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RESEARCH ARTICLE

Can temperature-dependent changes in myocardial contractility explain why fish only increase heart rate when exposed to acute warming?

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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^{-1}, 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, 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.

KEY WORDS: Fish, Thermal tolerance, Diastolic filling, Contraction frequency, Stroke volume, Lengthening work

INTRODUCTION

Temperature has been described as the 'ecological master factor' for fish (Brett, 1965; Farrell et al., 2007) as it affects numerous physiological processes including the functional capacity of the cardiovascular system (Eliasson and Anttila, 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 (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 the result of a combination of factors, including a loss of ventricular excitability due to disruptions in cardiomyocyte ionic currents (Haverinen and Vornanen, 2006, 2020), a decrease in the efficiency of mitochondrial oxidative phosphorylation (Christen et al., 2018; Gerber et al., 2020, 2021; Iftikar and Hickey, 2013; Penney et al., 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). These data suggest that there are physiological constraints that prevent increases in V_S as temperature and f_H increase, that fish preferentially increase f_H over V_S, or that increases in f_H are inescapable as a result of some aspect of the direct effect of temperature on the heart.

With regards to whether there is a reason why fish preferentially increase f_H over V_S (i.e. whether 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 (P_{O_2} values) whether tested at low f_H (35 beats min^{-1}) and high V_S (8% strain) versus 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 to 115 beats min^{-1}, the normal maximum f_H in this species is 75 beats min^{-1}). These data suggest that lengthening work (the work 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 fish, 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

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contribution of venous pressure (preload) to cardiac filling in fish 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 *Journal of Experimental Biology* (Predicting the Future: Species Survival in a Changing World), 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 the present study indicate 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 critical thermal maximum (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.

MATERIALS AND METHODS
Animal husbandry
All procedures were approved by the animal care committees of the Memorial University of Newfoundland and Labrador (MUN) and the University of Calgary, and followed CCAC guidelines. Adult steelhead trout, *Oncorhynchus mykiss* (Walbaum 1792), were sourced from Nova Fish Farms Inc. and initially housed in a circular, 3000 l, tank supplied with aerated 14°C seawater for approximately 2 months in the Dr Joe Brown Aquatic Research Building at the Ocean Sciences Centre (MUN; St John's, NL, Canada). A 12 h light:12 h dark photoperiod was maintained

throughout, and the fish were fed a commercial pelleted trout diet 3 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 mean±s.e.m.) 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 fibres 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, USA). 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, USA) 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 LabView (National Instruments, Austin, TX, USA) 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 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. 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 min^{-1}). The order in which the series of contraction rates was tested (low-to-high versus 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, as a percentage). A strain of 8% is the muscle strain measured at resting stroke volume (V_S=0.4 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

