THE THERMAL DEPENDENCE OF LOCOMOTOR PERFORMANCE AND MUSCLE CONTRACTILE FUNCTION IN THE SALAMANDER *AMBYSTOMA TIGRINUM NEBULOSUM*

BY P. L. ELSE AND A. F. BENNETT

*School of Biological Sciences, University of California at Irvine, Irvine,
CA 92717, USA*

SUMMARY

The thermal dependence of locomotor performance and *in vitro* muscle mechanical properties were studied after acclimation at 10° and 20°C in the salamander *Ambystoma tigrinum nebulosum* Hallowell. Performance measurements included burst and endurance capacities on land and in water. No significant differences in locomotor performance or muscle contractile properties were found between acclimation groups. Locomotor performance had only a slight thermal dependence, with Q_{10} values of 0·99–1·36 for running and swimming burst capacities (i.e. maximum speed and leg/tail cycling frequency). Running and swimming endurance capacities had thermal ratios of 1·58–1·66. Thermal dependence of muscle contractile rates was higher than that of locomotor performance: rates of force development for both isometric twitch and tetanus and maximal shortening velocity had Q_{10} values of 1·89–2·01. Maximal power output was also thermally dependent ($Q_{10} = 2·33$) and occurred at 38 % of maximal (tetanic) force output. Force-generating capacities in isometric twitch and tetanus were relatively temperature-independent.

INTRODUCTION

Poikilotherms by definition experience variable body temperatures. Most biological rate processes are positively modulated by higher body temperatures. Consequently, it might be expected that the physiological capacities of poikilotherms and the behaviour patterns that depend upon them (e.g. locomotion) may be variable and subject to depression in cold environments. A number of ecologically significant types of behaviour (for instance, prey capture, predator avoidance, courtship) might thus be affected adversely by low body temperatures.

There are two potential physiological solutions that would ameliorate the thermal effect on behavioural and physiological capacities. One is the evolution of physiological processes that are acutely thermally independent. The other is individual acclimation, compensation to long-term temperature exposure that tends to restore

rate processes to their original level. Poikilotherms often show alterations in biochemical activity in accordance with acclimation temperature (Prosser, 1973), and some behavioural modifications may also be seen. For example, in lower vertebrates thermal acclimation can alter metabolic rate (Feder, 1982), critical thermal maximum (Brattstrom & Lawrence, 1962), prolonged swimming speed (Fry & Hart, 1948; Rome, Loughna & Goldspink, 1985) and learning behaviour (Rahmann, Schmidt & Schmidt, 1980). However, in studies on anuran amphibians, in which changes in locomotor capacity and muscle contractile function have been examined after thermal acclimation, surprisingly little adjustment has been found (Putnam & Bennett, 1981; Renaud & Stevens, 1981a,b, 1983a,c, 1984; Rome, 1983). We have undertaken this study on the thermal dependence of muscle function and locomotor capacity in another amphibian, *Ambystoma tigrinum nebulosum*, a salamander that undergoes large changes in body temperature on a seasonal basis. We sought to determine whether acute or acclimatory adaptations exist in the functional capacities of the skeletal muscle of this animal to its variable temperature regime and whether these are reflected in locomotor capacities.

The salamander *Ambystoma tigrinum nebulosum* of northern Arizona is a native, widespread subspecies that occupies a variety of habitats (Collins, 1981). In central northern Arizona these animals experience seasonal temperature cycles with average minimal and maximal air temperatures ranging from $-1\,°C$ to $20\,°C$. In early spring as the snow melts, adult animals of this species migrate to breed in ponds that have water temperatures of $5°-10\,°C$. Courtship in ambystomatid salamanders is vigorous with multiple males attempting to displace each other from the female (Arnold, 1976). There is consequently a considerable premium for a capacity for both rapid and sustained activity at low body temperatures. These same ponds may warm to more than $25\,°C$ during the summer and metamorphosing animals will be exposed to these relatively high temperatures. To assay locomotor performance, we made quantitative measurements of burst speed and endurance on land and in water. To examine underlying muscle contractile performance, we measured isometric twitch force, time to peak twitch, maximal rate of twitch force development, twitch half-relaxation time, maximal tetanic force and rate of tetanic force development. Isotonic muscle measurements included maximal velocity of shortening and maximal power output. These experiments were performed on two groups of salamanders acclimated to $10°$ and $20\,°C$.

