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Effects of temperature and force requirements on muscle work and power output

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ABSTRACT

Performance of muscle-powered movements depends on temperature through its effects on muscle contractile properties. *In vitro* stimulation of Cuban treefrog (*Osteopilus septentrionalis*) plantaris muscles reveals that interactions between force and temperature affect the mechanical work of muscle. At low temperatures (9–17°C), muscle work depends on temperature when shortening at any force, and temperature effects are greater at higher forces. At warmer temperatures (13–21°C), muscle work depends on temperature when shortening with intermediate and high forces (≥30% peak isometric tetanic force). Shortening velocity is most strongly affected by temperature at low temperatures and high forces. Power is also most strongly affected at low temperature intervals, but this effect is minimized at intermediate forces. Effects of temperature on muscle force explain these interactions; force production decreases at lower temperatures, increasing the challenge of moving a constant force relative to the muscle's capacity. These results suggest that animal performance that requires muscles to do work with low forces relative to a muscle's maximum force production will be robust to temperature changes, and this effect should be true whether muscle acts directly or through elastic-recoil mechanisms and whether force is prescribed (i.e. internal) or variable (i.e. external). Conversely, performance requiring muscles to shorten with relatively large forces is expected to be more sensitive to temperature changes.

KEY WORDS: Cuban treefrog, *Osteopilus septentrionalis*, Elastic energy storage

INTRODUCTION

Temperature can have significant effects on whole-organism performance, especially in ectotherms (Angilletta, 2009). Muscle-powered movements are particularly susceptible to temperature changes (reviewed in Bennett, 1990) because of the effects of temperature on muscle contractile properties (Bennett, 1985; James, 2013). At lower temperatures, we see lower muscle shortening velocity (Coughlin et al., 1996; Hill, 1938; Johnston and Gleeson, 1984), lower rates of force generation (Herrel et al., 2007; Wilson et al., 2000), lower power output (Herrel et al., 2007; James et al., 2012; Ranatunga, 1998; Renaud and Stevens, 1984; Swoap et al., 1993) and, to a lesser extent, lower force production (James et al., 2012; Rall and Woledge, 1990). Here, we examine the effects of temperature on the work done by an ectothermic muscle during isotonic contractions with a range of forces.

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Many ectotherms bypass the limitations of muscle contraction and maintain performance at lower temperatures by using elastic-recoil mechanisms in their feeding movements (chameleons: Anderson and Deban, 2010; toads: Deban and Lappin, 2011; salamanders: Anderson et al., 2014; Deban and Richardson, 2011; Deban and Scales, 2016; Scales et al., 2016). These animals are able to use their muscles to stretch elastic connective tissue, storing energy that is later released rapidly when this tissue recoils (de Groot and van Leeuwen, 2004; Deban et al., 2007; Lappin et al., 2006). This increases the rate of energy release, effectively multiplying the power output of the muscle (Alexander, 1977). The storage and recovery of energy in elastic structures such as tendons and aponeuroses is relatively unaffected by temperature (Rigby et al., 1959); therefore, movements that use elastic recoil are not strongly affected by decreasing muscle power at lower temperatures. While the decoupling of the movement from muscle shortening afforded by the elastic mechanism diminishes the role of muscle power in these systems, the total amount of work done directly determines performance. Therefore, the thermal robustness of performance in elastic systems requires that the work done by a muscle be relatively independent of temperature.

The effects of temperature on most muscle contractile properties have been extensively studied (James, 2013), including temperature effects on the work done by a muscle during stretch–shortening cycles (Herrel et al., 2007; James et al., 2012; Swoap et al., 1993). While these work-loop experiments are useful for simulating cyclical movements, they are often optimized for net work or power rather than simulating a particular external load (Josephson, 1985). Many movements involved in feeding and locomotion require single muscle contractions, rather than a series of stretch–shortening cycles, and in these cases the work done by a muscle depends on the specific load being moved. Muscle contractions in simulated elastic-recoil mechanisms have revealed that work output is optimized when the muscle is shortening at intermediate loads (∼50% peak isometric tetanic force) (Sawicki et al., 2015). Therefore, the effects of temperature on muscle contraction in elastic-recoil mechanisms are most relevant under prescribed-load conditions, rather than cycles of imposed length changes and fluctuating load. The thermal robustness of performance has been well established in systems in which single muscle contractions are used to move prescribed forces, including the feeding mechanisms of salamanders, chameleons and toads (Anderson and Deban, 2010; Anderson et al., 2014; Deban and Lappin, 2011; Deban and Richardson, 2011; Deban and Scales, 2016; Scales et al., 2016).

The unusual morphology of the muscles in elastically powered feeding systems makes *in vitro* studies of muscle contractile properties such as shortening velocity, power output and work technically difficult (de Groot and van Leeuwen, 2004; Deban et al., 2007). Elastic recoil has been documented in several frog species including *Rana pipiens* and *Osteopilus septentrionalis* (Astley and Roberts, 2012; Peplowski and Marsh, 1997; Roberts et al., 2011; Roberts and Marsh, 2003), and temperature effects on jump performance in some

frog species (including *R. pipiens* and *Limnodynastes tasmaniensis*) are less than would be expected based on temperature effects on muscle (Hirano and Rome, 1984; Whitehead et al., 1989). In these species, energy is stored in the distal tendon of the plantaris muscle when the muscle shortens prior to the initiation of movement, and is subsequently released at high power (Astley and Roberts, 2012; Roberts and Marsh, 2003). Because of the large amount of power amplification in *O. septentrionalis* (Peplowski and Marsh, 1997; Roberts et al., 2011), the plantaris muscle of this species provides an ideal system in which to test the effects of temperature on muscle work. Given the similarities in function between these independently evolved feeding and locomotion systems, we expect thermal robustness to be a universal feature of elastic-recoil mechanisms. Here, we examine the interaction of temperature and force on muscle contractile properties using a series of after-loaded contractions of the frog plantaris at different temperatures to test the premise that muscle work is robust to changing temperature.

