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RESEARCH ARTICLE

Warmer is better: thermal sensitivity of both maximal and sustained power output in the iliotibialis muscle isolated from adult *Xenopus tropicalis*

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SUMMARY

Environmental temperature varies temporally and spatially and may consequently affect organismal function in complex ways. Effects of temperature are often most pertinent on locomotor performance traits of ectothermic animals. Given the importance of locomotion to mobility and dispersion, variability in temperature may therefore affect the current and future distribution of species. Many previous studies have demonstrated that burst muscle performance changes with temperature. However, less is known about the effects of temperature on sustained skeletal muscle performance. The iliotibialis muscle was isolated from eight male *Xenopus tropicalis* individuals and subjected to *in vitro* isometric and work-loop studies at test temperatures of 15, 24, 30 and 32°C. Work-loop power output (average power per cycle) was maximised at each temperature by altering stimulation and strain parameters. A series of 10 work loops was also delivered at each test temperature to quantify endurance performance. Warmer test temperatures tended to increase twitch stress (force normalised to muscle cross-sectional area) and significantly increased tetanic stress. Increased temperature significantly reduced twitch and tetanus activation and relaxation times. Increased temperature also significantly increased both burst muscle power output (cycle average) and sustained (endurance) performance during work loop studies. The increase in burst power output between 15 and 24°C yielded a high Q_{10} value of 6.86. Recent studies have demonstrated that the negative effects of inorganic phosphate accumulation during prolonged skeletal muscle performance are reduced with increased temperature, possibly explaining the increases in endurance found with increased test temperature in the present study.

Key words: burst, endurance, locomotion, temperature, work loop.

INTRODUCTION

Environmental temperature varies spatially and temporally, affecting many aspects of an organism's biology (Angilletta, 2009). Spatial variation in temperature may also affect species' distributions; for instance, a recent study demonstrated that low temperatures constrain the physiological systems that underlie locomotion in the cane toad, potentially limiting its invasion into regions of Australia with cooler climates (Seebacher and Franklin, 2011). As local temperatures alter, both burst and endurance locomotory performance changes in many ectotherms and endotherms (Bennett, 1990; Racinais and Oksa, 2010).

Skeletal muscle performance is sensitive to temperature change, often showing thermal patterns similar to those observed for whole-organism locomotor performance (Bennett, 1984; Rall and Woledge, 1990; Marsh, 1994). Rate-dependent mechanical processes, such as power production, force generation and force relaxation, have relatively high thermal sensitivity, with increased performance at higher temperatures (Rome and Swank, 1992; Swoap et al., 1993; Altringham and Block, 1997; Ranatunga, 1998; De Ruiter and De Haan, 2000; Herrel et al., 2007; Roots et al., 2009). In contrast, maximal isometric force production has low thermal sensitivity across ecologically relevant temperatures, with decreases in force production occurring only at relatively low temperatures. Although there is a wealth of information on thermal sensitivity of burst skeletal muscle performance, there is comparatively little information on sustained (endurance) performance of muscle and almost all previous work has tested muscle endurance while held at constant length (i.e. isometric studies).

The work-loop technique, which allows the evaluation of the mechanical work and power output of muscles *in vitro*, has previously been used to test muscle mechanics during cyclic length changes that represent *in vivo* usage for some muscles (Josephson, 1993). A number of previous studies have used the work-loop technique to investigate trade-offs between burst and endurance performance in skeletal muscle, demonstrating that the performance of a particular muscle varies between individuals within a species to reflect different positions on a sprint–endurance performance continuum (Wilson et al., 2002; Wilson and James, 2004; Wilson et al., 2004). Such a sprint–endurance trade-off reflects the constraints of skeletal muscle structure and composition, such that performance in a particular muscle cannot be simultaneously maximised for both sprint and endurance type activities (Goldspink, 2002; Navas et al., 2006). As temperature rises, muscle power output increases, primarily as a result of the increase in shortening velocity that is enabled by higher myosin ATPase activity (Barany, 1967). Many physiologists might expect such temperature increases to also reduce muscle fatigue resistance (*via* increased energetic cost, faster build-up of inorganic phosphate and other by-products of prolonged exercise or more rapid usage of energetic substrates), resulting in

a temperature-driven sprint–endurance trade-off. Indeed, earlier studies on skeletal muscle during repeated isometric contractions suggested that as temperature was increased endurance decreased (Segal et al., 1986; De Ruiter and De Haan, 2000). In contrast, one study found no change in endurance, with a 6°C change in temperature in mouse soleus fibre bundles subjected to isometric tetani (Place et al., 2009). However, more recently, muscle fibre bundles isolated from rat flexor hallucis brevis (predominantly fast-twitch), were subjected to repeated isometric (tetanus) or repeated isovelocity (isometric tetanus with ramp shortening within it) contractions (Roots et al., 2009). Increased temperature (10, 20 and 30°C test temperatures were used) increased relative tetanic force (stress; force normalised to fibre bundle cross-sectional area), relative power output (power normalised to fibre bundle volume) and fatigue resistance under both isometric and isovelocity conditions (Roots et al., 2009), i.e. there was no temperature-driven trade-off between burst and endurance performance.