<center>MATERIALS AND METHODS</center>

<center>*Animals and acclimation procedure*</center>

Tiger salamanders, *Ambystoma tigrinum nebulosum*, were collected by seining ponds on the Mogollon Rim, northern Arizona, during September, 1984, under Arizona Game and Fish Department Permit No. 85-5 to Dr James Collins, Arizona State University. The animals were transported to the University of California, Irvine, where all experiments were conducted. Salamanders rapidly metamorphosed when maintained at $20°-22\,°C$ in large ($42 \times 32 \times 90\,cm$) aquaria. Metamorphosed

animals were isolated and used in all experiments (body mass $\pm$ S.E.M. $= 8.62 \pm$ 1.14 g; snout–vent and total body length $\pm$ S.E.M. $= 6.59 \pm 0.25$ cm and $11.39 \pm$ 0.51 cm, respectively; $N = 21$). Animals were separated into two groups of similar mass and acclimated at either 10°C ($N = 11$) or 20°C ($N = 10$) for 3 weeks in temperature-controlled cabinets (12 h : 12 h light : dark photoperiod). Animals were fed mealworms and had constant access to fresh water. During the ensuing 8-week experimental period, animals were maintained at their acclimation temperature and were removed only for the short duration of the locomotor tests. Experiments were carried out at 10° and 20°C on consecutive days. The order of experimentation was (1) running burst, (2) running endurance, (3) swimming burst and (4) swimming endurance. Both acclimation groups for each locomotor behaviour were measured on the same day; the total experimental period of the locomotor measurements was 8 days. Muscle experiments began 9 days after the final locomotor experiment; one animal was drawn from each acclimation group on consecutive days.

Locomotor measurements

Animals were placed at test temperature 1 h before measurement of locomotor capacity. Individual burst and endurance capacities on land and in water were measured in a controlled temperature room at both 10° and 20°C (range ± 0.5°C) for both acclimation groups. Both running and swimming bursts were recorded in an open rectangular plastic trough (7 cm wide $\times$ 6 cm high $\times$ 2 m long), marked at 10-cm intervals. For running bursts, dampened plastic-backed paper towelling served as a substrate. The trough was filled with water to a depth of 5 cm for burst swimming. Animals were induced to run or swim by light pinches or prods at the base of the tail. Speeds were measured on three trials per individual, trials being separated by more than 1 h. Running and swimming bursts were videotaped (RCA Camera Model TC 1005, VHS Panasonic Recorder Model 8950) and measurements were made from a monitor (Hitachi Model VM 1290). The fastest speed over a 20-cm interval was recorded for each of the three trials and the fastest of the three is reported. Hindlimb and tail cycling frequencies were measured to the nearest quarter cycle during the fastest burst.

Running endurance was measured on a custom-designed, motorized, variable-speed treadmill. Initial speed was 0.10 km h^{-1}; speed was increased by 0.05 km h^{-1} every 5 min until exhaustion occurred (judged by the inability of an animal to right itself within 5 s after being placed on its back). Total time and apparent distance travelled were recorded. Animals were moistened frequently to prevent dehydration. Swimming endurance was measured in an open channel (7 cm wide $\times$ 6 cm high $\times$ 30 cm long) flume system. Water speed was controlled by gating water through the water pump with flow speeds starting at 0.20 km h^{-1} and increasing by 0.10 km h^{-1} every 5 min. Water temperature was controlled with an Endocal recirculating bath. Time to exhaustion and apparent distance travelled were used to measure endurance capacity. Running and swimming endurance at each temperature were measured once for each animal.

Muscle measurements

Body mass and snout–vent, total body and hind limb lengths were measured for each salamander. Animals were decapitated and spinally pithed. Muscle contractile performance was measured on the Musculus extensor iliotibialis pars anterior, a true extensor of the leg (Francis, 1934). Muscle length was measured *in situ* and the muscle was dissected with a portion of the pelvic bone that was used to attach the muscle to a stainless steel stand. The other end of the muscle was tied by its tendon with 5·0-gauge silk thread to an insect pin and attached to a servo-controlled muscle ergometer (Cambridge Instruments Model 300H). The ergometer was held in a custom-designed stand to permit fine adjustment of muscle length. The ergometer was calibrated in both its isometric and isotonic modes against an external strain-gauge force transducer (Pixie Model 8101) mounted on a custom-designed shaker motor with an optical position sensor and feedback circuit. Ergometer output was sent to a four-channel storage oscilloscope (Tektronix 5111). Stimulation was delivered from a Grass S48 stimulator through an amplifier (South West Technical Products Power Amp 275) to two 15×5 mm platinum electrodes mounted close to the muscle. The 90 % response time of the system with an attached muscle was 1·8 ms. The muscle and electrodes were surrounded by a Plexiglas chamber in which a saline solution was recirculated by a Masterflex dual-head pump from a thermostatted (range $\pm 0 \cdot 1 \degree$C) 900 ml reservoir. The saline solution contained 145 mmol l^{-1} NaCl, 4 mmol l^{-1} KCl, 20 mmol l^{-1} imidazole, 2·5 mmol l^{-1} CaCl$_2$, 2 g l^{-1} glucose and pH was adjusted to 7·0 at 20°C with HCl. The solution was oxygenated with 100 % O$_2$.