## MATERIALS AND METHODS
In vitro muscle experiments

Eight adult Cuban treefrogs [*Osteopilus septentrionalis* (Duméril & Bibron 1841); body mass=12.2 to 28.0 g] were wild-caught around Tampa, FL, and housed in laboratory facilities for 1–2 weeks prior to experiments. Frogs were housed in plastic containers with a water gradient and fed gut-loaded crickets *ad libitum* twice weekly. Frogs were humanely euthanized via rapid decapitation immediately prior to muscle experiments and destruction of the central nervous system was ensured through double-pithing. Plantaris muscles (muscle mass=0.217 to 0.309 g) were dissected from these frogs along with the intact sciatic nerve while being bathed with room-temperature amphibian Ringer's solution (Fischmeister and Hartzell, 1987). Kevlar thread (Weaverville Thread, Weaverville, NC, USA) was tied around the proximal region of the plantaris tendon, being careful not to include any muscle fibers in the knot. The origin of the plantaris at the knee was left intact and each muscle was connected to a dual mode servomotor (model 305C-LR, Aurora Scientific, Aurora, ON, Canada) by clamping the knee joint at the proximal end and tying the Kevlar thread from the tendon to the lever of the servomotor. Muscles were stimulated through the sciatic nerve using a bipolar pulse stimulator (model 701B, Aurora Scientific) controlled by a custom instrument in LabVIEW software (v9.0, National Instruments, Austin, TX, USA). The muscle preparation was submerged in a tissue-organ bath (model 805A, Aurora Scientific) filled with oxygenated (100% O_2) Ringer's solution. Temperature was controlled using a temperature-controlled water circulator (IsoTemp 1013S, Fisher Scientific, Waltham, MA, USA). All procedures were approved by the Institutional Animal Care and Use Committee of the University of South Florida.

Position and force of the muscle on the lever were recorded at 1000 Hz for 3 s as the muscle was activated during and relaxed following stimulation using a standard after-loaded protocol. Each muscle was allowed to rest initially for 30 min at a temperature of 17°C. Muscle were stimulated using 1 ms pulses at 20 V. Because voltage was specified, the system found its own current based on the resistance of the nerve tissue. Isometric twitches were used to find the whole-muscle length (L_0) that gave the highest force by manually altering the length of the muscle using a micropositioner. Optimum length measured using isometric twitches is larger than when measured using tetanic contractions; therefore, the muscles may have been operating on the descending limb of the force–length curve during tetanic contractions. Because this was done consistently for all muscles, the results should not be affected other than underestimating true peak isometric tetanic force (P_0) (Holt and Azizi, 2014). In all contractions,

the muscle was stretched ∼30 s prior to stimulation until passive tension was equal to that recorded at L_0. Length was periodically checked over the course of experiment to ensure that the correspondence of L_0 and passive remained unchanged. Passive tension was relaxed while the muscle rested between stimulations to prevent muscle damage.

Isometric contractions from preliminary studies found the stimulus frequency that yielded maximum force of tetanic contractions to be related to the rate of force development of a single twitch as 4400 divided by time (in ms) to peak twitch force. Using this relationship, a single isometric twitch was used to determine the stimulus frequency for tetanic contractions at each temperature for each muscle.

Experiments started at 17°C followed by either the sequence 9–13–21–25–17°C or 25–21–13–9–17°C so that half of the muscles experienced increasing temperatures through time, while the other half experienced decreasing temperatures. This was done to avoid confounding muscle fatigue with temperature effects. Muscles rested for 20 min at each new temperature, then P_0 was measured using previously determined L_0 and stimulus frequency. Isotonic contractions were measured by stimulating muscles to shorten with constant forces determined by the lever that resulted in relative forces from 10 to 90% of P_0 (Fig. 1). Stimulus duration lasted 1000
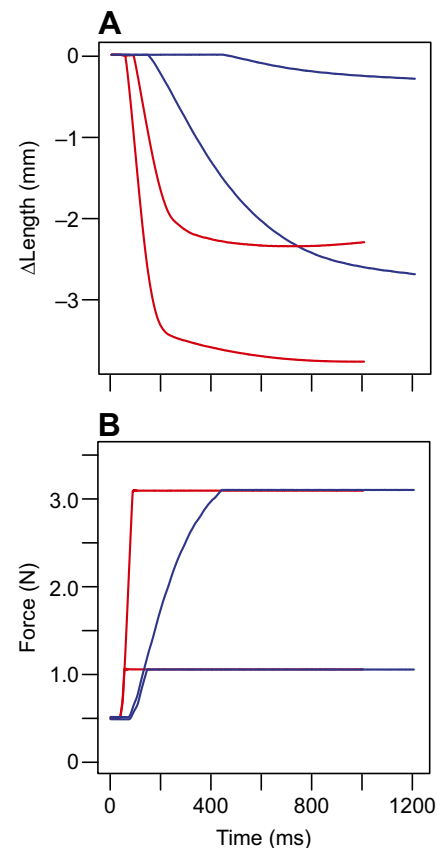

Fig. 1. Representative muscle length and force data for two different imposed forces at 9°C (blue) and 25°C (red). (A) Muscle length. (B) Force. Data are plotted for the duration of muscle stimulation. Following the onset of stimulation, muscle force (A) increased until reaching the level of force imposed by the lever. At this point, muscle shortened at high initial velocity, but shortening velocity decreased throughout the duration of stimulation until length no longer changed (B). For all trials at 9°C and most at 13°C, muscle shortening did not end before the end of stimulation, but predicted additional shortening in these trials (average 4.5%) does not significantly affect results (see Materials and methods for explanation).

to 1200 ms depending on temperature to allow muscles to fully shorten. Position of the lever and muscle force exerted on the lever were recorded during each contraction in LabVIEW.

Preliminary experiments found that a 10 min rest period was sufficient to observe repeatable measures of muscle work in isotonic contractions of a 0.342 g muscle, while a 5 min rest resulted in decreased work output (Fig. 2). Therefore, muscles were allowed to rest for 10 min between each tetanic isometric and isotonic contraction to reduce effects of fatigue. A second measure of P_0 was recorded following completion of isotonic contractions at each temperature to measure the change in performance across contractions at that temperature.