values estimated at maximum V_S in trout (1.3 ml g^{-1} ventricle, ~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 as a result of the inherent variability between fish and the varying orientation of trabeculae *in vivo* (Sanchez-Quintana et al., 1995). Experiments were performed at 14 and 22°C; however, a contraction rate of 105 min^{-1} 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 beats min^{-1} at the beginning of the experiment at 14°C, 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 composed 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 (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: 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: (total period of one cycle−period of shortening)/(2×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
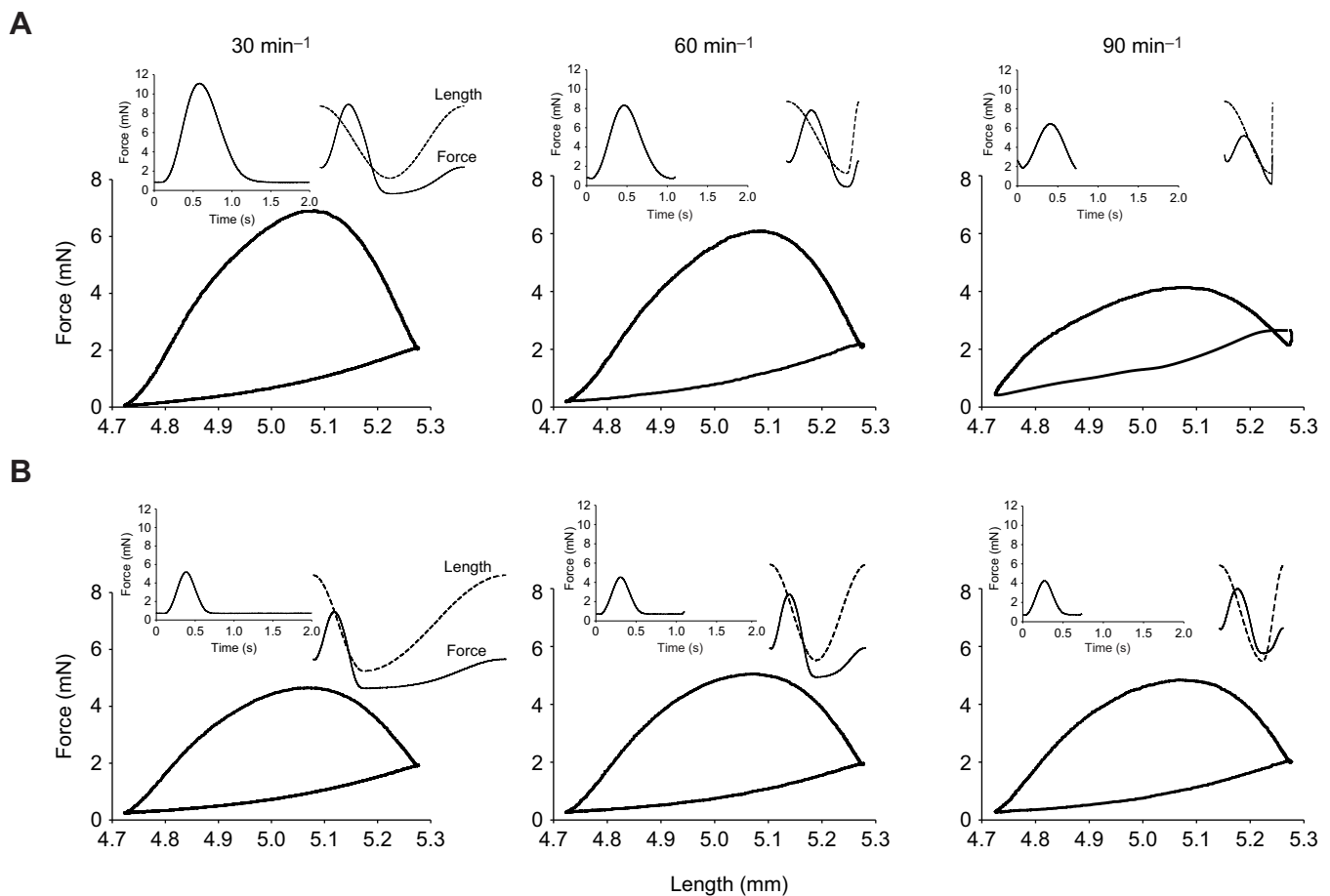

Fig. 1. Example work loops from the spongy ventricular myocardium of steelhead trout. Trabeculae were paced at (from left to right) 30, 60 and 90 contractions per minute at 14°C (A) and 22°C (B), all at a strain of 11%. Insets in the upper left of each panel show isometric twitch force at the attendant 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, dashed line) and force produced 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.

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 J kg^{-1} of muscle mass, and power as W 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, USA).

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 a contraction rate of 30 min^{-1} (i.e. to test for any loss of contractile performance over the experiment), were examined using one-way repeated measures ANOVA followed by Dunnett's tests (Fig. 2). Split-plot mixed general linear models, and the R statistical package (version 3.22; http://www.R-project.org/), 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, Figs 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.

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

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

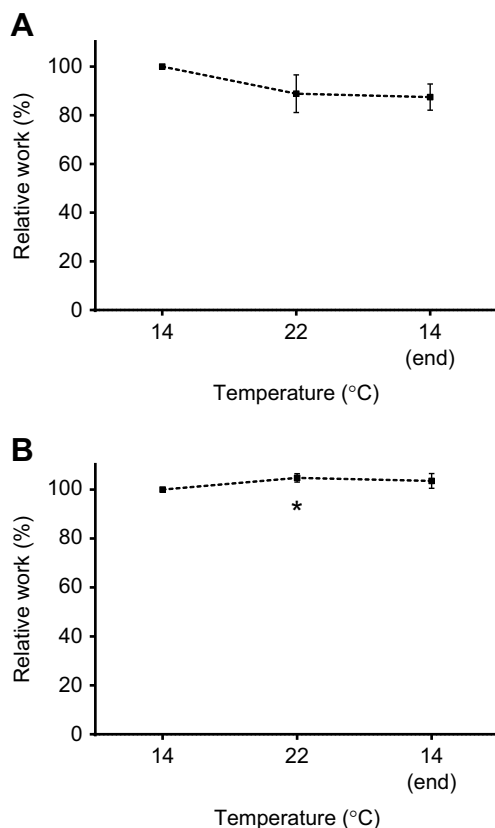The data are shown in Figs 2 and 3.

