig. 6), and assessed the combined effects of strain amplitude and contraction rate on the rate of muscle lengthening (strain rate) and lengthening work (Fig. 7). The time available for muscle lengthening, expressed either as a percentage of the total cardiac cycle or as time (in ms), decreased as contraction rate increased (Fig. 6). Of note, at 14°C the lengthening period became extremely brief at high contraction rates, only several percent of the entire cycle and a few milliseconds in duration, while at 22°C the lengthening period was significantly longer than at 14°C and remained a substantial portion of the entire cycle.

As a result of the decreased time available for lengthening with increased contraction rates, the rate of muscle lengthening (i.e., lengthening strain rate) also increased with contraction rate and strain amplitude because the muscle was lengthened a greater amount in the same period of time (Fig. 7). Conspicuously, the lengthening (strain) rates at 14°C (0.1 – 1%  $\text{ms}^{-1}$ ) were about 10-fold greater than at 22°C (0.01 – 0.1%  $\text{ms}^{-1}$ ) (compare x-axes in panels A and B of Fig. 7). This resulted in the effect of strain rate on lengthening work being decidedly (and significantly) negative at 14°C at all strain values (i.e., lengthening work approximately doubled). In contrast, when the muscle was working at 22°C, the relatively low rates of lengthening, and the relatively small increase in strain rate that occurred as contraction rate increased from 30 to 105 BPM, resulted in no effect of contraction rate on lengthening work.

## DISCUSSION

Fish increase  $f_H$  almost exclusively when exposed to acute increases in temperature, while  $V_S$  remains unchanged, even to the point of fatigue, regardless of whether the fish is resting or swimming (Farrell, 2009; Gollock et al., 2006; Joaquim et al., 2004; Leeuwis et al., 2019; Motyka et al., 2017; Steinhausen et al., 2008). In support of this observation, we report that while the increase in shortening power for a given relative increase in  $f_H$  at 22°C is only slightly greater than for the same relative increase in  $V_S$ , the relationships between strain,  $V_S$ ,  $f_H$ ,  $Q$  and myocardial power suggest that increases in strain alone would likely be inadequate to increase myocardial power and  $Q$ , while changes in  $f_H$  would. Further, at the warmer temperature (22°C) we found that the increase in work required to lengthen the myocardium (diastolic filling) was considerably greater when increasing  $V_S$  (strain) versus  $f_H$ . Thus, from a mechanical perspective, it would appear that increasing  $f_H$  is a preferable strategy over

increasing  $V_S$  at warm temperatures. In contrast, at cooler temperatures, even though the myocardium itself has the capacity to increase power until high rates of contraction are attained, the time available for cardiac filling/muscle lengthening quickly becomes limiting as  $f_H$  rises, and so diastolic filling time and increased lengthening work greatly impair the ability of the working heart to increase  $f_H$ . Thus,  $V_S$  can, and does, increase to promote increased  $Q$  when  $f_H$  remains low at cooler environmental / test temperatures (Steinhausen et al., 2008) or during warming with pharmacological blockade (Keen and Gamperl, 2012). While these results are based on measures from spongy trabecular muscle, and thus the effects of specific combinations of contraction rate and strain cannot necessarily be conferred to the compact layer, the twitch kinetics of the two layers differ by only about 10% (Roberts et al., 2021). This suggests that the same patterns of effect should occur in both layers, and broader conclusions regarding the impacts of  $f_H$  versus  $V_S$  will apply to the whole heart regardless of tissue type.

### ***Strain, contraction rate, myocardial power and cardiac output***

When the vectors of muscle force and length change are parallel, as they are in the experimental apparatus / conditions used in this study, work done by or on muscle is the product of force and the change in length (strain). Thus, as a first approximation, work output should be directly proportional to strain amplitude, and deviations from proportionality suggest additional impacts on the force produced by the muscle. This has important implications for the effectiveness of altering work or power through changes in strain amplitude (i.e.,  $V_S$ ). Changes in shortening work and power were somewhat less than proportional to changes in strain amplitude (e.g., ~33% increase in work with a 38% increase in strain from 8 to 11%, and an ~63% increase in work given a 75% increase in strain from 8 to 14%), such that changes in work or power averaged only about 86% of changes in strain (Fig. 3A and B, Fig. 4A and B). This indicates that force was depressed by increased strain amplitude, and resulted in less work done than anticipated from the increase in strain. This is a common observation, as increased strain results in increased velocities of shortening, which eventually limit work output (Altringham and Johnston, 1990; Johnson and Johnston, 1991; Syme and Stevens, 1989). As a consequence, changes in stroke work in a working heart (i.e., pressure-volume work) during each cardiac cycle would be proportionally less than the increase in strain amplitude (i.e., those associated with changes in  $V_S$ ).

Alternatively,  $f_H$  could be increased to meet the fish's demands for  $Q$  and oxygen delivery. Effects of contraction rate ( $f_H$ ) on  $Q$  are best considered by assessing power output (i.e., shortening power). At 14°C, shortening power first increased, but then approached or attained a plateau at higher rates of contraction (Fig. 4A). While at 22°C, power increased approximately in proportion to contraction rate up to the highest rates measured, and changes in power averaged about 93% of the change in contraction rate (Fig. 4B). This is somewhat higher than the gain in power attained by increasing strain noted above, suggesting that increasing  $f_H$  might be more effective than increasing strain ( $V_S$ ) to enhance myocardial power output when faced with higher temperatures.

In terms of implications for increasing  $Q$ , the myocardium must increase power output to at least the same extent as the increase in  $Q$ , otherwise stroke work and systemic pressure will be compromised.  $Q$  is directly proportional to  $f_H$ , as was myocardial shortening power output for the most part in these experiments (Fig. 4). Thus, increasing  $f_H$  as a means to increase  $Q$  would provide a good match between the power required to pump blood throughout the fish's circulation and that generated by the myocardium; particularly at 22°C where power was approximately proportional to  $f_H$  even at maximal  $f_H$ . In contrast, relationships between myocardial strain and  $V_S$  (and thus  $Q$ ) are not linear and dependent on heart volume (reviewed by Bijmens et al., 2012). Based on the approach of Syme et al. (2013), Carnevale (2019) measured myocardial strain in steelhead trout at rest (8% at a  $V_S$  of 0.4 ml kg<sup>-1</sup>) and estimated strain at maximal  $Q$  (12% at a  $V_S$  of 1.3 ml kg<sup>-1</sup>). Using these metrics, a 1.5-fold increase in strain would result in a 3.3-fold increase in  $V_S$  (from 0.4 to 1.3 mL kg<sup>-1</sup>), and thus, even if myocardial power was proportional to strain, the increase in power would be substantially less than the increase in  $V_S$  (and  $Q$ ). However, myocardial power was found to be less than proportional to changes in strain in this study, which would further exacerbate this disparity. Hence, the myocardium would clearly be challenged to generate enough power if strain (i.e.,  $V_S$ ) was the sole mechanism available to increase  $Q$ ; inotropy or other aspects of cardiac function would need to change as well. Thus, based on our analysis of muscle mechanics, increases in strain amplitude would appear to be less effective than tachycardia at increasing power output and  $Q$  at warm temperatures.