Analysis

Data were collected for complete temperature series for muscles from four individuals, but the Kevlar knot attaching the muscle to the lever broke free from the tendon before all temperatures could be collected for the other four muscles. For the four muscles that had a final 17°C treatment, the initial 17°C treatment was not included in the dataset in order to avoid confounding time (muscle fatigue) and temperature. The highest value of P_0 for a muscle at a temperature was chosen to represent the peak muscle force at that temperature. Values of lever force were divided by this P_0 at 17°C to calculate relative force imposed on each muscle during isotonic contractions. Peak rate of muscle force development and average rate of muscle force development from start of stimulation to P_0 were determined from the first time derivative of muscle force using a quintic spline in R (R Foundation for Statistical Computing, www.r-project.org). Velocity of muscle shortening was calculated as the first time derivative of lever position using a quintic spline. Maximum shortened distance of a muscle during isotonic contractions was determined at the point at which shortening velocity reached zero in graphs of velocity over time (plus or minus 10 ms). Power was calculated by multiplying instantaneous shortening velocity by instantaneous muscle force (which was constant) during isotonic contractions. Peak power was determined to be the highest value of instantaneous power for each isotonic contraction, which was also equivalent to the peak instantaneous shortening velocity times the
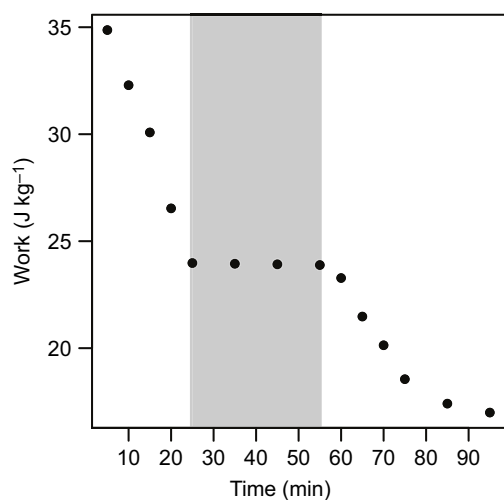
Fig. 2. The effect of rest period on performance in repeated bouts of muscle shortening. The muscle-mass-specific work done by a 0.342 g muscle shortening with a 6 N force decreased when rest period was 5 min, but stayed constant when rest period was 10 min (gray area). Based on these results, a 10 min rest period was used between tetanic contractions in all experiments.

constant muscle force. Average power was calculated from the start of shortening until shortening velocity reached zero for each isotonic contraction. Total distance shortened was multiplied by peak muscle force (=lever force) to calculate work done by a muscle. Values of power and work were divided by wet muscle mass to calculate muscle-mass-specific power and work.

All statistical analyses were performed using custom scripts in R. Statistical models of P_0, peak rate of muscle force development, and average rate of muscle force development included temperature as a continuous variable and individual as a random factor. When examining the effects of temperature on muscle performance, all dependent variables were $\log_{10}$-transformed because their relationship with temperature is assumed to be exponential. For each dependent variable, separate analyses were run for the entire temperature range and for three overlapping 8°C temperature intervals: 9–17°C, 13–21°C and 17–25°C. The (partial) regression coefficients of temperature were used to calculate temperature coefficients (Q_{10}) using the following equation: $Q_{10}=10^{(\text{regression coefficient}\times10)}$. Temperature coefficients were considered significantly different from 1.0 if based on a regression coefficient with a P-value <0.05.

For each muscle, temperature effects on force-dependent variables (work, power, velocity and distance shortened) were examined by fitting temperature–force surfaces to the raw (i.e. not $\log_{10}$-transformed) data that included a quadratic equation for temperature and a quadratic equation for relative force. A cubic polynomial equation was used for relative force in surfaces with velocity as the dependent variable. Interpolated values of dependent variables from the temperature–force surfaces for each muscle were calculated at all combinations of 9, 13, 17, 21 and 25°C and 10, 20, 30, 40, 50, 60 70, 80 and 90% relative force. $\log_{10}$-transformed interpolated values of force-dependent variables for each muscle were used to calculate Q_{10} values for each relative force using regressions similar to those described above. Values of shortening velocity were measured at the level of the whole muscle and are therefore lower than shortening velocity measured from muscle fascicles because of the pennate structure of the plantaris muscle.

To examine the force–velocity characteristics of these muscles at different temperatures, a third order polynomial function was fit to force–velocity curves separately for each muscle at each temperature. Regressions similar to those described above were used to examine the effects of temperature on force–velocity variables. These include maximum unloaded shortening velocity ($V_{\max}$), peak power output ($W_{\max}$), power ratio [PR=$W_{\max}$/ ($V_{\max}P_0$)], shortening velocity at which peak power occurs (V_{power}) and force at which peak power occurs (F_{power}). Out of eight muscles, two muscles were missing data from the 25°C treatment (last in the series) because of failure of the connection between the Kevlar thread and plantaris tendon, and one muscle only had measurements for only two different forces at the 9°C treatment.

Effects of stimulus duration

Although 1200 ms of total stimulation appeared to allow for complete muscle shortening based on examination of the muscle length traces, examination of muscle shortening velocity traces revealed that muscle had not always fully shortened by the end of stimulation. This effect occurs in all isotonic contractions at 9°C and most contractions at 13°C (N=68), introducing a potential confounding factor of stimulation duration that would tend to underestimate muscle work at these low temperatures. In trials in which shortening velocity was greater than zero at the end of

stimulation, a logarithmic function (velocity=a+b×log[time]) was fit to the last 200 ms of velocity data prior to the end of stimulation. This function was used to predict values of velocity out to the x-intercept (zero velocity), and the integral of this extrapolated region of the curve was measured to determine the amount of shortening that would occur during this time. This distance was added to the measured distance the muscle shortened and used to calculate work.

Data for these 68 trials were analyzed using the additional predicted work, but these did not significantly change the results. On average, the predicted additional shortening added 4.5% additional muscle work. In only four cases this procedure predicted additional work greater than 11%. The most severe case, a ∼3 N force at 9°C, is represented in Fig. 1. In this trial, predicted additional shortening changed the muscle-mass-specific work from 4.04 to 7.27 J kg^{-1} (an 80% increase). While muscle may have not reached zero shortening velocity in these cases, they did reach low velocities at which even several hundred additional milliseconds of stimulation would not result in enough work to significantly change the results in most cases. This additional shortening does not affect measurement of peak velocity or peak power, which occur early in shortening, or the force–velocity characteristics, which are calculated from peak velocities. Measurements of average velocity and average power were changed in these 68 trials to include the predicted additional shortening velocity, and resulting power when multiplied by force, until the x-intercept was reached.

RESULTS

Peak muscle force ranged from 3.34 to 10.29 N and increased with increasing temperature. For the four muscles with repeated measures of 17°C, peak muscle force decreased to 78% of initial measurement on average over the entire experiment, though this long-term fatigue is not confounded with temperature because of the balanced number of muscles that experienced increasing or decreasing temperatures. On average, peak muscle force decreased to 97, 99 and 93% of initial measurement across the 9, 17 and 21°C treatments, respectively. Peak muscle force increased to 101% of initial values across the 13°C treatment on average. At 25°C, P_0 decreased to 86% of initial values on average, but because forces were measured in decreasing order at each temperature, the effect of muscle fatigue would lead to underestimates of work at lower forces at 25°C. The temperature coefficient was highest for P_0 across the 9–17°C temperature interval (Q_{10}=1.34) and decreased at the higher intervals (Table 1). Peak rate of muscle force development ranged from 12 to 83 N s^{-1} and average rate of muscle force development ranged from 2 to 27 N s^{-1} (Table S1). The peak and average rates of muscle force development during isometric contraction both increased with increasing temperature, with higher Q_{10} values across the 9–17°C temperature interval (Table 1) compared with the warmer intervals.