Fig. 2. Work performed by the muscle over the experiment. Shortening (A) and lengthening (B) work from rainbow trout ventricular trabeculae at 30 contractions 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. Preparations were then returned to 14°C at the end of the experiment after the measurements at all temperatures, strains and rates were complete, to assess their stability. The asterisk indicates a significant difference (P<0.05) as compared with initial values. This statistical analysis was performed on the absolute values of work at each time point. Values are means±s.e.m., N=10.

Values of lengthening work done per unit strain amplitude at each temperature (Table 2, Fig. 5) were analysed using a two-way repeated measures ANOVA (with controlled variables contraction rate and strain). This was followed by one-way repeated measures ANOVA and Tukey's HSD tests. Differences in the relationship between lengthening work and strain rate at the three different strain amplitudes (8%, 11%, 14%; 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 ANOVA 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 min^{-1} and 8% strain (*in vivo* conditions), as compared with two particular strain/contraction rate combinations at 22°C, using repeated measures ANOVAs followed by Holm–Šidák tests (Table 3). These strain/rate combinations reflected possible *in vivo* conditions at warmer temperatures. The ANOVA, Tukey's HSD tests and t-tests were performed using Prism 8 (GraphPad Software, San Diego, CA, USA). $P<0.05$ was used as the level of statistical significance in all analyses, and all values in the text, tables and figures are means±s.e.m.
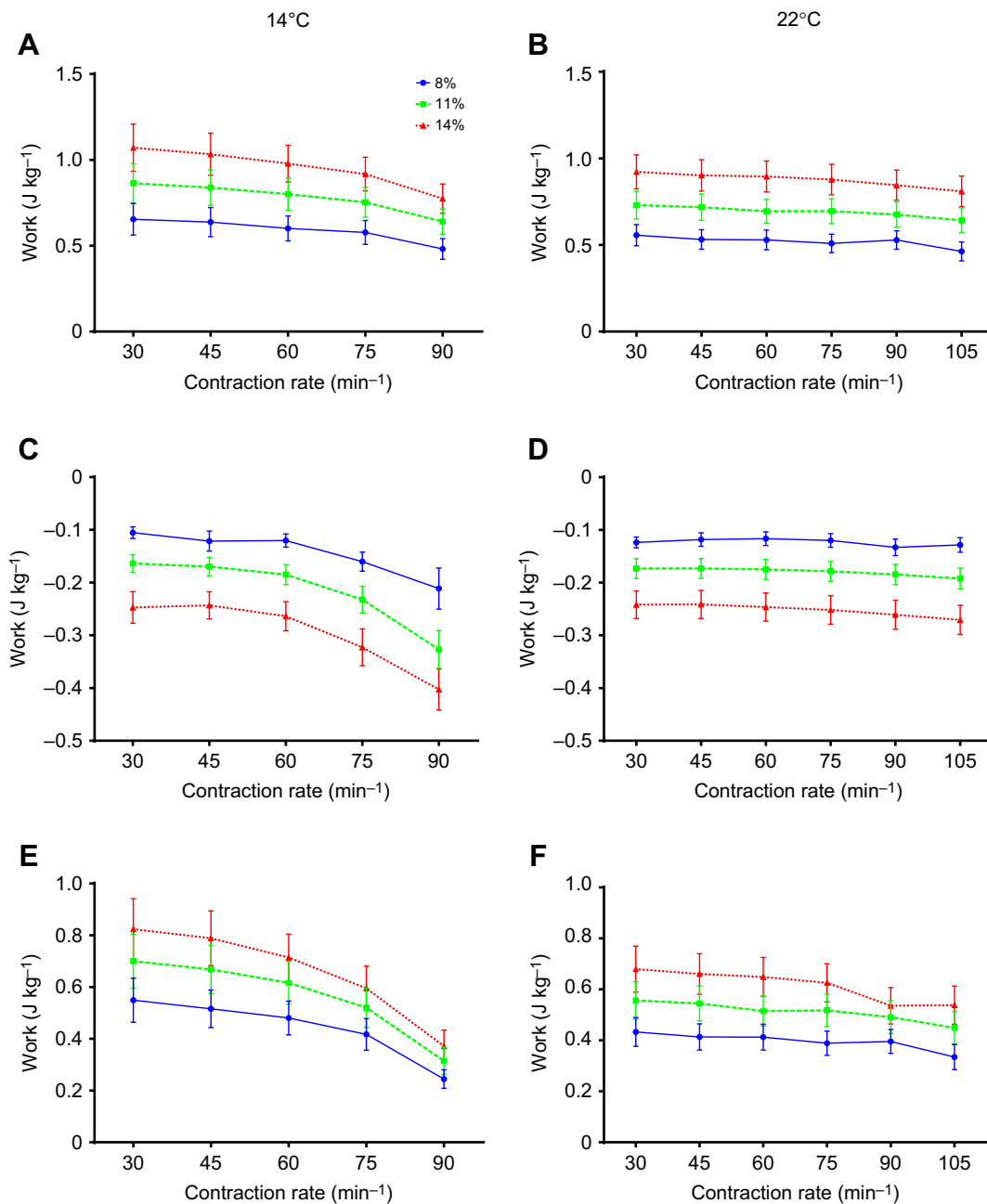