The work required to fill the ventricle (lengthen the ventricular myocardium) must also be considered in assessing the effects of temperature on  $f_H$  vs.  $V_S$ . Lengthening work and power increased considerably more than the change in strain amplitude (i.e., 100-150% increase in

work with a 38-75% increase in strain) (Figs. 3 and 4C and D, Fig. 5, Table 2), and this indicates that there is enhanced resistance to muscle lengthening with increased strain. This is likely a result of increased rates of stretch at higher strain amplitudes (Fig. 7), which result in increased resistance to stretch through viscous resistance (Syme, 1990), and through residual cross-bridge activity (particularly if the myocardium is not fully relaxed at the onset of lengthening) (Katz, 1939). Of relevance to *in vivo* cardiac function, diastolic filling (lengthening) work would be predicted to rise disproportionately to the increase in muscle strain amplitude, and hence,  $V_S$ . In contrast, lengthening power was simply proportional to contraction rate at 22°C (Fig. 4D), as evident by the lack of a change in lengthening work per cycle as the myocardium was paced at higher rates (Fig. 3D), and was much less than the considerable (and disproportionate) increase that occurred when increasing strain. This is likely the result of contraction rate having a very small effect on rates of lengthening at warm temperatures (Fig. 7 B), and that there was considerable time available for diastolic filling/lengthening regardless of contraction rate when warm (Fig. 1, lower panels; Fig. 6), while the effects of strain are always directly proportional regardless of temperature. Hence, changes in  $f_H$  at warm temperatures have only limited effects on lengthening work, while the impact of strain is large (Fig. 3D). Thus, again, increased strain (and thus  $V_S$ ) appears to be disadvantageous as a mechanism to increase  $Q$  from the perspective of diastolic filling work.

To further test the prediction that increasing  $f_H$  might be advantageous with regard to myocardial performance than increasing strain (i.e.,  $V_S$ ) when temperature is increased, we compared: 1) measures of myocardial shortening power and lengthening work under conditions estimated to mimic what occurs *in vivo* in trout at rest at 14°C (8% strain and 60 BPM); to 2) that when temperature is increased from 14 to 22°C (i.e.,  $f_H$  increasing from 60 to 105 BPM while strain remains unchanged at 8%) (Keen and Gamperl, 2012; Motyka et al., 2017); and to 3) what would occur under conditions where  $V_S$  increases to its maximum (1.3 ml  $\text{kg}^{-1}$ , 12% strain amplitude) but  $f_H$  remains unchanged at 60 BPM [similar to what occurs when fish are exercised at cool temperatures (Steinhausen et al., 2008), or when  $f_H$  is pharmacologically prevented from increasing when temperature is raised (Keen and Gamperl, 2012)]. There was a significant, and similar, increase in shortening power (which would support increased  $Q$ ) upon warming to 22°C when increasing either contraction rate or strain amplitude (Table 3). In contrast, while there was no significant change in lengthening work on warming to 22 °C when  $f_H$  was increased and strain remained constant, there was a substantial and

significant (~65%) increase in lengthening work when strain was increased and  $f_H$  remained low (Table 3). This supports the conclusion that increased  $f_H$  may be preferable, mechanically, to promote diastolic filling of the heart and support increased  $Q$  at warmer temperatures. However, we note that the specific values of strain employed in this comparison were estimates based on the assumption that the heart is a sphere, and thus, may not be completely accurate. Because lengthening work is quite sensitive to strain (e.g., see Fig. 3C and D), it would be important to confirm these assumptions with empirical measures of relationships between changes in stroke volume and strain in trout hearts before we can be confident in this particular comparison.

Other factors may also contribute to the observed relationships between temperature,  $f_H$  and  $V_S$ . Increased  $f_H$  with temperature has been suggested to be an obligatory response, and likely associated with the effects of temperature on the cardiac pacemaker (e.g., see Steinhausen et al., 2008). The increase in  $f_H$ , and resultant increase in  $Q$ , may actually preclude the need for increased  $V_S$  if the increase in  $Q$  is adequate to satisfy metabolic needs. In fact, this would be the anticipated outcome if both  $f_H$  and metabolic rate exhibit a similar  $Q_{10}$ . Hence, the lack of an increase in  $V_S$  with temperature may simply reflect the lack of need for any compensation in addition to that achieved by increased  $f_H$ . Alternatively, the lack of an increase in  $V_S$  with temperature could also have a basis in changes in plasma pH, potassium and  $PO_2$  with warming that limit inotropy, and thus, the capacity to elevate  $V_S$  [see Steinhausen et al. (2008) for a discussion]. However, when Sockeye salmon are exercised at 15°C,  $f_H$  increases by only about ~20%, while  $V_S$  increases by ~80% (Steinhausen et al., 2008), and in steelhead trout, when  $f_H$  is pharmacologically blocked from increasing,  $V_S$  increases with temperature to the extent that  $Q$  matches what occurs when  $f_H$  is allowed to increase (Keen and Gamperl, 2012). These observations suggest that  $V_S$  can increase, and thus, there must be additional physiological constraints and/or mechanistic explanations as to why increases in  $f_H$  versus  $V_S$  are used (favoured) when fish are exposed to various biotic and/or abiotic challenges. Neural and humoral influences (e.g., the release of catecholamines and the stimulation of cardiac  $\beta$ -adrenoreceptors) on the heart would also impact relationships between temperature and the ability of the heart to increase  $Q$  via  $f_H$  vs  $V_S$ . Their effects would ultimately be to modify the same mechanical mechanisms discussed here, including the availability of power for cardiac ejection, the work associated with diastolic filling, and the time available for diastolic filling as described below. In fact, Ask et al. (1981) showed that adrenergic stimulation is particularly

relevant with regards to adjusting trout cardiac performance at lower temperatures, and Keen et al. (1993) showed that *in situ* hearts from trout acclimated to 8°C were approximately 10-fold more sensitive to adrenaline than were hearts from fish acclimated to 18°C. If a similar temperature-dependent effect is observed in fish acutely exposed to increased temperature (e.g., over several hours), this decrease in inotropic support could also partially explain why  $V_S$  does not increase *in vivo* when fish are exposed to rising temperatures.

### ***Duration of diastole***

The duration of the cardiac twitch is dependent on  $f_H$  (e.g., see Shiels and Farrell, 1997), but did not decrease in direct proportion to the increase in  $f_H$  (Fig. 1). Thus, the duration of the cardiac cycle that comprised shortening became an increasingly larger fraction of the cycle period, while the duration of lengthening decreased (Figs. 1 and 6). As a consequence, at 14°C, where the duration of the twitch was relatively long, there was very little time available for lengthening at higher  $f_H$  (Figs. 1 and 6). In fact, at higher  $f_H$ s, the time available for lengthening was unrealistically brief for a functioning heart, and this suggests that there is a constraint on the ability of the trout heart to function at cool temperatures and high  $f_H$ . However, at 22°C, the duration of the cardiac twitch was much briefer than at 14°C (Fig. 1). Thus, the duration of the cardiac cycle that comprised shortening was relatively brief, and this provided a longer timeframe for muscle lengthening (Figs. 1 and 6); even at the highest contraction rates the heart could sustain. This very short period of time available for lengthening at the cooler temperature (14°C) also had the effect of imposing very high rates of muscle lengthening, as compared to those at 22°C (Fig. 7); to the extent that there was no effect of  $f_H$  on lengthening work at 22°C, but a very large effect at 14°C (Fig. 3). As a result, when warm, there is considerable scope to increase  $f_H$  up to the maximum that the myocardium can sustain, with little impact on lengthening work or diastolic filling time. In contrast, when the fish is at cooler temperatures, one might expect that the limited time available for cardiac filling, and the resultant high amounts of work required to lengthen the myocardium (fill the ventricle), would greatly limit maximal  $f_H$ , despite an ability of the myocardium itself to contract at higher rates. Thus, increasing strain ( $V_S$ ) might be an alternative to elevating  $f_H$  as a means to increase  $Q$  at cooler temperatures (assuming that filling pressures are sufficient to accommodate an increase in end diastolic volume). This hypothesis is supported by observations that salmon increase  $V_S$  to a much greater extent than  $f_H$  when exercised at 'cool'/lower temperatures (Steinhausen et



al., 2008), and steelhead trout increase  $V_S$  with increased temperature when  $f_H$  is pharmacologically limited to 60 BPM (Keen and Gamperl, 2012). Thus, while fish are capable of increasing  $Q$  exclusively through increased  $f_H$  when exposed to an acute temperature increase, they can use increases in  $V_S$  to elevate  $Q$  when  $f_H$  is constrained at lower temperatures or by pharmacological intervention.