No previous study has investigated the effects of temperature on burst work-loop power output, sustained work loop performance and the relationship between the two, yet these types of data are likely most pertinent in a whole-organism context as they best reflect the conflicting demands imposed on locomotor muscle in ecologically relevant tasks. We isolated iliotibialis muscle from wild-caught *Xenopus tropicalis* to test the following hypotheses: (1) increased temperature increases maximal power output (burst performance) under work-loop conditions; and (2) increased temperature prolongs sustained muscle performance during a series of work loops (endurance performance). We predict qualitatively similar changes to those found by Roots and co-workers (Roots et al., 2009), in that we expect both burst power output (cycle average) and endurance performance in isolated muscle to increase as temperature rises.

MATERIALS AND METHODS

Tissue samples

Xenopus (*Silurana*) *tropicalis* Gray 1864 were captured in December 2009 in Cameroon and transported to the laboratory at the field station of the Centre d'Ecologie Expérimentale du CNRS in Moulis, France. Individuals were housed at 24°C. Eight males were transported to Coventry University in July 2010, and were killed by pithing and transection of the spinal cord. Body mass was determined to the nearest 0.01 g using an electronic balance. The skin was removed from the hindlimbs and the animal was transferred to an oxygenated (95% O_2; 5% CO_2) Ringer solution (composition in mmol l^{-1}: 115 NaCl, 2.5 KCl, 2.15 Na_2HPO_4, 0.85 NaH_2PO_4, 10.0 glucose and 1.8 $CaCl_2$, pH 7.4 at 22°C prior to oxygenation) (Altringham et al., 1996). The iliotibialis muscle (sometimes referred to as the gluteus magnus) was dissected from the left hindlimb with a piece of bone left at the end of both the proximal and distal tendons. The iliotibialis is one of the largest knee extensors and is activated during both swimming and jumping, playing a key role in powering the kick that initiates a swimming sequence (Olson and Marsh, 1998; Gillis and Biewener, 2000).

Isometric studies

Isometric studies were used to determine the twitch and tetanus kinetics of isolated iliotibialis muscle. The bone at one end of the muscle preparation was clamped *via* a crocodile clip to a strain gauge (UF1, Pioden Controls Ltd, Canterbury, Kent, UK), whereas the bone at the other end was clamped *via* a crocodile clip to a motor arm (V201, Ling Dynamics Systems, Royston, Hertfordshire, UK) attached to a linear variable displacement transformer (LVDT) (DFG 5.0, Solartron Metrology, Bognor Regis, Sussex, UK). The LVDT was used to monitor the length changes delivered to the muscle preparation. The whole of the muscle, tendon and bone preparation was then allowed to equilibrate within the bath at 24±0.5°C for 10 min in circulating, oxygenated (95% O_2, 5% CO_2) frog Ringer solution. The preparation was then held at constant length and stimulated *via* parallel platinum electrodes to generate a series of twitches. Stimulus amplitude and muscle length were adjusted to determine the stimulation parameters and muscle length corresponding to maximal isometric twitch force. Time to peak twitch and time from peak twitch to half relaxation were measured *via* a storage oscilloscope. An isometric tetanic force response was elicited by subjecting the muscle to a 200 ms train of electrical stimulation. Stimulation frequency was altered (120 to 170 Hz) to determine maximal tetanic force. Time to half peak tetanic force and time from last stimulus to half tetanic force relaxation were measured. A rest period of 5 min was allowed between each tetanic response.