Fig. 3. Effects of contraction rate, strain amplitude and temperature on myocardial work. Shortening (A,B), lengthening (C,D) and net (E,F) work done per cycle by rainbow trout ventricular trabeculae at different contraction rates and strain amplitudes (8%, 11% and 14%) at 14°C (left) and 22°C (right). There were significant strain and contraction rate effects as summarized in Table 1. Values are means±s.e.m., $N=10$.

Table 2. Lengthening work per unit strain amplitude per cycle from rainbow trout ventricular trabeculae at different contraction rates and strain amplitudes at 14 and 22°C

Temperature	Strain	Lengthening work (J kg⁻¹ % strain⁻¹ cycle⁻¹)					
		30 min⁻¹	45 min⁻¹	60 min⁻¹	75 min⁻¹	90 min⁻¹	105 min⁻¹
14°C	8%	−0.0131±0.001[aA]	−0.0152±0.002[abcAB]	−0.0151±0.002[bA]	−0.0200±0.002[cdA]	−0.0296±0.005[dA]	
	11%	−0.0149±0.002[aB]	−0.0154±0.002[aA]	−0.0168±0.002[bB]	−0.0211±0.002[cB]	−0.0297±0.003[dA]	
	14%	−0.0177±0.002[abcB]	−0.0174±0.002[aB]	−0.0189±0.002[bC]	−0.0231±0.002[cC]	−0.0288±0.003[dA]	
22°C	8%	−0.0154±0.001[abcdAB]	−0.0148±0.002[abcdA]	−0.0146±0.002[acA]	−0.0150±0.002[bcA]	−0.0166±0.002[cdAB]	−0.0161±0.002[dA]
	11%	−0.0158±0.002[abcA]	−0.0157±0.002[aA]	−0.0159±0.002[aB]	−0.0162±0.002[bB]	−0.0168±0.002[cA]	−0.0175±0.002[dB]
	14%	−0.0173±0.002[abcdB]	−0.0172±0.002[aB]	−0.0176±0.002[bC]	−0.0180±0.002[cC]	−0.0186±0.002[dB]	−0.0193±0.002[eC]

Values obtained at the indicated contraction rates are means±s.e.m., N=10. Within a strain, values without a lowercase letter in common are significantly different ($P<0.05$). Within a contraction rate/temperature combination, values without a capital letter in common are significantly different between strains.