A caveat with the ability of the heart to increase  $V_S$  as a means to enhance  $Q$  is that CVP (which is responsible for 2/3 of cardiac filling in fishes; Farrell and Smith, 2017) would need to increase, or at least remain constant, to support increased  $Q$  as temperature increases. CVP has only been measured in salmonids from 10 – 16°C (Sandblom and Axelsson, 2006). Clearly, additional measurements related to the effects of altering  $V_S$  and  $f_H$  on cardiac filling are needed before we can fully understand the relationship between changes in temperature and the contributions of  $f_H$  and  $V_S$  to changes in  $Q$ . For example, we expect that the slowing of  $f_H$  following zatebradine injection (Keen and Gamperl, 2012) and the concomitant increase in filling time and CVP, are critical to the capacity of fish treated with this pharmacological agent to increase  $V_S$  when exposed to acute increases in temperature. An additional limitation of interpreting the effects of  $f_H$  and  $V_S$  on the work required to extend the myocardium in this study is that, even though the duration of the lengthening trajectory imposed on the myocardial strips was adjusted to reflect changes in the duration of the twitch at different temperatures and contraction rates, it was still a sinusoidal trajectory. Therefore, it would not reflect phasic filling of the heart if it occurred (e.g., venous filling vs. active atrial contraction vs. *visa fronte* filling). Rapid phasic filling would likely augment the limitations imposed by increased filling/lengthening work, but active atrial contraction would provide some reprieve from filling limitations, including reduced times required for diastolic filling at cooler temperatures. We aren't aware of any studies that would provide further insight into these questions.

### ***Perspectives and Significance***

This study provides novel, and important, mechanistic information that addresses the question of why fish only increase  $f_H$  as a means to elevate  $Q$  when faced with acute increases in temperature to their  $CT_{Max}$ . Further, it provides several testable hypotheses about the role of increases in  $V_S$  versus  $f_H$  in enabling  $Q$  to meet increased  $O_2$  requirements in temperate fish species at cold temperatures, and likely other ectotherms where temperature would have

similar impacts on myocardial contractile mechanics. However, additional studies/measurements related to the effects of altering  $V_S$  and  $f_H$  on cardiac filling pressure, and on the effects of acute temperature changes on circulating catecholamine levels and cardiac  $\beta$ -adrenergic sensitivity/responsiveness, are needed before we can fully understand the relationship between changes in temperature and how  $Q$  is modulated. Such information is critical to improving our understanding of how cardiac function and blood  $O_2$  transport will/may be constrained in fish exposed to heat waves and/or 'cold shocks'. Both of these environmental perturbations are increasing in frequency and severity with climate change, and can impact fish survival and distribution (Szekeres et al., 2016; Johnson et al. 2018; Frölicher et al., 2018, Cheung and Frölicher, 2020).

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**DATA AVAILABILITY:** The raw data is available from the authors upon request

**AUTHOR CONTRIBUTIONS:** A.K.G. and D.A.S. conceived and designed the research; A.L.T. and D.A.S. performed the experiments and analyzed the data; A.K.G and D.A.S. interpreted the results of the experiments; drafted the manuscript, and edited and revised the manuscript. All authors approved the final version of manuscript.

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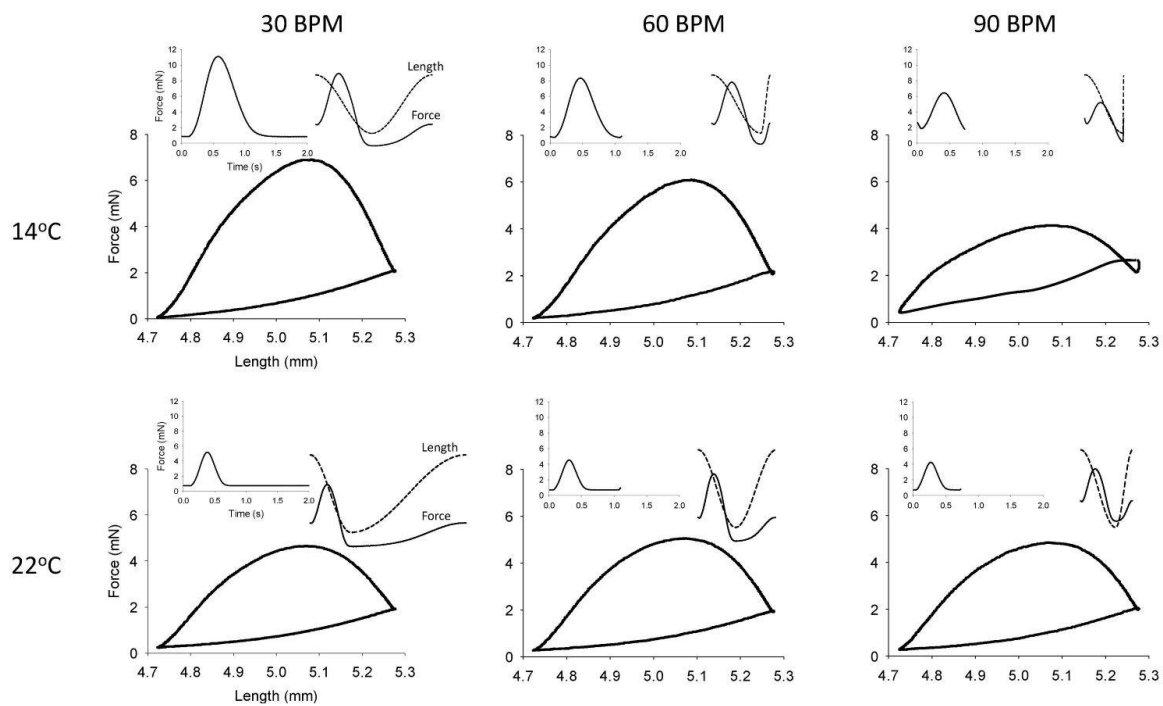
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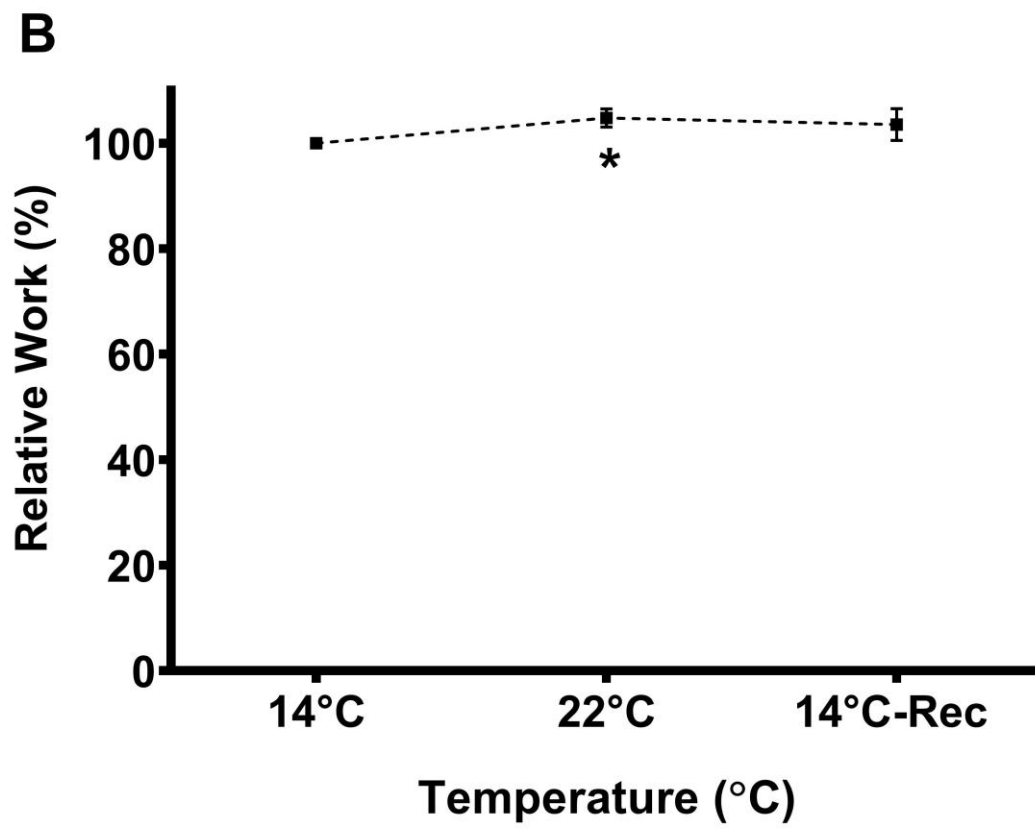
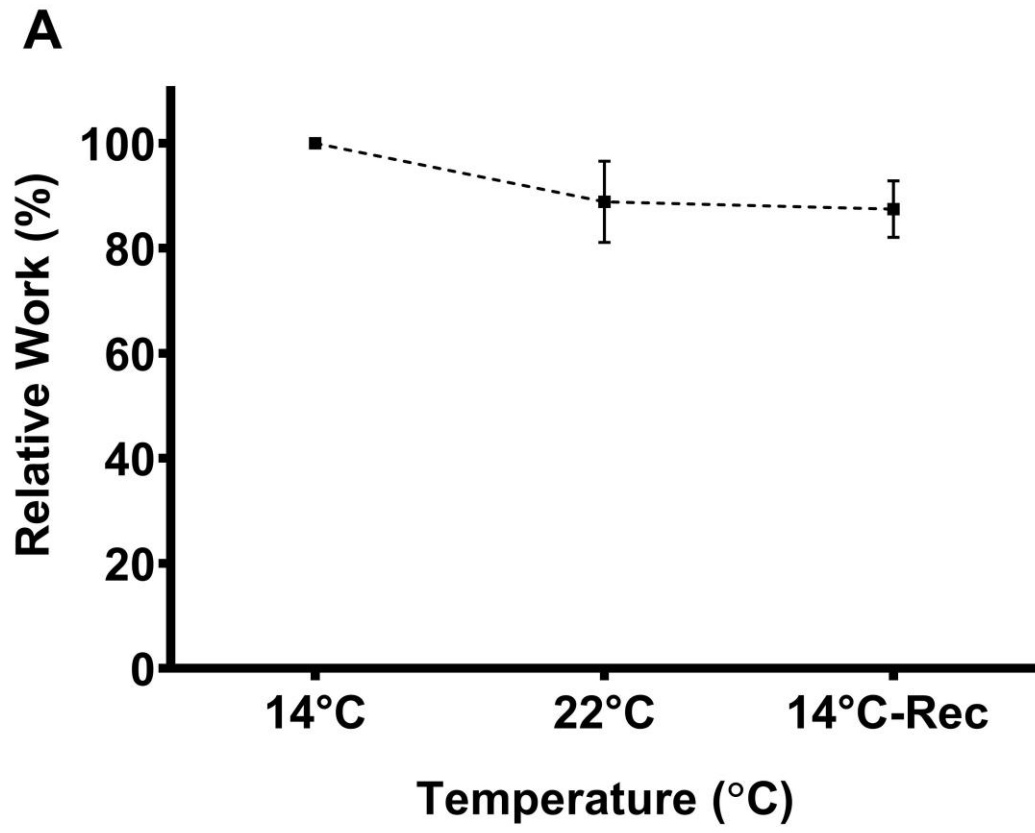
## Figures