Work-loop analysis

The work-loop technique was used to determine the power output (average of each work-loop cycle) of muscles during cyclical length changes (Josephson, 1993). Unlike fixed-length isometric studies and fixed-load isotonic studies of muscle performance, the work-loop technique allows measures of muscle power output under length and activation changes that are generally more indicative of *in vivo* contractile performance (Caiozzo, 2002; James et al., 1996). In the absence of *in vivo* strain data for iliotibialis muscle in *X. tropicalis*, each muscle preparation was subjected to a set of four sinusoidal length changes symmetrical around the length found to generate maximal twitch force. Previous research on *Bufo marinus* (Gillis and Biewener, 2000) suggests that sinusoidal length changes likely represent a simplification of *in vivo* strain patterns. The muscle was stimulated using the stimulation amplitude and stimulation frequency found to yield maximal isometric force. Electrical stimulation and length changes were controlled *via* a data acquisition board (KUSB3116, Keithley Instruments, OH, USA) and a custom-designed program developed with TestPoint software (CEC TestPoint version 7, Measurement Computing, Norton, MA, USA). Muscle force was plotted against muscle length for each cycle to generate a work loop, the area of which equated to the net work produced by the muscle during the cycle of length change (Josephson, 1993). Instantaneous power output was calculated for every data point in each work loop (2000 data points per work loop), and then these instantaneous power output values were averaged to generate a net work value for each work loop. The net work produced was multiplied by the frequency of length change cycles to calculate net power output (average power per cycle). The cycle frequency of length change was altered in 1 Hz increments between 3 and 9 Hz to determine the cycle frequency for maximal power output. Muscle strain was altered between 0.09 and 0.13 at each cycle frequency to maximize power output (where a strain of 0.10 represents ±5% of resting muscle length). Every 5 min, the muscle was subjected to a further set of four work-loop cycles with cycle frequency, strain, stimulation duration and stimulation phase parameters being altered in between each set until maximum net work was achieved at each cycle frequency. On completion of the maximal power output determination (burst performance test) at 24°C, the muscle was subjected to a short, sustained, high-intensity (endurance) test whereby 10 work loops were delivered to the muscle using the strain, cycle frequency and stimulation parameters found to elicit maximal power output. After the endurance test, the temperature of the Ringer solution bathing the muscle was increased to 30°C over 10–20 min, allowing at least a further 10 min for the

muscle to equilibrate to the new test temperature. The above isometric and work-loop studies were then repeated at 30°C. In a similar manner, muscle temperature was then changed to 15°C, 24°C, 32°C and then back again to 24°C, with power output (average power per cycle) maximized, and endurance was determined at each temperature. In brief, at lower temperatures, muscle power output (average power per cycle) was maximized *via* lower cycle frequency, lower strain and fewer stimuli. A set of control sinusoidal length change and stimulation parameters were imposed on the muscle every three to five sets of work loops, whenever the muscle was at 24°C, to monitor variation in the muscle's ability to produce power/force over the time course of the experiment. Any variation in power (average power per cycle) was found to be due to a matching change in ability to produce force. Therefore, the power produced by each preparation at each temperature was corrected to the control run at 24°C that yielded the highest power output (average power per cycle), assuming that alterations in power-generating ability were linear over time between the control runs delivered at 24°C.

At the end of the isometric and work-loop experiments, the bones and tendons were removed and each muscle was blotted on absorbent paper to remove excess Ringer solution. Wet muscle mass was determined to the nearest 0.0001 g using an electronic balance (Mettler-Toledo B204-S, Greifensee, Switzerland). Mean muscle cross-sectional area was calculated from muscle length and mass assuming a density of 1060 kg m^{-3} (Méndez and Keys, 1960). Maximum isometric muscle stress (kN m^{-2}) at each test temperature was then calculated as maximum tetanic force divided by mean cross-sectional area. Normalised muscle power output (W kg^{-1}) at each test temperature was calculated as average power output per cycle divided by wet muscle mass. Q_{10} values were calculated for each measure of mechanical performance to indicate the rate of change in performance with temperature changes from 15 to 24°C and from 24 to 32°C. Note that a Q_{10} value of 2 indicates a doubling of performance over a nominal 10°C change in temperature, whereas a Q_{10} value of 0.5 indicates a halving of performance over a nominal 10°C change in temperature.

Statistics

Univariate ANOVA was used to investigate the effects of test temperature on isometric force rise times, isometric force relaxation times, isometric stress, maximal power output (burst performance; average power per cycle) and sustained (endurance) performance. A Bonferroni *post hoc* test was used where applicable. The truncated product method (Zaykin et al., 2002) was used to combine all the *P*-values in this study to determine whether there was a bias from multiple hypothesis testing. The truncated product method *P*-value was <0.001, showing that the results were not biased. Significance was taken at the level of $P<0.05$. Data are presented as means ± s.e.m.

RESULTS

Isometric force

There was a tendency for increased test temperature to raise iliotibialis twitch stress ($F=2.62$, $P=0.071$; Fig. 1A). Increased test temperature caused a significant increase in iliotibialis tetanic stress ($F=3.79$, $P=0.022$; Fig. 1B, Fig. 2). There was a significant rise in tetanic stress between 15 and 32°C (Bonferroni *post hoc* $P=0.038$). Q_{10} values indicated that both twitch and tetanus mean stress values increased from 15 to 24°C, but there was limited change in stress from 24 to 32°C (Table 1).