RESULTS

The shortening work performed by myocardial strips (at a reference strain of 11% and 30 contractions min⁻¹) at 14°C at the end of the experiment was not significantly different from 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% versus 8% strain, and about 63% greater at 14% versus 8% strain (Figs 3A,B and 4A,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 3C,D and 4C,D). For example: at 14°C, lengthening work and power were about 150% greater at 14% versus 8% strain at a contraction rate of 30 min⁻¹ and about 100% greater at 90 min⁻¹; and at 22°C, lengthening work and power were about 100% greater at 14% versus 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 was 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 3E,F and 4E,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 strain.

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 versus 22°C, particularly at higher contraction rates (Fig. 3A,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 min⁻¹, but then approached a plateau at 90 min⁻¹ (Fig. 4A), whereas at 22°C, power continued to increase in approximate proportion to contraction rate up to 105 min⁻¹ (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% versus 60%, respectively; Fig. 3C,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,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 min⁻¹), 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 versus 22°C (Fig. 3E,F, Table 1). As a result, net power at 14°C initially increased with contraction rate, but then attained a maximum at ∼75 min⁻¹ and subsequently declined. In contrast, net power at 22°C continued to increase with contraction rate, with little evidence of it approaching a maximum (Fig. 4E,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 per cent of the entire cycle and a few milliseconds in duration, while at 22°C, the lengthening period was significantly longer than that 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% ms⁻¹) were about 10-fold greater than at 22°C (0.01–0.1% ms⁻¹) (compare x-axes in Fig. 7A,B). This resulted in the effect of strain rate on lengthening work being decidedly (and significantly) negative at 14°C at all
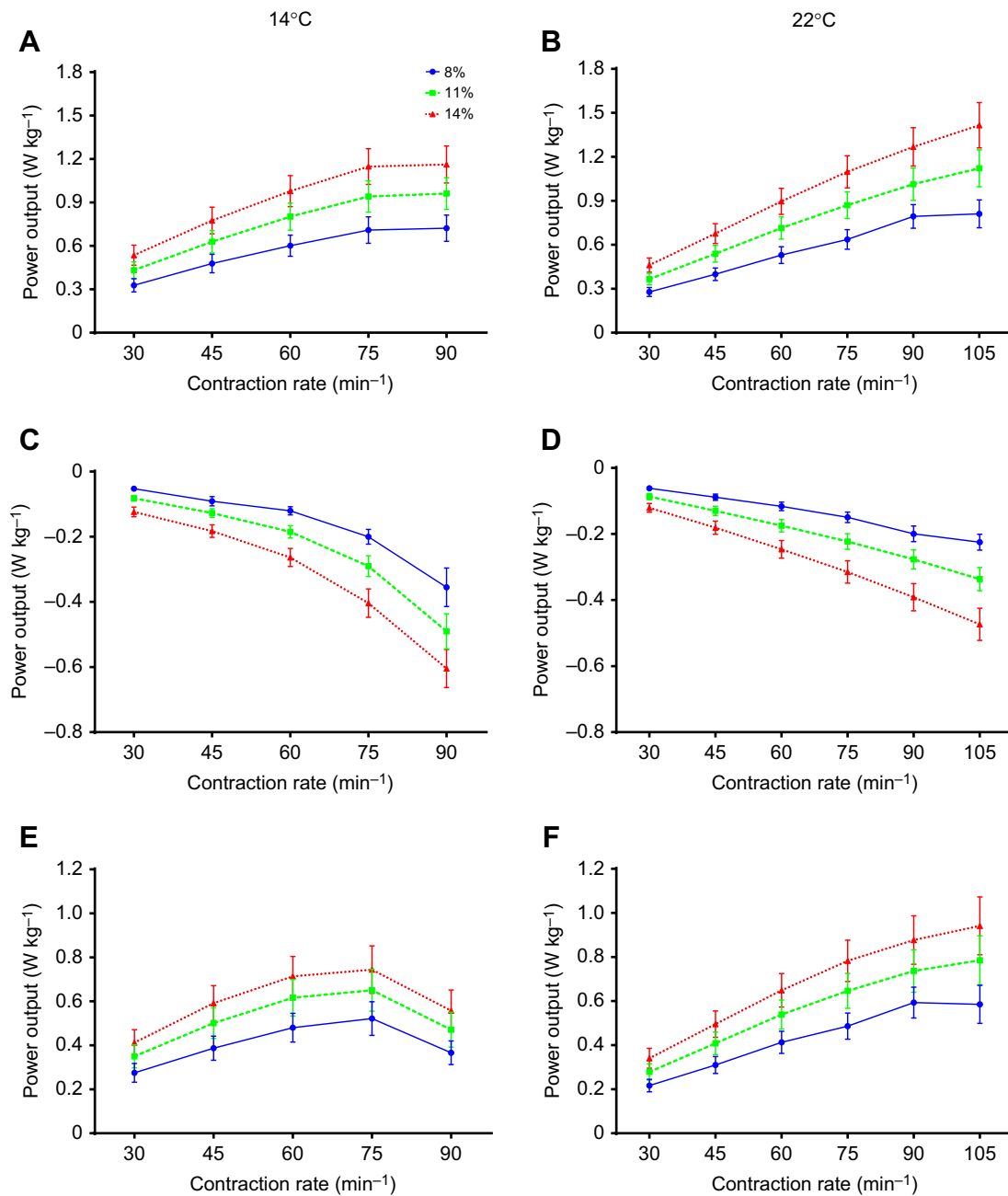
Fig. 4. Effects of contraction rate, strain amplitude and temperature on myocardial power. Shortening (A,B), lengthening (C,D) and net (E,F) power from rainbow trout ventricular trabeculae at different contraction rates and strain amplitudes (8%, 11% and 14%) at 14°C (left) and 22°C (right). There were significant strain and contraction rate effects as summarized in Table 1. Values are means±s.e.m., N=10.