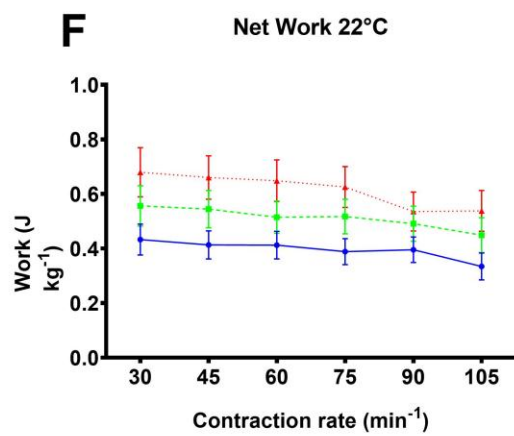
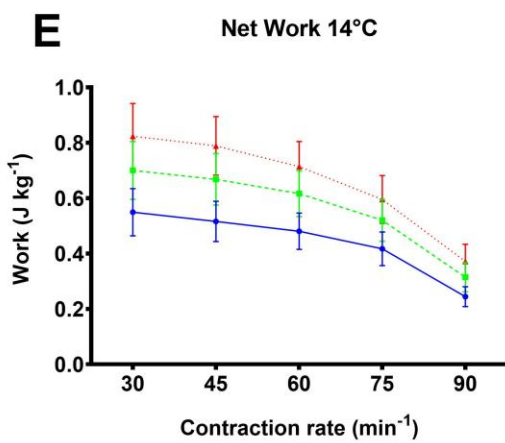
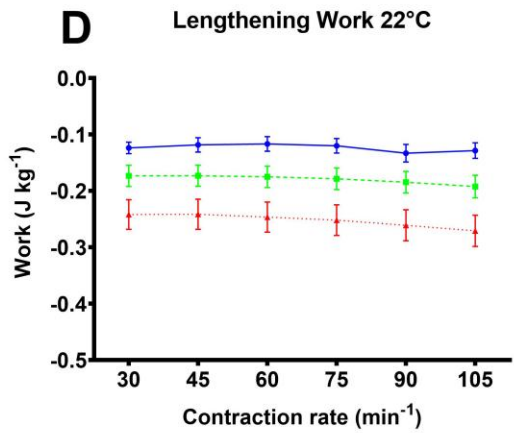
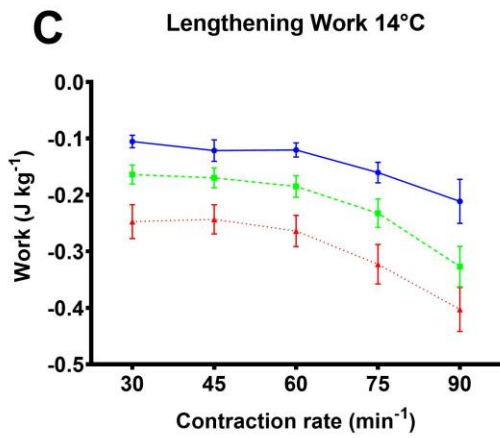
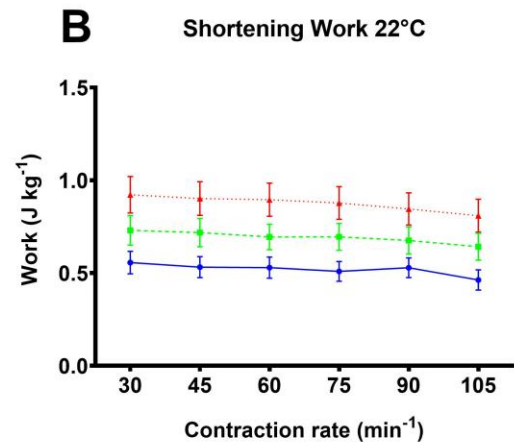
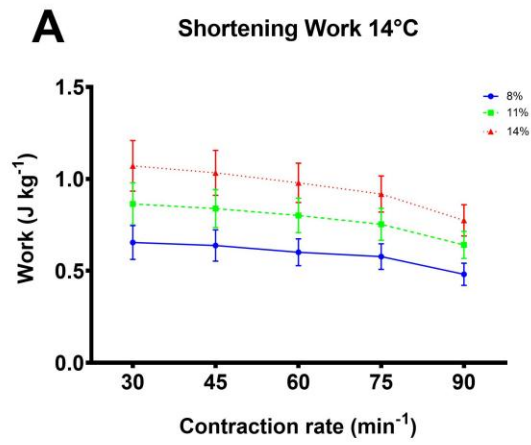
**Figure 1. Example work loops from the spongy ventricular myocardium of steelhead trout.**

Trabeculae were paced at 30 (left), 60 (centre) and 90 (right) contractions per minute at 14°C (upper row) and 22°C (lower row), all at a strain of 11%. Insets at upper left of each panel show isometric twitch force at the attendant combination of temperature and contraction rate. These were used for calculation of the strain trajectory imposed during the work loops. Insets in the upper right of each panel are the imposed strain trajectory (i.e., muscle length, broken line) and force produce by the muscle (solid line) during the work loop shown. For the example shown, resting muscle length was 5.0 mm and the mass of the muscle preparation was 1.89 mg.