Force–velocity characteristics of the muscles followed similar patterns. Values of V_{max} ranged from 0.60 to 2.16 L_0 s^{-1} and W_{max} ranged from 27.9 to 327.5 W kg^{-1}. Both V_{max} and W_{max} increased significantly with increasing temperature across all temperature ranges, and Q_{10} values were largest across the 9–17°C temperature interval compared with the warmer temperature intervals (Table 1). PR ranged from 0.09 to 0.22, V_{power} ranged from 21% to 55% V_{max} and F_{power} ranged from 22% to 67% P_0. Power ratio increased significantly with increasing temperature across the 9–17°C interval and total 9–25°C range (Table 1). V_{power} and F_{power} increased with increasing temperature across the 17–25°C interval and the total 9–25°C range (Table 1).

Muscle-mass-specific work ranged from 0.33 to 57.76 J kg^{-1} across different forces and temperatures; the highest work was obtained at an intermediate force (50% P_0) at 21°C. Effects of temperature on the work done by a muscle depended on the force with which the muscle was shortening (Fig. 3, Table 1). Values of Q_{10} were lowest at relatively low forces (e.g. 10% P_0), but increased at higher forces (e.g. 80% P_0), and these effects were significant across the 9–17°C interval at all forces. At a force of 10% P_0, work decreased with increasing temperature across the 13–21 and 17–25°C intervals and the total 9–25°C range. At a force of 20% P_0, temperature had no significant effect on work across the 13–21°C interval and 9–25°C range, but work did decrease significantly with increasing temperature across the 17–25°C interval at this force. For all other forces, work increased significantly with increasing temperature across the 13–21°C interval and total 9–25°C range (Table 1).

Peak shortening velocity ranged from 0.001 to 1.61 L_0 s^{-1} and increased significantly with increasing temperature, but these effects were greatest at the 9–17°C interval and at higher forces (Fig. 3, Table 1). Peak power during muscle shortening ranged from 3.7 to 303.2 W kg^{-1} across all temperatures and forces, with the highest values at intermediate forces (50% P_0) at 25°C. Temperature effects on peak power were strongest at the 9–17°C interval and low and high forces, but weakest at intermediate forces (Table 1). Similar trends were seen when examining average rather than peak velocity and power (Tables S2, Fig. S1). Values and temperature effects for variables at high forces (80 and 90% P_0) at the 9–17°C interval are reported with a small sample size (Table S1), which makes the calculated regression coefficients unreliable.

DISCUSSION

The effects of temperature on frog muscle contractile properties including work, power and velocity during isotonic shortening depend on the force with which the muscle is shortening (Fig. 3). The forces at which velocity, power and work are optimized are not necessarily the same forces at which temperature effects on each property are lowest, thus there may be trade-offs between these different aspects of muscle contractile performance. Extending to the whole-animal level, the specific conditions of the movements (i.e. force and temperature) determine not only muscle performance, but also the thermal robustness of this performance. These factors may impact performance when muscles are contracting with or without in-series elastic structures and moving internal or external loads.

Thermal effects on isotonic contractile properties are largely explained by the significant effects of temperature on the force of contraction and the relationship of that force to both muscle length and contractile velocity. A muscle shortening with a constant force will shorten at a velocity dictated by the force–velocity relationship of that muscle. As shortening results in decreased muscle length and lower force capacity according to the force–length relationship of that muscle, the shortening velocity will also decrease because the force capacity of the muscle is now closer to the force imposed by the lever. The muscle will continue to shorten until it reaches a length where force capacity equals the force imposed by the lever, at which point shortening velocity will be zero. Because the force imposed by the lever determines the shortening velocity and the decreased length that results from shortening determines force capacity, the change in muscle length is effectively a function of the force of the lever and the height of the force–length curve, which is assumed to be represented by the amplitude of the peak isometric force of the muscle, P_0. Therefore, as P_0 decreases with decreasing