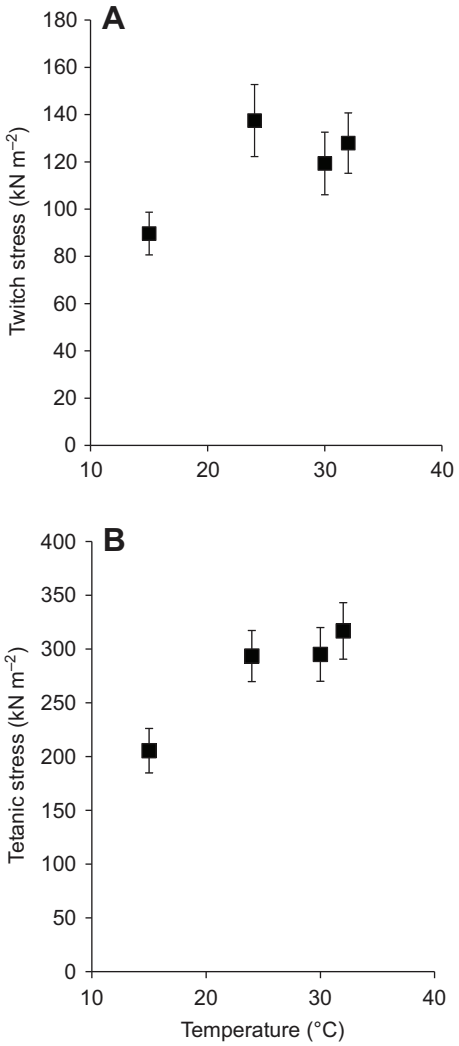

Fig. 1. Effect of test temperature on *Xenopus tropicalis* iliotibialis muscle isometric stress. (A) Increased test temperature tended to raise twitch stress ($F=2.62$, $P=0.071$), and (B) caused a significantly higher tetanic stress ($F=3.79$, $P=0.022$). Data are means ± s.e.m., $N=8$.

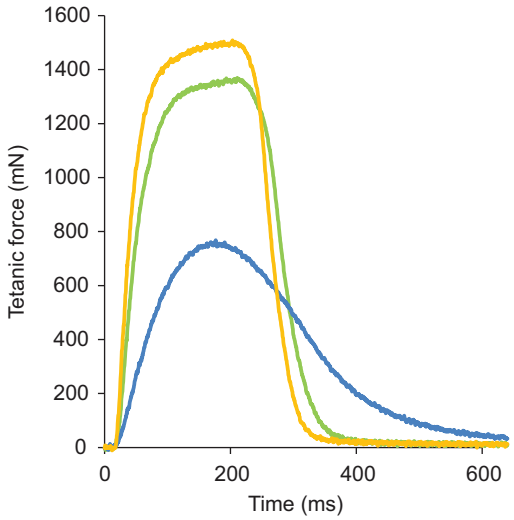

Fig. 2. Increased test temperature increased tetanic force and increased tetanus force rise and relaxation rates in *Xenopus tropicalis* iliotibialis muscle. Typical tetanus responses at 15°C (blue), 24°C (green) and 30°C (orange) are shown.

Table 1. Q_{10} values for measures of isolated iliotibialis muscle performance in *Xenopus tropicalis*

	Q_{10} 15–24°C	Q_{10} 24–32°C
Maximum twitch stress	1.61	0.91
Maximum tetanus stress	1.49	1.10
Time to peak twitch	0.47	0.51
Time from peak twitch to half relaxation	0.37	0.46
Time to half peak tetanus	0.44	0.98
Last stimulus to half tetanus relaxation	0.42	0.66
Maximal work loop power output	6.86	1.82

Isometric force rise and relaxation times

Test temperature had a significant effect on each twitch and tetanus force rise time and relaxation time measured in this study. Increased test temperature caused a significant decrease in time to peak twitch (F=253, P<0.001; Fig. 3A), time from twitch peak to half relaxation (F=43.4, P<0.001; Fig. 3B), time to half peak tetanus (F=33.4, P<0.001; Fig. 3C) and time from last stimulus to half tetanus relaxation (F=14.8, P<0.001; Fig. 3D). Time to peak twitch was significantly different between all test temperatures (Bonferroni *post hoc* P<0.001), except for between 30 and 32°C. Time from twitch peak to half relaxation, time to half peak tetanus and time from last stimulus to half relaxation were each significantly different between 15°C and all other test temperatures (Bonferroni *post hoc* P<0.001). Q_{10} values indicated that both twitch and tetanus mean force rise times and force relaxation times decreased rapidly between 15 and 24°C (Table 1). Q_{10} values indicated that between 24 and 32°C mean values for twitch force rise times, twitch relaxation times and tetanus relaxation times continued to decrease at similar rates; however, time to half peak tetanus was unchanged over this temperature range (Table 1).