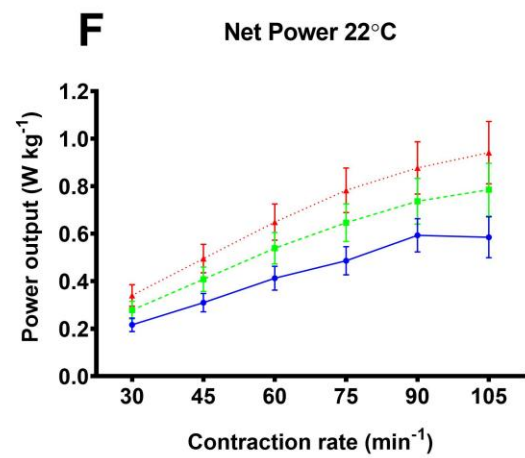
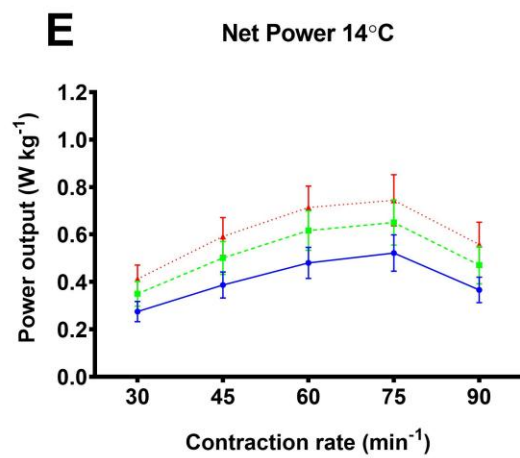
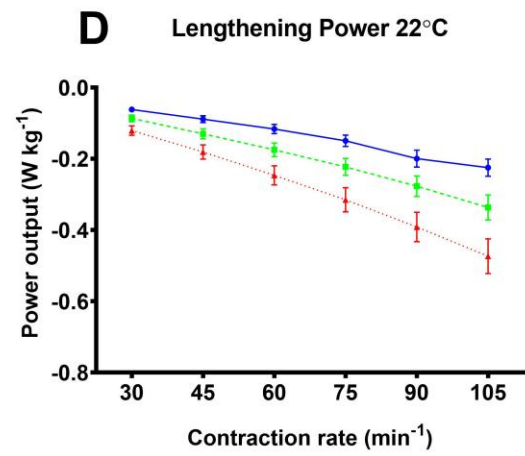
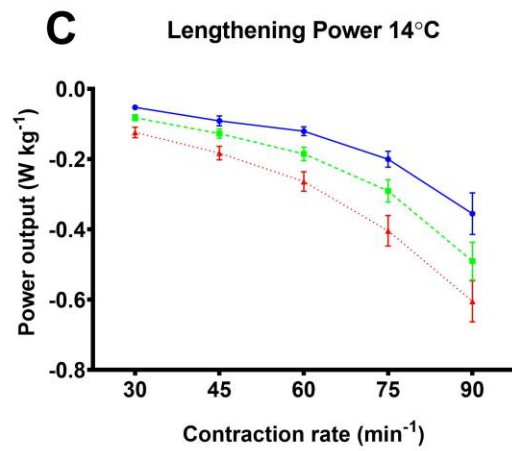
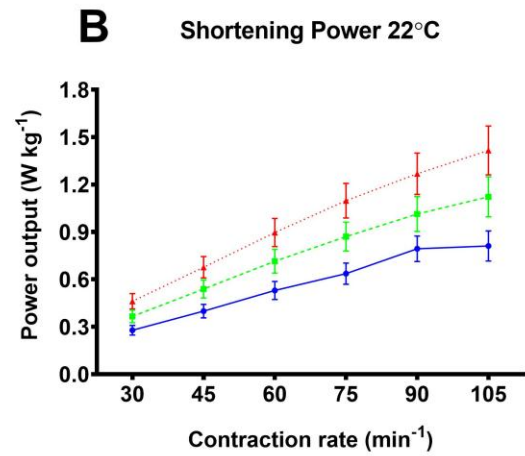
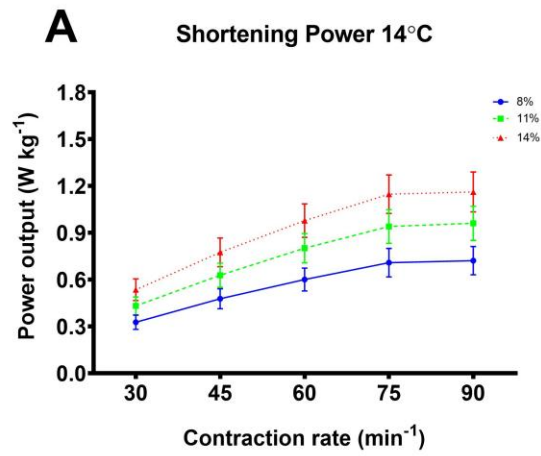