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 min^{-1}, 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

Table 3. Comparison of the effects of contraction rate and strain amplitude on lengthening work and shortening power generated by trout myocardial strips

Temperature (°C)	Test condition	Lengthening work (J kg^{-1} beat^{-1})	Shortening power (W kg^{-1})
14	60 min^{-1}, 8% strain	−0.120±0.013	0.601±0.073
22	105 min^{-1}, 8% strain	−0.128±0.014	0.810±0.095*
22	60 min^{-1}, 12% strain	−0.199±0.022*,‡	0.774±0.080*

Strips performed at a contraction rate of 60 min^{-1} and 8% strain at 14°C (resting cardiac function at this temperature), and at 22°C under two specific conditions: contraction rate of 105 min^{-1} and 8% strain, representing normal cardiac function at 22°C; and contraction rate of 60 min^{-1} and 12% strain, representing cardiac function at 22°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 Materials and Methods) was interpolated from the 11% and 14% values at this temperature. *Significant difference between values at 22°C and 14°C. ‡Significant difference between values at 105 min^{-1} and 8% strain versus 60 min^{-1} and 12% strain at 22°C. Significant ($P<0.05$) differences were identified using repeated measures ANOVA and Holm–Šidák *post hoc* tests. Values are means±s.e.m., N=10.

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 impact 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
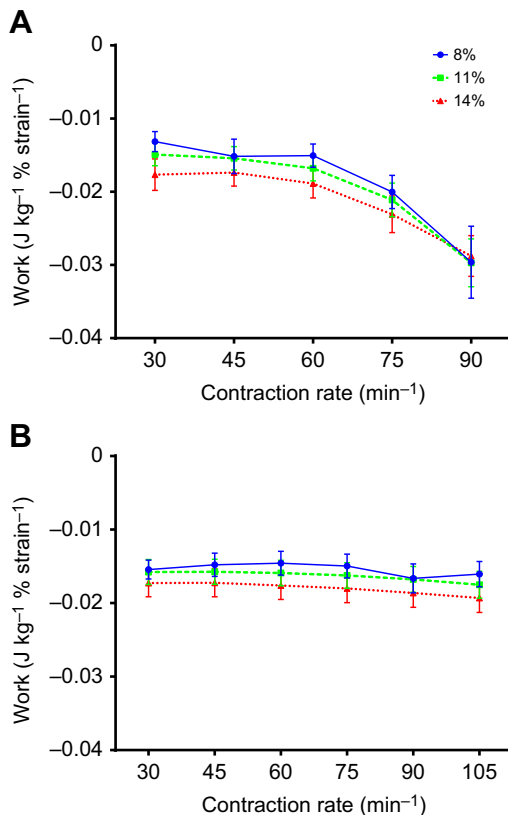