Work-loop power output

There was a significant rise in maximal work loop power output (burst performance; average power output per cycle normalised to muscle mass) with increased test temperature (F=39.1, P<0.001; Fig. 4A). Increased temperature raised muscle power output by increasing the work done per length change cycle and by increasing the optimal cycle frequency for power generation; e.g. from 15 to 24°C, the mean optimal cycle frequency for maximal power output doubled from 3.5 to 7 Hz. A comparison of work-loop shapes demonstrates that the large increase in work at higher temperatures was due to higher force generation and greater maintenance of force during shortening (Fig. 4B). Power output was significantly lower at 15°C when compared with all other test temperatures (Bonferroni *post hoc* P<0.001). The highest Q_{10} value in this study was 6.86 for the large increase in power output between 15 and 24°C (Table 1). There was a significant difference in power output between 24 and 32°C (Bonferroni *post hoc* P=0.001; Table 1).

There was a significant rise in endurance of work-loop power output (average power per cycle) as test temperature increased (F=23.1,

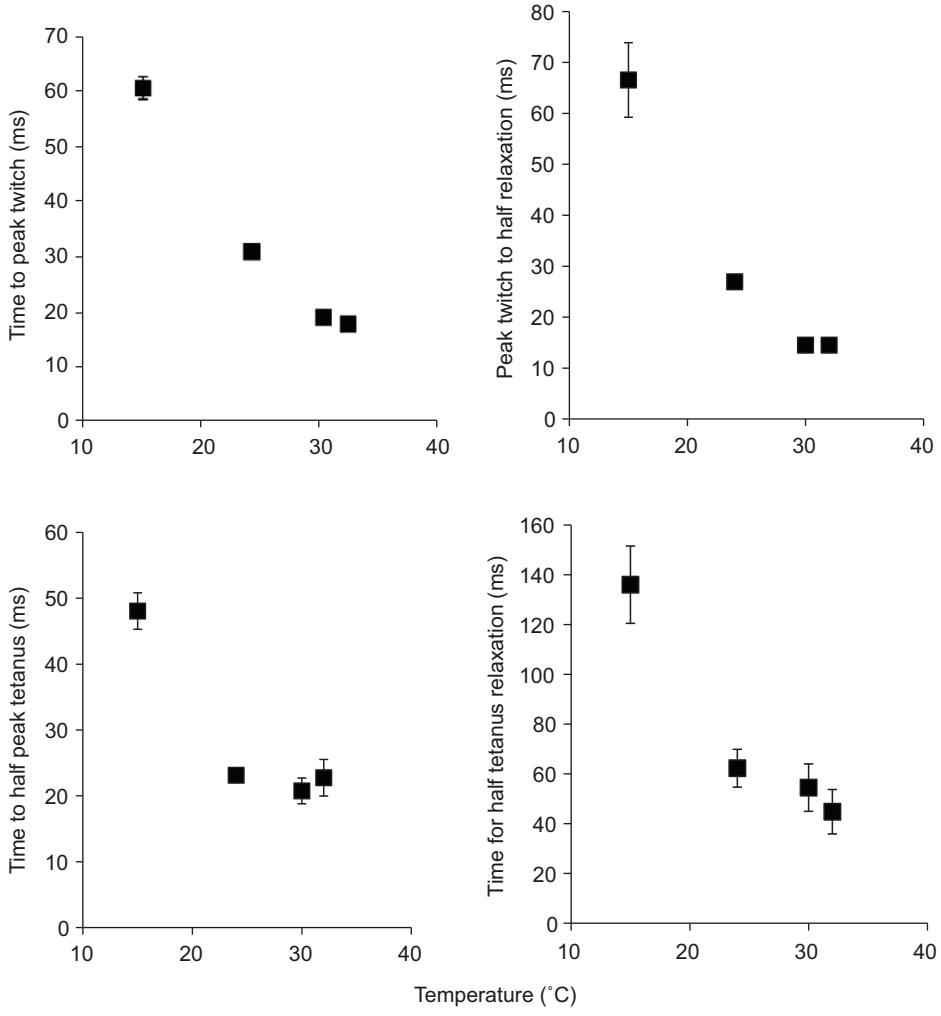

Fig. 3. Increased test temperature caused significant decreases in *Xenopus tropicalis* iliotibialis muscle twitch and tetanus force rise and relaxation times (F>14.8, P<0.001 in each case). (A) Time to peak twitch; (B) time from peak twitch to half relaxation; (C) time to half peak tetanus; (D) time from last stimulus to half tetanus relaxation. Data are means ± s.e.m., N=8.