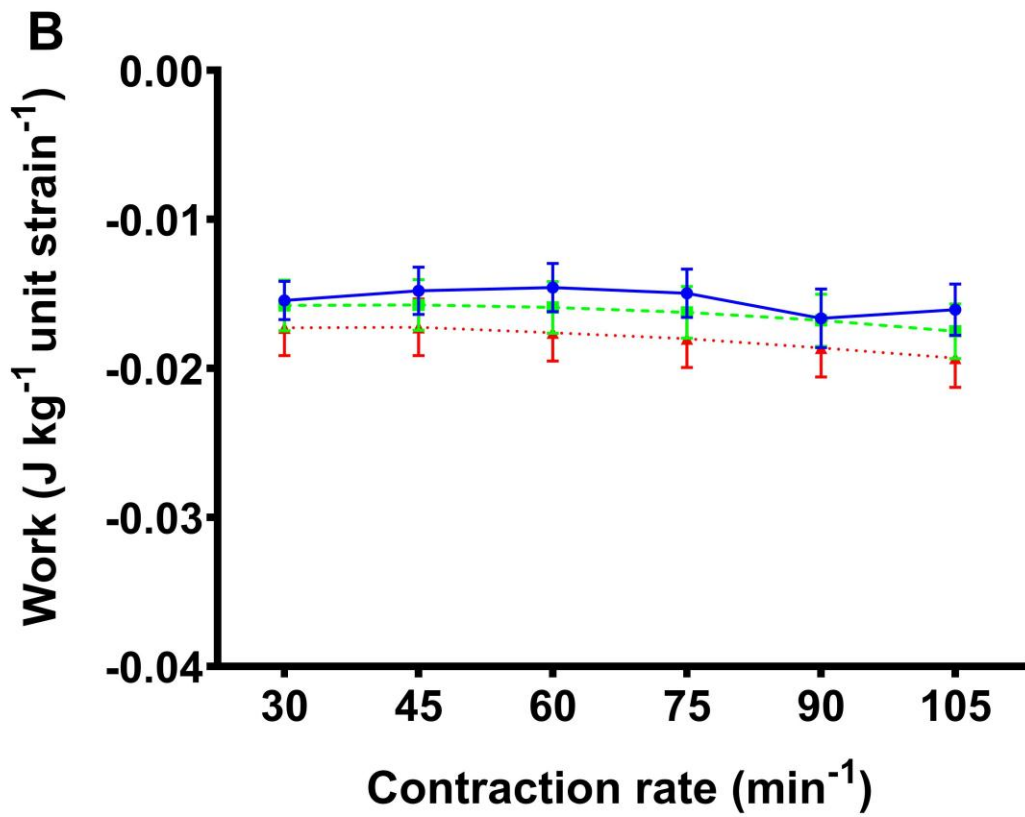
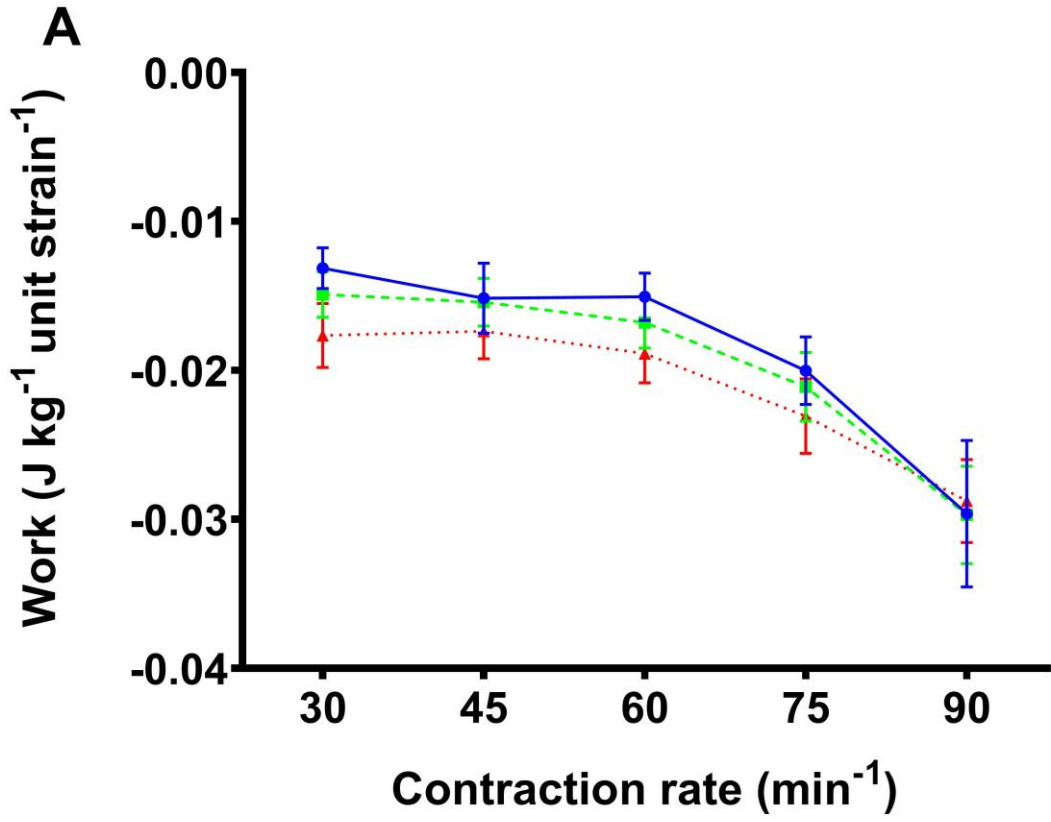
**Figure 2: Work Performed by the muscle over the experiment.** Shortening (A) and lengthening (B) work from rainbow trout ventricular trabeculae at 30 contractions  $\text{min}^{-1}$  and 11% strain amplitude over the duration of the experiment. Data are shown relative to the work done at 14°C during the initial phase of the experiment. Values at 22°C were recorded midway through the experiment, and 14°C-Rec indicates measurements at 14°C that were made at the end of the experiment after the measurements at all temperatures, strains and rates were complete. An asterisk indicates a significant difference ( $P < 0.05$ ) as compared to initial values. This statistical analysis was performed on the absolute values of work at each time point. Values are means  $\pm$  SEM, N = 10.



**Figure 3: Effects of contraction rate, strain amplitude and temperature on myocardial work.** Shortening (A and B), lengthening (C and D) and net (E and F) work done per cycle by rainbow trout ventricular trabeculae at different contraction rates and strain amplitudes (8%, blue solid; 11%, green dashed; and 14%, red dotted) at 14°C and 22°C. There were significant strain and contraction rate effects, summarized in Table 1. Values are means  $\pm$  SEM, N = 10.

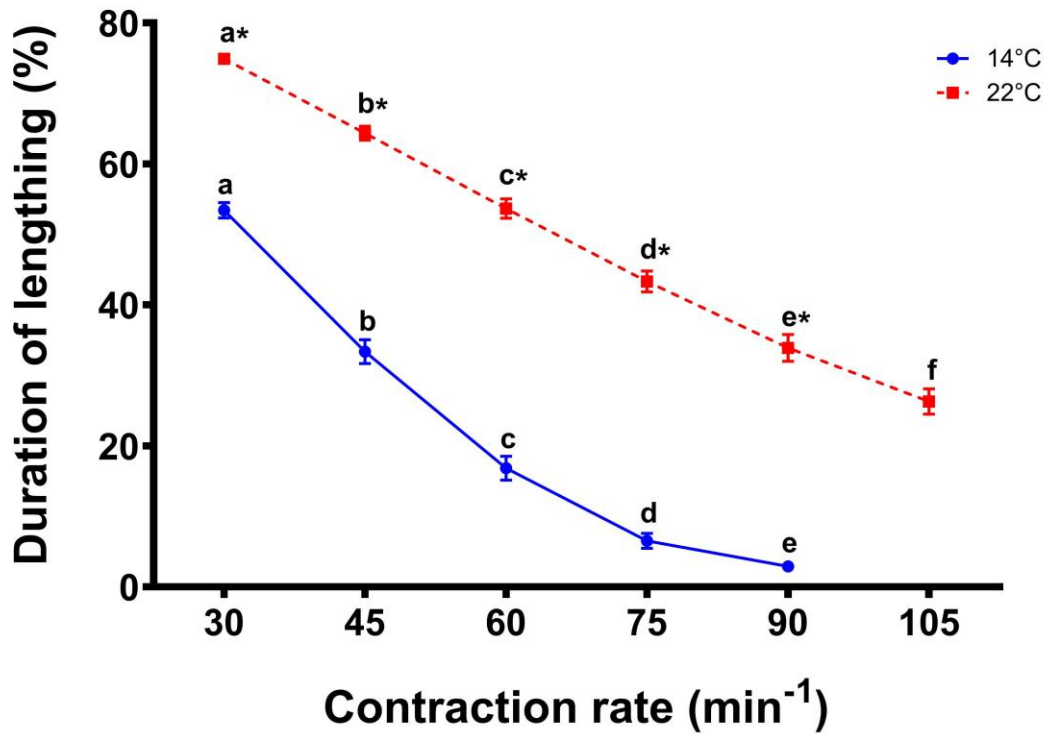
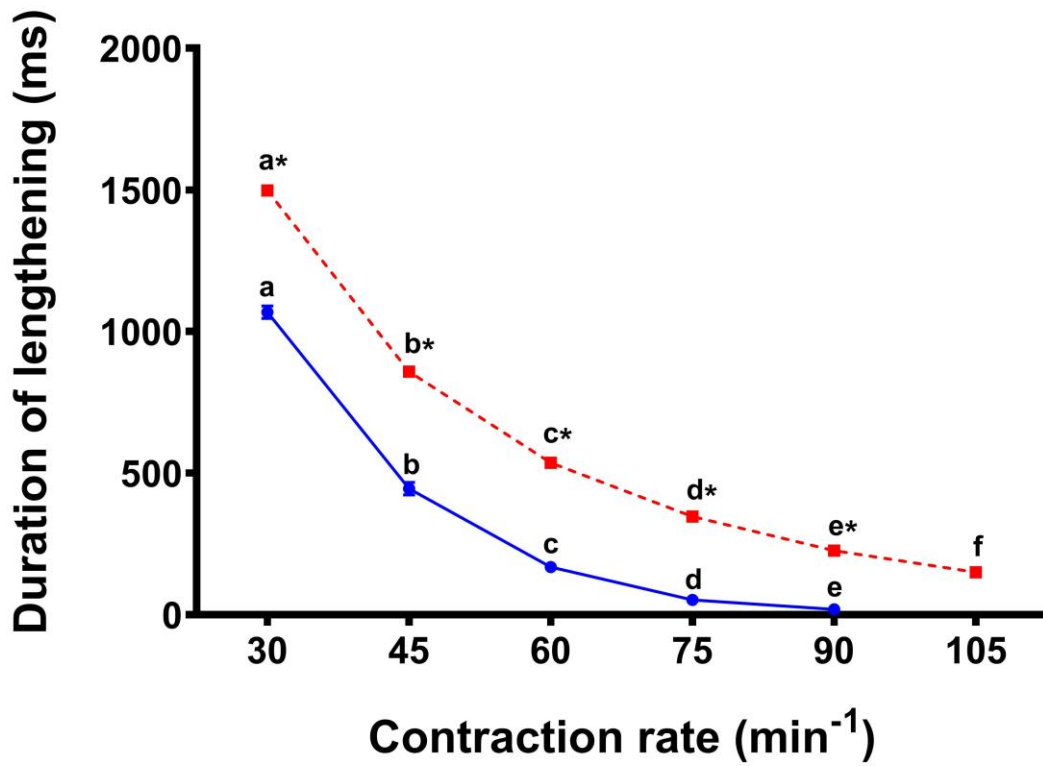