**Table 1. Regression results from statistical models of contractile variables**

Variable	9–17°C			13–21°C			17–25°C			9–25°C		
	Coef.	P	Q_{10}	Coef.	P	Q_{10}	Coef.	P	Q_{10}	Coef.	P	Q_{10}
P_0 (N)	0.013	0.002	**1.34**	0.008	0.020	**1.21**	0.010	0.025	**1.25**	0.011	<0.001	**1.28**
Peak rate of force (N s^{-1})	0.047	<0.001	**2.98**	0.030	<0.001	**1.98**	0.026	<0.001	**1.83**	0.036	<0.001	**2.27**
Average rate of force (N s^{-1})	0.043	<0.001	**2.68**	0.027	0.019	**1.85**	0.029	0.027	**1.97**	0.035	<0.001	**2.21**
W_{max}	0.058	<0.001	**3.84**	0.035	<0.001	**2.25**	0.029	<0.001	**1.94**	0.043	<0.001	**2.67**
V_{power}	0.000	0.902	**1.00**	−0.001	0.720	0.98	−0.010	0.038	**0.80**	−0.004	0.023	**0.91**
F_{power}	0.007	0.067	1.18	0.006	0.098	1.15	0.014	0.014	**1.37**	0.010	<0.001	**1.25**
PR	0.007	0.024	**1.18**	0.005	0.126	**1.13**	0.004	0.366	**1.09**	0.006	0.001	**1.14**
V_{max} (L_0 s^{-1})	0.035	<0.001	**2.23**	0.022	<0.001	**1.65**	0.020	<0.001	**1.57**	0.027	<0.001	**1.84**
10% P_0												
Peak velocity (L_0 s^{-1})	0.036	<0.001	**2.31**	0.026	<0.001	**1.82**	0.020	<0.001	**1.57**	0.028	<0.001	**1.89**
Peak power (W kg^{-1})	0.084	<0.001	**6.87**	0.036	<0.001	**2.28**	0.014	0.173	1.38	0.046	<0.001	**2.90**
Work (J kg^{-1})	0.020	0.001	**1.58**	−0.005	0.035	**0.89**	−0.040	0.001	**0.40**	−0.009	0.061	0.81
20% P_0												
Peak velocity (L_0 s^{-1})	0.045	<0.001	**2.82**	0.030	<0.001	**2.00**	0.022	<0.001	**1.65**	0.033	<0.001	**2.12**
Peak power (W kg^{-1})	0.048	<0.001	**3.02**	0.027	<0.001	**1.87**	0.014	0.039	**1.38**	0.030	<0.001	**2.01**
Work (J kg^{-1})	0.019	<0.001	**1.53**	0.002	0.218	1.06	−0.014	0.008	**0.72**	0.002	0.359	1.05
30% P_0												
Peak velocity (L_0 s^{-1})	0.057	<0.001	**3.71**	0.035	<0.001	**2.23**	0.024	<0.001	**1.73**	0.039	<0.001	**2.47**
Peak power (W kg^{-1})	0.043	<0.001	**2.71**	0.026	<0.001	**1.82**	0.015	0.013	**1.40**	0.028	<0.001	**1.92**
Work (J kg^{-1})	0.020	<0.001	**1.59**	0.006	0.006	**1.16**	−0.006	0.120	0.86	0.007	0.003	**1.17**
40% P_0												
Peak velocity (L_0 s^{-1})	0.074	<0.001	**5.49**	0.040	<0.001	**2.51**	0.026	<0.001	**1.80**	0.048	<0.001	**3.00**
Peak power (W kg^{-1})	0.046	<0.001	**2.86**	0.027	<0.001	**1.87**	0.016	0.005	**1.44**	0.030	<0.001	**2.00**
Work (J kg^{-1})	0.024	<0.001	**1.73**	0.010	0.001	**1.25**	−0.002	0.588	0.95	0.011	<0.001	**1.28**
50% P_0												
Peak velocity (L_0 s^{-1})	0.102	<0.001	**10.39**	0.045	<0.001	**2.84**	0.027	<0.001	**1.85**	0.060	<0.001	**4.02**
Peak power (W kg^{-1})	0.055	<0.001	**3.53**	0.031	<0.001	**2.04**	0.018	0.002	**1.51**	0.035	<0.001	**2.25**
Work (J kg^{-1})	0.030	<0.001	**1.98**	0.013	<0.001	**1.36**	0.001	0.788	1.03	0.015	<0.001	**1.41**
60% P_0												
Peak velocity (L_0 s^{-1})	0.140	<0.001	**25.28**	0.051	<0.001	**3.22**	0.027	0.002	**1.86**	0.076	<0.001	**5.71**
Peak power (W kg^{-1})	0.083	<0.001	**6.71**	0.038	<0.001	**2.40**	0.021	0.001	**1.62**	0.049	<0.001	**3.09**
Work (J kg^{-1})	0.042	<0.001	**2.62**	0.018	<0.001	**1.52**	0.004	0.387	1.10	0.022	<0.001	**1.66**
70% P_0												
Peak velocity (L_0 s^{-1})	0.141	<0.001	**25.57**	0.056	<0.001	**3.67**	0.024	0.038	**1.72**	0.070	<0.001	**4.98**
Peak power (W kg^{-1})	0.128	<0.001	**18.85**	0.053	<0.001	**3.42**	0.026	0.001	**1.82**	0.069	<0.001	**4.94**
Work (J kg^{-1})	0.067	0.001	**4.72**	0.027	<0.001	**1.85**	0.007	0.210	1.18	0.034	<0.001	**2.18**
80% P_0												
Peak velocity (L_0 s^{-1})	0.106	0.001	**11.52**	0.066	<0.001	**4.55**	0.026	0.080	1.81	0.058	<0.001	**3.77**
Peak power (W kg^{-1})	0.146	0.011	**29.10**	0.097	<0.001	**9.39**	0.036	0.001	**2.27**	0.069	<0.001	**4.87**
Work (J kg^{-1})	0.109	<0.001	**12.42**	0.048	<0.001	**2.99**	0.011	0.188	1.27	0.052	<0.001	**3.34**
90% P_0												
Peak velocity (L_0 s^{-1})	0.079	0.014	**6.12**	0.041	0.177	2.59	0.033	0.208	2.15	0.054	0.003	**3.45**
Peak power (W kg^{-1})	–	–	–	0.090	0.001	**7.96**	0.034	0.275	2.20	0.034	0.275	2.20
Work (J kg^{-1})	0.207	0.009	**116.17**	0.119	<0.001	**15.33**	0.003	0.929	1.07	0.061	0.030	**4.06**

Analyses included temperature as a continuous variable and individual as a random factor. For each variable, separate analyses were run for the entire temperature range and for three overlapping 8°C temperature intervals: 9–17°C, 13–21°C and 17–25°C. The (partial) regression coefficients from each regression were transformed into temperature coefficients (Q_{10}) using the following equation: $Q_{10} = 10^{(\text{regression coefficient} \times 10)}$. Temperature coefficients were considered significantly different from 1 if based on a regression coefficient with $P < 0.05$ (significant Q_{10} values in bold). P_0, peak isometric force; W_{max}, peak power from force–velocity curves; V_{power}, velocity at which peak power occurred; F_{power}, force at which peak power occurred; PR, power ratio; V_{max}, unloaded contractile velocity.

temperature, the force–length relationship has a lower amplitude and less work can be done with a particular force because less shortening can occur before force capacity is equal to the force of the lever (Fig. 4A).

The challenge imposed by a large force relative to P_0 is also affected more strongly by temperature than the challenge imposed by a small force: as P_0 decreases with decreasing temperature, a given absolute force becomes larger relative to P_0 (Fig. 4). For example, when P_0 drops from 7 N at 17°C to 6 N at 13°C, a force of 6 N changes from 84% of P_0 to 100% of P_0. However, a force of 1 N would be 14% of P_0 of 17°C, and 17% of P_0 at 13°C. The muscle experiences a 16% change in relative force with the 6 N force, but only a 3% change with the smaller 1 N force across the same