Fig. 5. Temperature-dependent effects on lengthening work. Lengthening work per unit strain amplitude per cycle from rainbow trout ventricular trabeculae at different contraction rates and strain amplitudes (8%, 11% and 14%) at 14°C (A) and 22°C (B). There were significant strain and contraction rate effects, summarized in Table 2. Values are means±s.e.m., N=10.
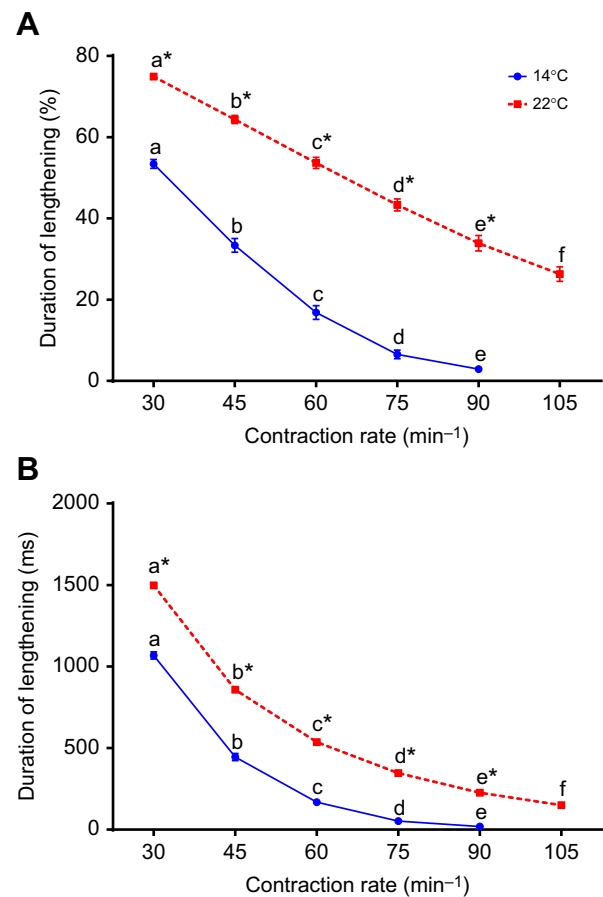

Fig. 6. Duration of the strain cycle attributed to lengthening. Data are expressed as a percentage of the whole cycle (A) and in milliseconds (B) at different contraction rates and two different temperatures (14 and 22°C) for rainbow trout ventricular trabeculae. Within a temperature, symbols without a letter in common are significantly different ($P<0.05$). Asterisks indicate significant differences between temperatures at a specific contraction rate. Values are means±s.e.m., N=10.
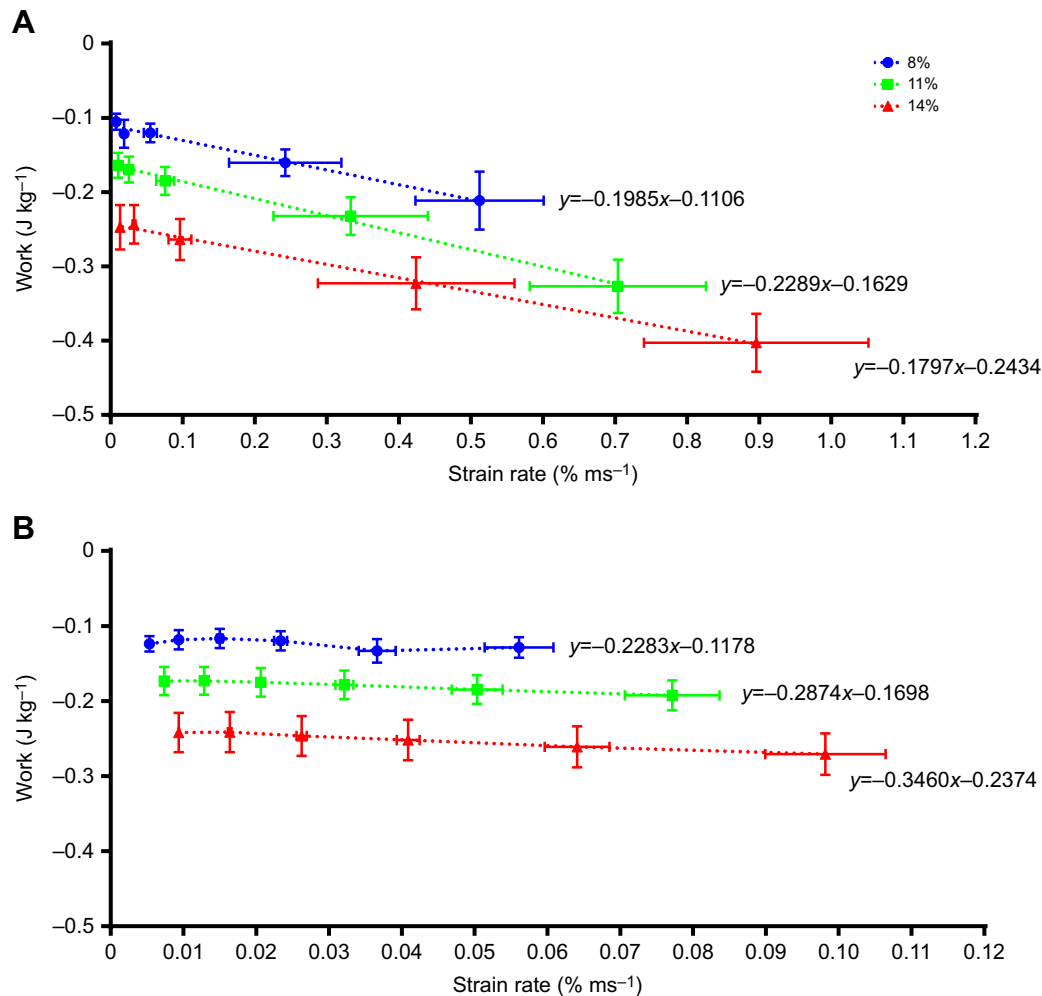
Fig. 7. Effects of lengthening strain rate on lengthening work of rainbow trout ventricular trabeculae. Strips were tested at different contraction rates and strain amplitudes (8%, 11% and 14%) at 14°C (A) and 22°C (B). Note the order of magnitude difference between the strain rate axes at the two temperatures. The equations are linear regressions based on the individual data points. In 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 B, none of the regressions have significant slopes, but the intercepts are significantly different. Values are means±s.e.m. of data pooled at each contraction rate, $N=10$.