**Figure 4: Effects of contraction rate, strain amplitude and temperature on myocardial power.** Shortening (A and B), lengthening (C and D) and net (E and F) power from rainbow trout ventricular trabeculae at different contraction rates and strain amplitudes (8%, blue solid; 11%, green dashed; and 14%, red dotted) at 14°C and 22°C. There were significant strain and contraction rate effects, summarized in Table 1. Values are means  $\pm$  SEM, N = 10.

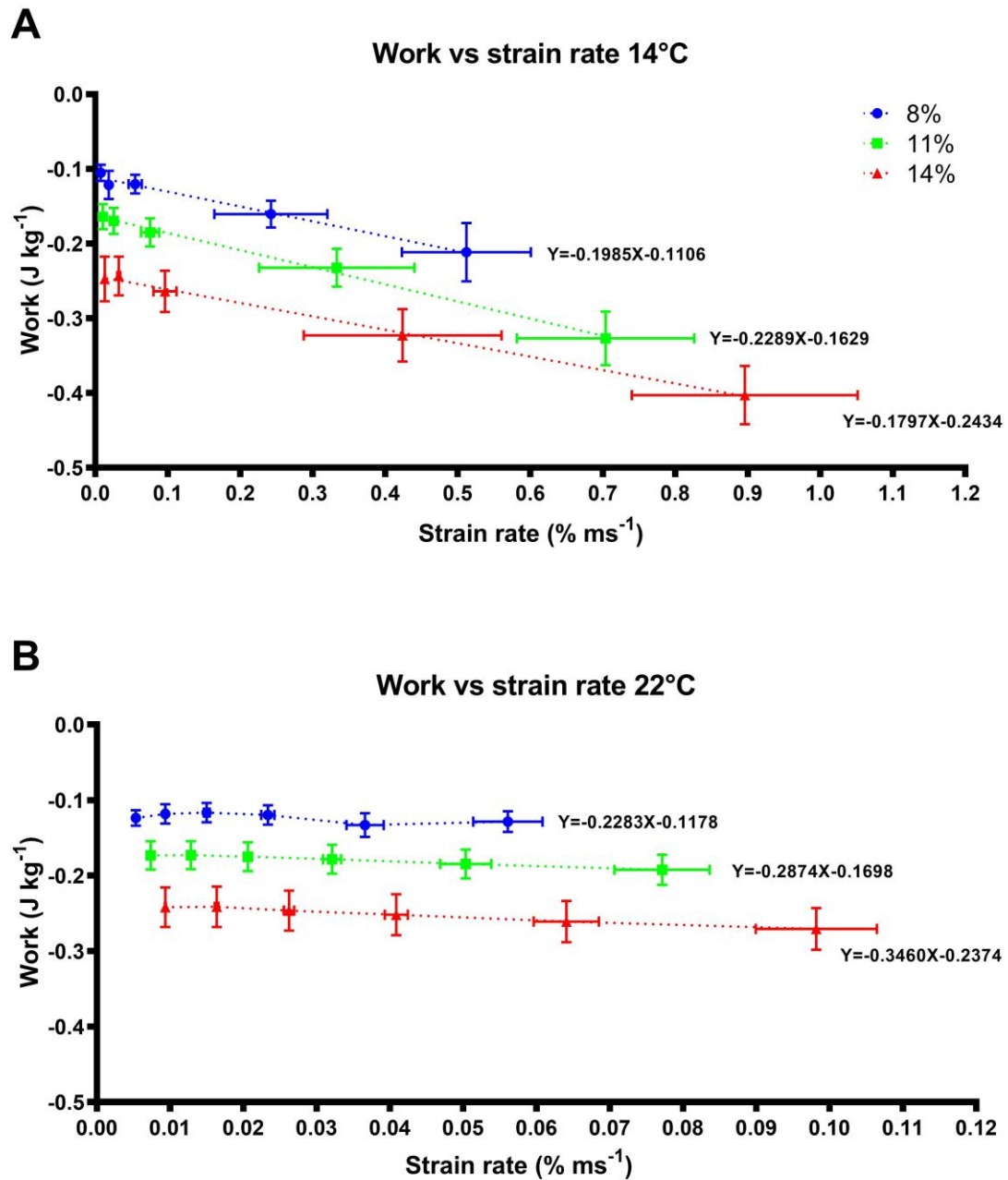


**Figure 5: Temperature-dependent effects on lengthening work.** Lengthening work / unit strain amplitude per cycle from rainbow trout ventricular trabeculae at different contraction rates ( $\text{min}^{-1}$ ) and strain amplitudes (8%, blue solid; 11%, green dashed, and 14 %, red dotted), at 14°C (A) and 22°C (B). There were significant strain and contraction rate effects, summarized in Table 2. Values are means  $\pm$  SEM, N = 10.



**A****B**

**Figure 6: Duration of the strain cycle attributed to lengthening.** Data are expressed as a percentage of the whole cycle (A) and in milliseconds (B) at different contraction rates and two different temperatures (14°C blue solid and 22°C red dashed) from rainbow trout ventricular trabeculae. Within a temperature, symbols without a letter in common are significantly different ( $P < 0.05$ ). Asterisks (\*) indicate significant differences between temperatures at a specific contraction rate. Values are means  $\pm$  SEM, N = 10.



**Figure 7: Effects of lengthening strain rate (in  $\% \text{ ms}^{-1}$ ) on lengthening work of rainbow trout ventricular trabeculae.** Strips were tested at different contraction rates and strain amplitudes (8% blue, 11% green and 14% red) at 14°C (A) and 22°C (B). Note the order of magnitude difference between the strain rate axes at the two temperatures. The equations in both panels are linear regressions based on the individual data points. In panel A all the slopes are significant ( $P < 0.01$ ), and while the intercepts of the lines are significantly different between strains, the slopes are not different. In panel B, none of the regressions have significant slopes, but the intercepts are significantly different. Values are means  $\pm$  SEM of data pooled at each contraction rate,  $N = 10$ .