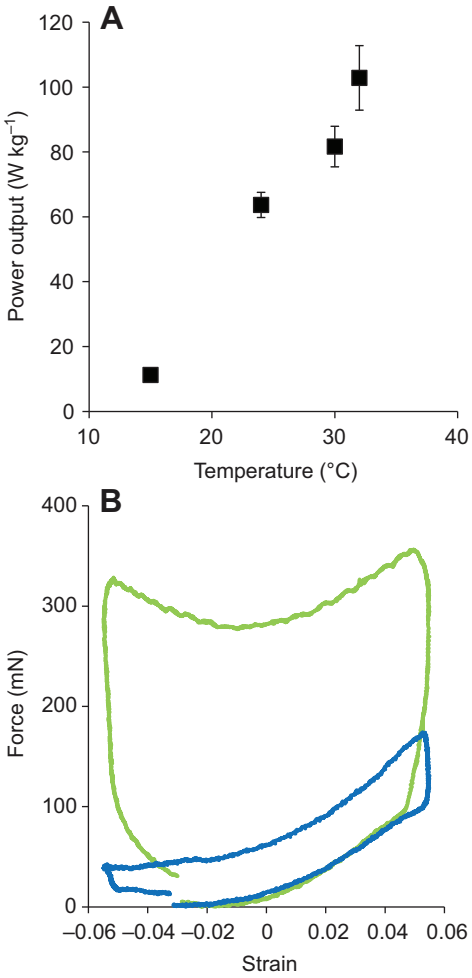

Fig. 4. Increased test temperature caused a significant rise in maximal (burst) *Xenopus tropicalis* iliotibialis muscle power output (average power per cycle) when determined using the work-loop technique (F=39.1, P<0.001). (A) Power output data has been normalised to muscle mass and plotted as means ± s.e.m., N=8. (B) Typical work-loop shapes at 15 (blue, 5 Hz cycle frequency) and 24°C (green, 7 Hz cycle frequency).

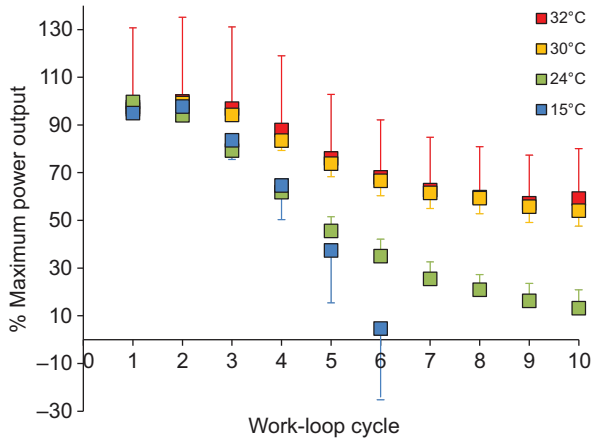

Fig. 5. Increased test temperature caused a significant rise in sustained (endurance) *Xenopus tropicalis* iliotibialis muscle performance when determined using the work-loop technique (F=23.1, P<0.001). Each datum for each individual has been normalised to a percentage of the maximal power output achieved in the endurance protocol for that individual. Data are plotted as means ± s.e.m., N=8.

P<0.001; Fig. 5). Endurance was significantly different between all test temperatures (Bonferroni *post hoc* P<0.02), except for between 30 and 32°C. Therefore, when burst power output was plotted against endurance performance for each test temperature, there was no temperature-driven burst–endurance trade-off, as both power output and endurance performance increased as temperature rose (Fig. 6).

DISCUSSION

In general, at higher test temperatures there was enhanced mechanical performance. In agreement with hypotheses 1 and 2, increased test temperature enhanced iliotibialis muscle performance in *X. tropicalis*, increasing maximal power production (burst) and prolonging sustained (endurance) activities as assessed by the work-loop technique.

Isometric force

Mean isometric tetanic stress values in the present study on *X. tropicalis* (e.g. 294±24 kN m^{-2} at 24°C) were comparable to, or higher than, those found in previous experiments using *Xenopus laevis* at comparable temperatures [e.g. sartorius, 215 kN m^{-2} at 20°C (Altringham et al., 1996); gastrocnemius, 250 kN m^{-2} at 23–24°C (Putnam and Bennett, 1983) and 275 kN m^{-2} at 25°C (Wilson et al., 2000); and ileofibularis, 199 kN m^{-2} at 23–24°C (Putnam and Bennett, 1983) and ~300 kN m^{-2} at 21–23°C (Lännergren and Westerblad, 1989)]. In the present study, warmer test temperatures tended to increase twitch force and significantly increased tetanus force. However, the thermal sensitivity of stress production changed with temperature. Q_{10} values indicated that both twitch and tetanus stress values increased from 15 to 24°C, but there was limited change in stress from 24 to 32°C. Broadly similar findings have previously been found in other ectotherms (Bennett, 1984; Rall and Woledge, 1990; Marsh, 1994). For instance, measurements of twitch and tetanus stress in gastrocnemius muscle from *X. laevis* demonstrated high thermal sensitivity between 5 and 20°C, with limited change from 20 to 30°C (Wilson et al., 2000). Previous work on *Rana esculenta* demonstrated that raising the temperature of single tibialis anterior muscle fibres from 2 to 12°C increased force output by a Q_{10} of approximately 2 by increasing the number of cross-bridges that were in a force-producing state, without affecting the number of cross-bridges formed between the thick and thin filaments (Piazzesi et al., 2003). Such findings on single fibres help to explain the relatively large effects of temperature on whole-muscle force production during isometric activity at low temperatures in the present and previous studies.