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 ~63% increase in work with a 75% increase in strain from 8% to 14%), such that changes in work or power averaged only about 86% of changes in strain (Figs 3A,B, 4A,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 velocity of shortening, which eventually limits 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), whereas 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 Bijnens 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^{-1}) and estimated strain at maximal Q (12% at a V_S of 1.3 ml kg^{-1}). 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^{-1}), 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 versus 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 3C,D, 4C,D and 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 disproportionally 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. 7B), and the fact that there was considerable time available for diastolic filling/lengthening regardless of contraction rate when warm (Fig. 1B, 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 more 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 f_H of 60 beats min^{-1}) with (2) measures when temperature is increased from 14 to 22°C (i.e. f_H increasing from 60 to 105 beats min^{-1} while strain remains unchanged at 8%) (Keen and Gamperl, 2012; Motyka et al., 2017); and with (3) what would occur under conditions where V_S increases to its maximum (1.3 ml kg^{-1}, 12% strain amplitude) but f_H remains unchanged at 60 beats min^{-1} [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 (see Fig. 3C,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. 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 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 P_{O_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 abiotic and/or biotic challenges. Neural and humoral influences (e.g. the release of catecholamines and the stimulation of cardiac β-adrenoreceptors) on the heart would also impact relationships between temperature and the ability of the heart to increase Q via f_H versus 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. 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, 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 that at 14°C (Fig. 1). Thus, the duration of the cardiac cycle that comprised shortening was relatively brief, and this provided a longer time frame 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 with 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 beats min^{-1} (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 to 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 versus active atrial contraction versus *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 are not 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 β-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 are potentially 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).

Author contributions

Conceptualization: A.K.G., D.A.S.; Methodology: A.K.G., A.L.T., D.A.S.; Formal analysis: A.L.T., D.A.S.; Investigation: A.L.T., D.A.S.; Resources: A.K.G.; Writing - original draft: A.K.G., A.L.T., D.A.S.; Writing - review & editing: A.K.G., D.A.S.; Supervision: A.K.G., D.A.S.; Project administration: A.K.G.; Funding acquisition: A.K.G, D.A.S.

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