temperature change. Muscle work depends on the relative force, as reasoned above; therefore, the effect of temperature on the work done by a muscle is greater for the larger force. These results are supported by measurements of work from sartorius muscles in *Hyla aurea* and *Bufo marinus* at 10 and 20°C (Gibbs and Chapman, 1974). Satorius mass-specific work in *H. aurea* increases from 4.19 to 4.24 mcal g^{-1} from 10 to 20°C ($Q_{10} = 1.01$) when shortening with a force that is 20% of peak isometric force, but increases from 3.79 to 5.49 mcal g^{-1} across the same temperature interval ($Q_{10} = 1.45$) when moving an 80% force (Gibbs and Chapman, 1974). Results for *B. marinus* are similar: work increases from 5.20 to 5.90 mcal g^{-1} ($Q_{10} = 1.14$) at 20% force and from 3.09 to 5.50 mcal g^{-1} ($Q_{10} = 1.78$) at 80% force from 10 to 20°C (Gibbs and Chapman, 1974).
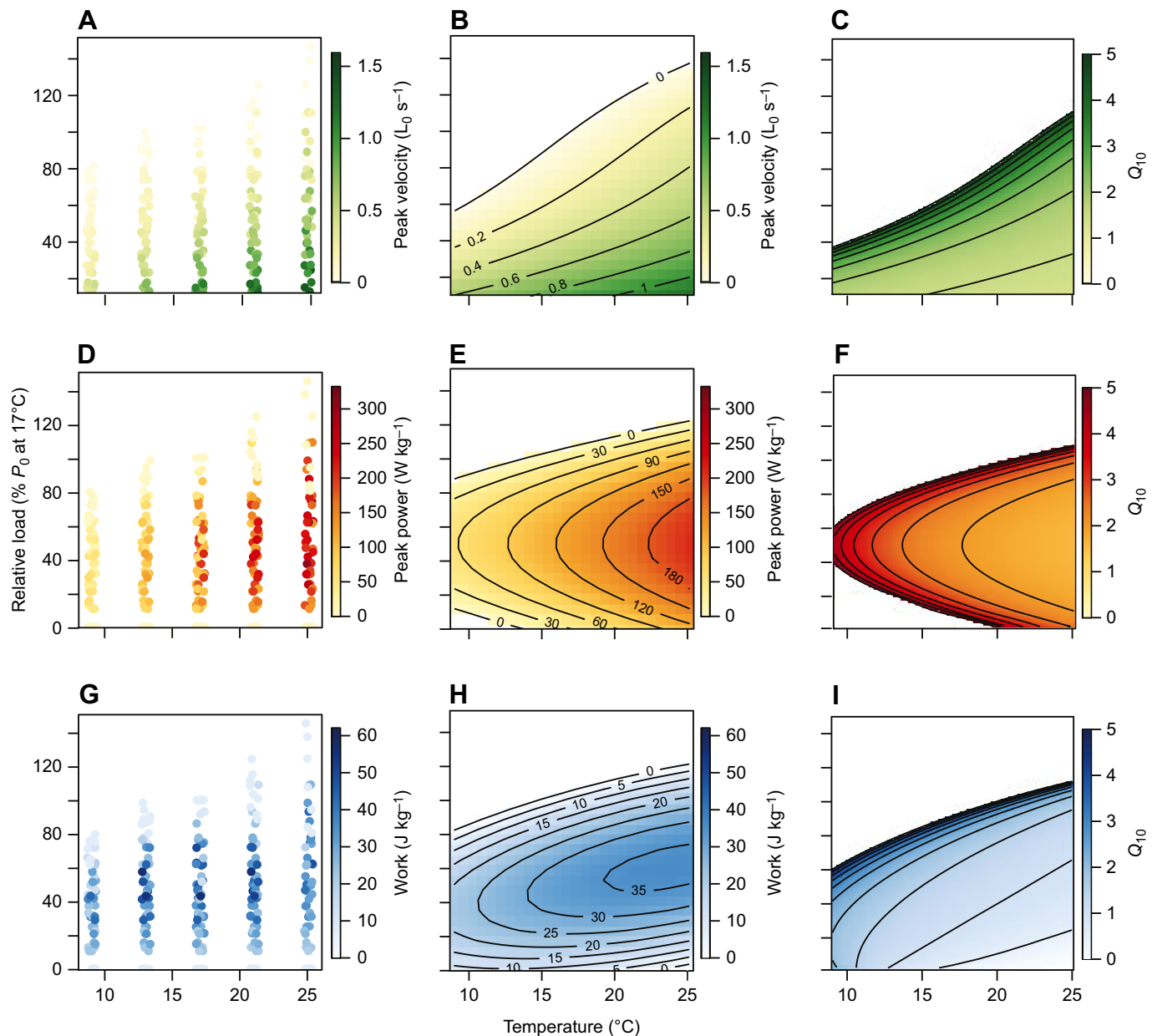
Fig. 3. The interaction of temperature and force on contractile properties. Force in this case is calculated relative to P_0 at 17°C, hence load can exceed 1.0 at warmer temperatures. Actual (A) and interpolated (B) values of peak shortening velocity increase at high temperatures and low forces. Actual (D) and interpolated (E) values of muscle-mass-specific peak power increase with greater temperature and at intermediate forces. Actual (G) and interpolated (H) values of muscle-mass-specific work are greatest at higher temperatures and intermediate forces. Which forces are intermediate depends on temperature. Interpolated values were found by fitting temperature–force surfaces that included either a quadratic equation for relative force in models of work (H) and power (E), or a cubic polynomial equation for relative force in surfaces of velocity (B). To visualize temperature coefficients (Q_{10}) on temperature–force plots, predicted values were extracted from surfaces of velocity, power and work at constant forces ranging from 0 to 150% P_0. These values were log$_{10}$-transformed and the first derivative with respect to temperature was calculated. Values of the first temperature derivative at each temperature step were transformed into Q_{10} values using the formula: $Q_{10} = 10^{(\text{variable} \times 10)}$. Because Q_{10} values rise to infinity as values approach zero, only regions with Q_{10} values less than 5 were plotted to aid in visualization. Temperate effects are lowest at high temperatures and low forces for velocity (C) and at intermediate forces and higher temperatures for power (F). For work (I), temperature effects were lowest at low forces and higher temperatures. Random jitter was added to plots of actual values to improve visualization of the data. Note that fitted surfaces in B, D and H are based on combined data from all muscles to allow visualization of the general temperature–force effects on contractile properties. Separate surfaces were fit for each individual muscle (not shown) to extract values used for statistical analyses.

The work performed by a muscle tends to be greatest at intermediate forces at any temperature (Fig. 3) because work is the product of force and distance shortened. However, which absolute forces are 'intermediate' depends on temperature, because P_0 decreases with decreasing temperature (Table 1). A force that results in the most work at a warm temperature may be too great for doing maximum work at a cooler temperature. This reasoning can be extended to explain the effects of temperature and force interactions on power, as well. When force is calculated relative to P_0 at each temperature rather than relative to a single value of P_0 at 17°C, the interaction between force and temperature is significantly diminished for both work and power (Table 2, Fig. 5).