Isometric force rise and relaxation times

In the present study, tetanus force rise and relaxation times approximately halved with each 10°C increase in temperature across the thermal range studied, except for time to half peak tetanus, which was constant between 24 and 32°C. Such high thermal sensitivity of force rise and relaxation rates is consistent with previous studies (Bennett, 1984; Rall and Woledge, 1990; Rome and Swank, 1992; Swoap et al., 1993; Marsh, 1994; Altringham and Block, 1997). Wilson and co-workers (Wilson et al., 2000) found that increased temperature caused large reductions in time to peak twitch force and time from last stimulus to half tetanus relaxation in gastrocnemius muscle from *X. laevis*. Such increases in rates of force rise and relaxation are linked with temperature-related increases in both myofibrillar ATPase activity and the rate of calcium binding to parvalbumin (Barany, 1967; Stein et al., 1982; Rall and Woledge, 1990).

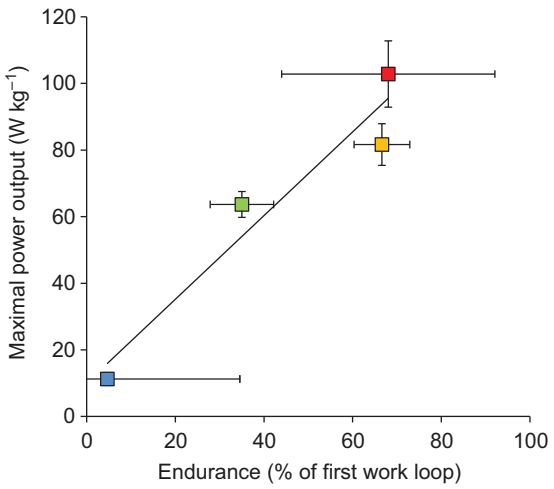

Fig. 6. Increased test temperatures led to an increase in both maximal power output (burst; average power per cycle) and sustained (endurance) performance of *Xenopus tropicalis* iliotibialis muscle when determined using the work-loop technique (F>23.0, P<0.001 in each case). For each individual, the power output generated in the sixth work loop of the endurance test has been normalised to a percentage of the power output achieved in the first work loop. Maximal power output data has been normalised to muscle mass. Data are plotted as means ± s.e.m., N=8. The line has been fitted to the mean values *via* first-order polynomial regression analysis, y=1.26x+10.1, r^2=0.93.

Work loop power output

Work-loop power output values (average power per cycle) in the present study (e.g. 63.7±3.9 W kg^{-1} at 24°C) were comparable to those found in previous studies using frog fast twitch muscles subjected to sinusoidal length changes at similar temperatures [e.g. *X. laevis* sartorius, 65 W kg^{-1} at 20°C (Altringham et al., 1996), and *Cyclorana alboguttata* sartorius, 67 W kg^{-1} at 25°C (Symonds et al., 2007)].

In the present study, maximal power output (average power per cycle) increased very rapidly between 15 and 24°C (Q_{10}=6.86) because of more rapid force generation, an enhanced ability to generate peak force, an increase in ability to maintain force during shortening, more rapid force relaxation and a rise in the number of work-loop cycles performed per second (Fig. 4B). There was a continued but lower rate of increase in power output between 24 and 32°C (Q_{10}=1.82). In comparison, Navas and co-workers (Navas et al., 1999) found that maximal work-loop power output (average power per cycle) increased with a Q_{10} of 1.63 between 10 and 25°C in lateral gastrocnemius muscle from *Rana temporaria*. The higher thermal sensitivity of *X. tropicalis* at the lower temperatures used in the present study may be due to adaptive differences (i.e. adaptation to the warmer climate that *X. tropicalis* originates from; the mean annual temperature in Cameroon is 23.6°C, compared with 10.4°C in the UK; www.climatetemp.info) and/or thermal acclimation (*R. temporaria* had been kept at 15°C, *X. tropicalis* at 24°C). Further work-loop studies on muscle power output have also demonstrated a decrease in Q_{10} with increased temperature, in yellowfin tuna and bonito red muscle fibre bundles (Altringham and Block, 1997), desert iguana ileofibularis fast muscle fibre bundles (Swoap et al., 1993) and tobacco hawkmoth flight muscle (Stevenson and Josephson, 1990). Studies using force–velocity measurements have also found higher Q_{10} values for muscle power output at lower than physiological temperatures, for example in rats (Ranatunga, 1998), humans (De Ruiter and De Haan, 2000) and Pacific blue marlin (Johnston and Altringham, 1985).