**Table 1:** Results of the split plot mixed general linear model analysis that examined the effects of contraction rate, strain amplitude and test temperature on myocardial shortening, lengthening and net work and power. This data is shown in Figures 2 and 3.

<b>Parameter</b>	<b>Factor(s)</b>	<b>F</b>	<b>d.f.</b>	<b>P</b>
<b>Shortening Work</b>	Temperature	32.309	1	$3.51 \times 10^{-8}$
	Strain	321.445	2	$< 2 \times 10^{-16}$
	Rate	17.965	4	$4.8 \times 10^{-13}$
	Temperature*Strain	0.108	2	0.898
	Strain*Rate	0.578	8	0.796
	Temperature*Rate	7.604	4	$8.27 \times 10^{-6}$
	Temperature*Strain*Rate	0.152	8	0.996
<b>Lengthening Work</b>	Temperature	83.756	1	$< 2 \times 10^{-16}$
	Strain	370.252	2	$< 2 \times 10^{-16}$
	Rate	56.029	4	$< 2 \times 10^{-16}$
	Temperature*Strain	2.193	2	0.114
	Strain*Rate	0.507	8	0.85
	Temperature*Rate	38.079	4	$< 2 \times 10^{-16}$
	Temperature*Strain*Rate	0.369	8	0.936
<b>Net Work</b>	Temperature	5.921	1	0.0156
	Strain	122.914	2	$< 2 \times 10^{-16}$
	Rate	46.327	4	$< 2 \times 10^{-16}$
	Temperature*Strain	0.174	2	0.8406
	Strain*Rate	0.907	8	0.5111
	Temperature*Rate	23.955	4	$< 2 \times 10^{-16}$
	Temperature*Strain*Rate	0.237	8	0.9837
<b>Shortening Power</b>	Temperature	11.893	1	0.000657
	Strain	240.237	2	$< 2 \times 10^{-16}$
	Rate	265.171	4	$< 2 \times 10^{-16}$
	Temperature*Strain	0.086	2	0.917239
	Strain*Rate	4.756	8	$1.82 \times 10^{-5}$
	Temperature*Rate	5.547	4	0.000267
	Temperature*Strain*Rate	0.116	8	0.998632
<b>Lengthening Power</b>	Temperature	74.827	1	$5.33 \times 10^{-16}$
	Strain	167.149	2	$< 2 \times 10^{-16}$
	Rate	283.838	4	$< 2 \times 10^{-16}$
	Temperature*Strain	1.278	2	0.28
	Strain*Rate	7.005	8	$2.26 \times 10^{-8}$
	Temperature*Rate	35.816	4	$< 2 \times 10^{-16}$
	Temperature*Strain*Rate	0.347	8	0.946
<b>Net Power</b>	Temperature	0.562	1	0.454
	Strain	105.035	2	$< 2 \times 10^{-16}$
	Rate	99.873	4	$< 2 \times 10^{-16}$
	Temperature*Strain	0.472	2	0.624
	Strain*Rate	1.229	8	0.282
	Temperature*Rate	31.752	4	$< 2 \times 10^{-16}$
	Temperature*Strain*Rate	0.319	8	0.958

**Table 2.** Lengthening work / unit strain amplitude ( $\text{J kg}^{-1} \% \text{ strain}^{-1} \text{ cycle}^{-1}$ ) from rainbow trout ventricular trabeculae at different contraction rates and strain amplitudes at 14 and 22°C.

	Contraction rate ( $\text{min}^{-1}$ )					
	30	45	60	75	90	105
<b>14°C</b>						
Strain 8%	-0.0131±0.001 <sup>aA</sup>	- 0.0152±0.002 <sup>abcA</sup> B	- 0.0151±0.002 <sup>ba</sup>	-0.0200±0.002 <sup>cdA</sup>	-0.0296±0.005 <sup>dA</sup>	
11%	-0.0149±0.002 <sup>aB</sup>	-0.0154±0.002 <sup>aA</sup>	-0.0168±0.002 <sup>bB</sup>	-0.0211±0.002 <sup>cB</sup>	-0.0297±0.003 <sup>dA</sup>	
14%	- 0.0177±0.002 <sup>abc</sup> B	-0.0174±0.002 <sup>aB</sup>	-0.0189±0.002 <sup>bC</sup>	-0.0231±0.002 <sup>cC</sup>	-0.0288±0.003 <sup>dA</sup>	
<b>22°C</b>						
Strain 8%	- 0.0154±0.001 <sup>abc</sup> dAB	- 0.0148±0.002 <sup>abcd</sup> A	- 0.0146±0.002 <sup>acA</sup>	-0.0150±0.002 <sup>bcA</sup>	-0.0166±0.002 <sup>cdAB</sup>	- 0.0161±0.002 <sup>dA</sup>
11%	- 0.0158±0.002 <sup>abc</sup> A	-0.0157±0.002 <sup>aA</sup>	-0.0159±0.002 <sup>aB</sup>	-0.0162±0.002 <sup>bB</sup>	-0.0168±0.002 <sup>cA</sup>	-0.0175±0.002 <sup>dB</sup>
14%	- 0.0173±0.002 <sup>abc</sup> dB	-0.0172±0.002 <sup>aB</sup>	-0.0176±0.002 <sup>bC</sup>	-0.0180±0.002 <sup>cC</sup>	-0.0186±0.002 <sup>dB</sup>	-0.0193±0.002 <sup>eC</sup>

Values are means  $\pm$  SEM, N = 10. Within a strain, values without a lower-case letter in common are significantly different ( $P < 0.05$ ). Values without a capital letter in common, within a contraction rate/temperature combination, indicate a significant difference between strains.

**Table 3:** Comparison of the effects of contraction rate and strain amplitude on lengthening work ( $\text{J kg}^{-1} \text{ beat}^{-1}$ ) and shortening power ( $\text{W kg}^{-1}$ ) generated by trout myocardial strips.

$^{\circ}\text{C}$	Test Condition	Lengthening Work	Shortening Power
14	60 $\text{min}^{-1}$ , 8% Strain	$-0.120 \pm 0.013$	$0.601 \pm 0.073$
22	105 $\text{min}^{-1}$ , 8% Strain	$-0.128 \pm 0.014$	$0.810 \pm 0.095^*$
22	60 $\text{min}^{-1}$ , 12% Strain	$-0.199 \pm 0.022^{*+}$	$0.774 \pm 0.080^*$

Strips performed at 60 contractions  $\text{min}^{-1}$  and 8% strain at  $14^{\circ}\text{C}$  (resting cardiac function at this temperature), and at  $22^{\circ}\text{C}$  under two specific conditions: 105 contractions  $\text{min}^{-1}$  and 8% strain representing normal cardiac function at  $22^{\circ}\text{C}$ ; and 60 contractions  $\text{min}^{-1}$  and 12% strain representing cardiac function at  $22^{\circ}\text{C}$  when heart rate is prevented from increasing. The value for 12% strain (which we calculate is the strain at maximum stroke volume in rainbow trout; see methods) was interpolated from the 11 and 14% values at this temperature. Asterisks (\*) indicate a significant difference between values at  $22^{\circ}\text{C}$  and the value at  $14^{\circ}\text{C}$ . A plus sign (+) indicates a significant difference between values at 105 contractions  $\text{min}^{-1}$  and 8% strain vs. 60  $\text{min}^{-1}$  and 12% strain at  $22^{\circ}\text{C}$ . Significant ( $P < 0.05$ ) differences were identified using repeated measures ANOVAs and Holm-Sidak post-hoc tests. Values are means + SEM,  $N = 10$ .