Temperature effects on muscle shortening velocity are greatest when muscle forces are large. In high-force movements, muscle
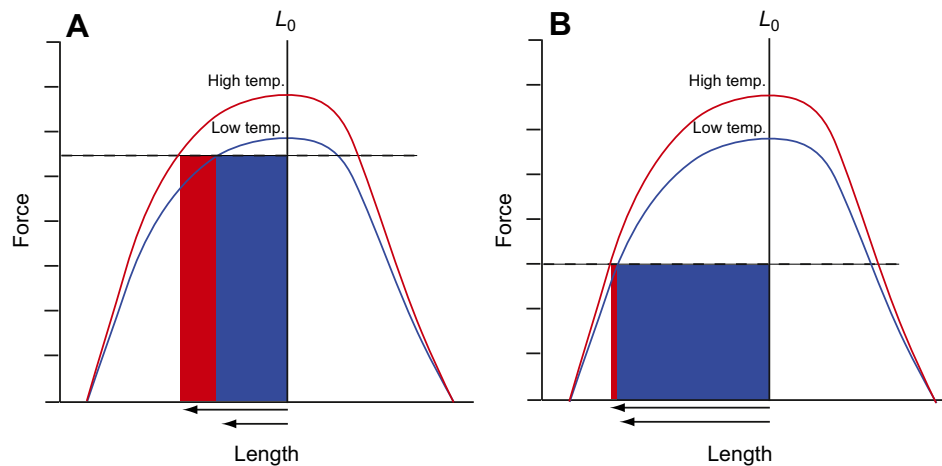
Fig. 4. Force determines shortening velocity and the decreased length that results from shortening determines force capacity, thus muscle work is effectively a function of the resisting force and the shape of the muscle force–length curve. (A) At a high temperature, a relatively high force (dashed line) is well below the isometric muscle force (P_0) at optimum length (L_0). The muscle can shorten at a velocity dictated by the force–velocity relationship, doing work (red plus blue shaded regions). Muscle length will continue to shorten until the muscle force capacity at that length is equal to the load, shortening velocity is zero, and no more work can be done. At a low temperature, less work (blue shaded region) can be done by the same muscle shortening with the same force. (B) The effect of temperature on muscle work depends on the relative force with which the muscle is shortening. The same muscle challenged with a relatively low force is able to do more work at both temperatures, but the relative difference between the work done at high and low temperatures is small compared that of the high-force condition (A).

force will be closer to P_0 and thus at the low end of shortening velocities, while in low-force movements, muscle force is far from P_0 and thus at the high end of the velocity range. Because decreasing temperature causes a greater increase in force relative to P_0 for larger absolute forces (see above), there will be a greater decrease in velocity for a muscle shortening with high forces at lower temperatures. For a muscle shortening with a low force compared with P_0, the increase in relative force with decreasing temperatures is small and thus results in a smaller change in shortening velocity.

Impacts on elastically and muscle-powered movements

The temporal decoupling of muscle shortening from movement in elastic-recoil mechanisms minimizes the effects of muscle shortening velocity and power on the performance of the movement, yet the ability of muscle to do work can have impacts on performance. *In vivo* studies have found that performance of feeding behaviors that utilize elastic-recoil mechanisms is maintained despite changing temperature and the effects on muscle contractile physiology (Anderson and Deban, 2010; Anderson et al., 2014; Deban and Lappin, 2011; Deban and

Richardson, 2011; Deban and Scales, 2016; Scales et al., 2016). Because these salamanders, toads and chameleons are maintaining performance at lower temperatures, their muscles must be shortening to do work with relatively low forces, provided that P_0 is affected by temperature as we have shown in the present study. Relatively low muscle-mass-specific energy requirements (0.08–8.18 J kg^{-1}) of tongue projection in some salamanders (Deban and Scales, 2016) suggest that projector muscles are relatively large compared with the forces they experience.

Elastic systems in which relatively large muscles are moving small loads are optimized for power amplification, but not muscle work (Sawicki et al., 2015). In contractions of a muscle tendon unit with simulated loads, low loads relative to P_0 (~17.5% P_0) tend to produce the greatest amount of muscle power amplification (Sawicki et al., 2015). These relatively low forces that maximize performance as measured by power amplification (Sawicki et al., 2015) also maximize thermal performance, as we have shown (Table 1, Fig. 3). However, shortening with intermediate forces relative to P_0 (~50% P_0) will increase performance measured as muscle work (Fig. 3) (Sawicki et al., 2015). Therefore, there is an

Table 2. Regression results from statistical models of muscle-mass-specific work with force calculated relative to P_0 at each temperature

Relative load (% P_0)	9–17°C			13–21°C			17–25°C			9–25°C		
	Coef.	P	Q_{10}	Coef.	P	Q_{10}	Coef.	P	Q_{10}	Coef.	P	Q_{10}
10	0.027	<0.001	**1.86**	0.011	0.011	**1.29**	−0.005	0.509	0.90	0.011	0.003	**1.29**
20	0.014	<0.001	**1.39**	0.006	0.010	**1.16**	−0.002	0.660	0.96	0.006	0.002	**1.16**
30	0.011	<0.001	**1.29**	0.005	0.009	**1.12**	−0.001	0.725	0.97	0.005	0.002	**1.12**
40	0.010	<0.001	**1.25**	0.004	0.009	**1.11**	−0.001	0.764	0.98	0.004	0.001	**1.11**
50	0.009	<0.001	**1.24**	0.004	0.008	**1.10**	−0.001	0.789	0.98	0.004	0.001	**1.10**
60	0.010	<0.001	**1.26**	0.005	0.008	**1.11**	−0.001	0.803	0.98	0.005	0.001	**1.11**
70	0.012	<0.001	**1.31**	0.005	0.008	**1.13**	−0.001	0.805	0.98	0.005	0.001	**1.13**
80	0.016	<0.001	**1.45**	0.007	0.008	**1.19**	−0.001	0.781	0.97	0.007	0.001	**1.19**
90	0.036	<0.001	**2.28**	0.015	0.008	**1.41**	−0.003	0.664	0.92	0.016	0.001	**1.44**