The ability of the iliotibialis muscle to maintain power output over a series of work loops increased at higher temperatures in the present study. These findings are broadly similar to those of Roots and co-workers (Roots et al., 2009), who demonstrated an increase in fatigue resistance (maintenance of peak tetanic force over a series of tetani) with increased test temperature in rat muscles subjected to either repeated isometric tetani or repeated tetani that each included an isovelocity release. A major cause of local skeletal muscle fatigue is accumulation of inorganic phosphate, which affects force production, e.g. *via* reducing calcium release from the sarcoplasmic reticulum and decreasing myofibrillar calcium sensitivity (Allen et al., 2008; Fitts, 2008). Evidence suggests that increased test temperature reduces the force depression caused by high inorganic phosphate concentrations (Coupland et al., 2001; Debold et al., 2004), providing one possible mechanism by which endurance increased with higher test temperatures in the previous (Roots et al., 2009) and present studies. Frog skeletal muscle can convert lactate to glycogen (Petersen and Gleeson, 2009); therefore, it is likely that significant recovery of glycogen stores will have occurred between each of our fatigue tests. *Xenopus laevis* have also been shown to rely much less on glycogen to fuel sprinting than do ranid frogs (Miller and Camilliere, 1981). The order of temperatures we used to test for endurance was 24, 30, 15 and 32°C, yet we found that endurance increased in the order 15, 24, 30 and 32°C. Considering these results, and the literature evidencing glycogen resynthesis in frog muscle, we believe that the use of multiple short endurance tests in the present study has produced valid and reliable results. A further possible reason for decreased fatigue resistance at lower temperatures is that slowing of relaxation because of fatigue had a greater effect at lower temperatures, where relaxation already constituted a much larger proportion of the work-loop cycle.

The sinusoidal strain waveform used in the present study is probably an oversimplification of the *in vivo* muscle strain waveform. Sonomicrometry measurements in swimming toads (*B. marinus*) suggest that muscles involved in powering leg extension, including the iliotibialis, often spend less of the cycle in shortening than in lengthening (Gillis and Biewener, 2000). However, iliotibialis muscle strain waveforms during swimming are unknown for *X. tropicalis* and we do not think that the exact choice of strain waveform used in the present study would affect the observed effects of temperature on skeletal muscle performance.

Implications of the present study

In the present study, the high Q_{10} for muscle power output (average power per cycle) between 15 and 24°C suggests a potential limiting factor on burst performance (swimming and jumping), which may affect the geographical distribution of this species. For example, the muscle power output at 15°C may be too low to enable the animal to escape predation or to move between ponds when water levels are low. Low temperatures have previously been shown to restrict aerobic performance in cane toads, placing a limit on their distribution in Australia (Seebacher and Franklin, 2011). As global climate change will continue to affect local environmental conditions, causing warmer climates in some regions (Ganguly et al., 2009), this could open up new geographical regions for tropical amphibian species to move into. Differences in thermal sensitivity of locomotor performance have been found between populations of frogs from different latitudes and/or altitudes (Navas, 1996; Wilson, 2001) (but see John-Alder et al., 1988); however, it remains unclear in many cases as to how much of such populational differences are due to acclimation and how much reflect genetic adaptation. Indeed, latitudinally different populations of killifish, from different thermal

environments, did not differ in thermal sensitivity of sustained swimming performance (U_{crit}) once acclimated to a common temperature (Fangue et al., 2008), suggesting a plastic component to thermal sensitivity. In contrast, similar experiments on cod found that the population from the warmer latitude had higher sustained swimming performance at both high and low test temperatures (Sylvestre et al., 2007). Future studies on populational differences in thermal sensitivity of locomotor performance could use work-loop muscle mechanics experiments to investigate some of the potential reasons for differences in locomotor performance. However, it is important to establish the link between *in vitro* muscle work-loop experiments and whole-organism performance traits, such as burst performance or sustained locomotor capacity, if one is to make inferences on the potential ecological and evolutionary ramifications of changes in temperature.

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