Analyses included temperature as a continuous variable and individual as a random factor. For each variable, separate analyses were run for the entire temperature range and for three overlapping 8°C temperature intervals: 9–17°C, 13–21°C and 17–25°C. The (partial) regression coefficients from each regression were transformed into temperature coefficients (Q_{10}) using the following equation: $Q_{10}=10^{(\text{regression coefficient}\times10)}$. Temperature coefficients were considered significantly different from 1 if based on a regression coefficient with $P<0.05$ (significant Q_{10} values in bold).
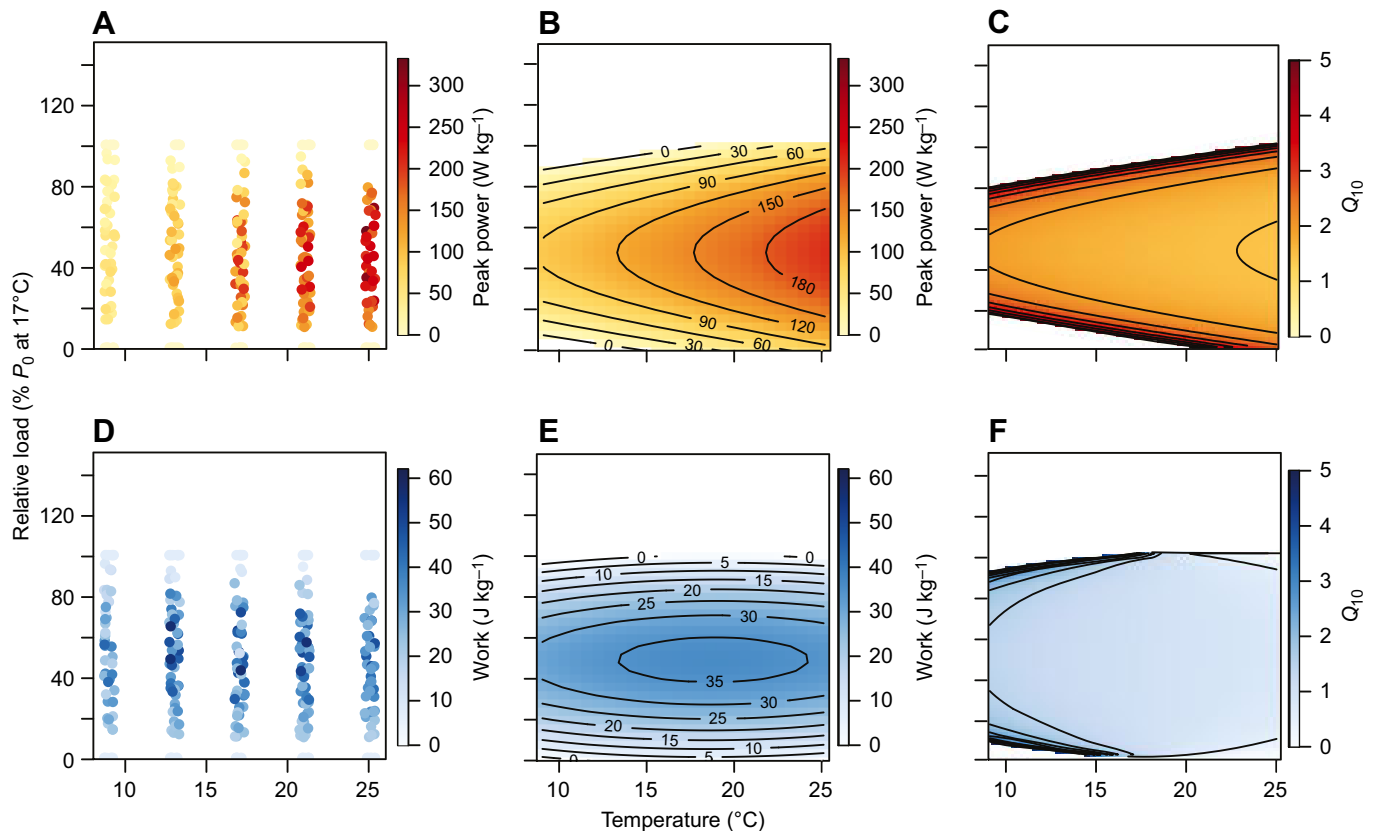
Fig. 5. Calculating force relative to peak muscle force (P_0) measured at each temperature minimizes the interactions of temperature and force (see Fig. 3D–I). Interaction of temperature and force on actual (A) and interpolated (B) values muscle-mass-specific power (A,B) and work (D,E). Interpolated values were found by fitting temperature–load surfaces that included a quadratic equation for relative force. Temperature coefficients (Q_{10} values) of power (C) and work (F) were calculated as in Fig. 3. Random jitter was added to plots of actual values (A,D) to improve visualization of the data.

apparent performance trade-off in elastic mechanisms in which tuning of force relative to P_0 will permit either net work or power amplification and thermal robustness to be maximized. In an elastic-recoil mechanism, the muscle must have a large P_0 relative to the stiffness of the elastic structure to operate at low forces and maximize power, which can be accomplished by having relatively compliant elastic structures in series with the muscle. The 'cost' of a system tuned in this way would be in maintaining a relatively large muscle that is capable of more force and work than is ever used. Measuring force *in vivo* in many elastic systems is technically challenging, but modeling of elastic-recoil mechanisms based on morphology of the muscle and elastic structure may reveal systems that are 'tuned' to work at low forces relative to P_0.

The impacts of the temperature and force interaction on muscle contractile properties are not limited to elastic-recoil systems. If temperature effects on frog muscle are similar to other systems, then any musculoskeletal mechanism that must maintain performance while experiencing ranges of relatively low temperatures (e.g. 9–17°C) may benefit from muscle contractions at relatively low forces. When the loads being moved are prescribed (i.e. internal work through motion of body parts), low forces could result through muscles with large physiological cross-section or through high mechanical advantage. Therefore, the force at which a muscle operates, either directly measured or calculated from morphology, could predict power, work and the thermal robustness of these contractile properties.

In conclusion, the work done by a muscle decreases significantly with decreasing temperatures when shortening at intermediate and

high force relative to P_0, but not when shortening with relatively low force. Additionally, the increases in muscle velocity and power with increasing temperature that have been previously described (reviewed in Bennett, 1985; James, 2013) are also found to be dependent on force. The performance of skeletal muscle during concentric contractions depends not only the temperature of the muscle, but also on the load that is being moved; thus the performance of both muscle-powered and elastically powered movements is determined by not only the mechanical demand on the muscle but also its thermal environment.

Author contributions
J.P.O. and S.M.D. conceived of the project and designed the experiment. J.P.O. and S.M.D. conducted the experiments and J.P.O. analyzed the results. J.P.O. wrote the manuscript with help from S.M.D. Both authors gave final approval for publication.

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