The experimental protocol used for isometric contractile measurements was that of Putnam & Bennett (1982). Muscle length and stimulation regime were adjusted to elicit maximum developed twitch tension at the initial test temperature (20°C). Static tension was less than 0·05 g. Isometric measurements consisted of a twitch followed by a tetanic stimulus. Representative oscillograph traces are shown in Fig. 1. The measurements were repeated as muscle temperature was lowered from 20° to 0°C by 5° intervals with 2 min equilibration at each temperature (± 1°C). Each muscle was then returned to 20°C, allowed 10 min to equilibrate, and contractile performance was remeasured to determine any decrement in muscle performance: the mean reduction in maximum isometric tension (P$_0$) was 4·1 % of initial P$_0$. The preparation was then warmed and measured at 2·5° intervals up to failure of contraction. To obtain maximal tetanic tension at each temperature, train duration (range = 100–800 ms) was increased and stimulation frequency (range = 40–400 Hz) was reduced at low temperatures. Isometric properties determined were peak twitch force (P$_t$), time to peak twitch force (TPT), time from peak twitch force to half-relaxation ($\frac{1}{2}$RT), maximal rate of force development during a twitch (dP$_t$/dt) and during a tetanus (dP$_0$/dt), and peak tetanic force (P$_0$).

After measurement of isometric variables, isotonic properties were measured on the contralateral iliotibialis muscle of each individual at 10° and 20°C. Maximal isometric tension was determined for each muscle and the rate of muscle shortening

($mm\,s^{-1}$) as a function of imposed load (1.5–95% P_0) was measured with the Cambridge ergometer system. A representative oscillograph trace is shown in Fig. 2. The maximal velocity of shortening (V_{max}) was calculated for each muscle from the relationship between shortening velocity (muscle lengths s^{-1}, $L\,s^{-1}$) and P/P_0. Force–velocity data were fitted to the following hyperbolic–linear equation using the non-linear least-squares regression method NLLSQ (CET Research Group, Ltd, Norman OK): $V = [B(1-X)/(A+X)]+C(1-X)$, where X equals P/P_0. This equation yields a better approximation to the data than does the Hill equation (Hill, 1938; Marsh & Bennett, 1985, 1986a). Power output was calculated for each muscle by multiplying force (in N) by shortening velocity (in $m\,s^{-1}$) and dividing by muscle mass (in kg) to normalize power output ($W\,kg^{-1}$). Muscle power curves were fitted with least-squares cubic polynomial regressions.

At the end of each experiment, the length of the unstimulated muscle in the apparatus was measured with Vernier calipers. This closely approximated muscle length *in vivo*. The muscle was released from the apparatus, the bone and connective tissue were dissected away, and the muscle was blotted and weighed to the nearest 0.1 mg. Cross-sectional area of each muscle was calculated by dividing muscle mass by *in vitro* muscle length. All forces are expressed as kilonewtons per square metre of muscle cross-sectional area. All muscles used generated greater than $200\,kN\,m^{-2}$ of force at $20\,°C$.

Statistics

The effects of acclimation and measurement temperature were tested on all locomotor performance and muscle contractile properties at $10°$ and $20\,°C$, and additionally on isometric muscle properties at $0°$ and $35\,°C$ to examine any shifts in
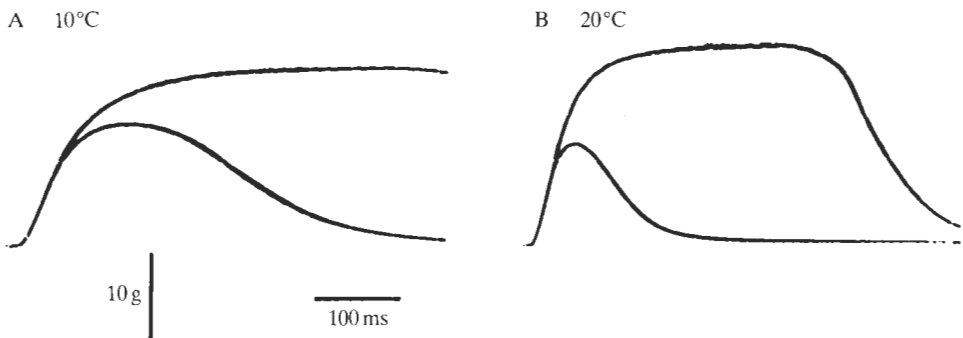

Fig. 1. Isometric twitch and tetanic contractions of the M. extensor iliotibialis pars anterior of *Ambystoma tigrinum nebulosum* at $10°$ and $20\,°C$. (A) $10\,°C$: isometric tension (P_0) = $481\,kN\,m^{-2}$, rate of force development for a tetanus (dP_0/dt) = $6.10\,kN\,m^{-2}\,ms^{-1}$, twitch force ($P_t$) = $327\,kN\,m^{-2}$, rate of force development for a twitch (dP_t/dt) = $5.72\,kN\,m^{-2}\,ms^{-1}$, time to peak twitch force (TPT) = 135 ms, time from peak twitch force to half-relaxation ($\frac{1}{2}RT$) = 170 ms. (B) $20\,°C$: P_0 = $538\,kN\,m^{-2}$, dP_0/dt = $9.15\,kN\,m^{-2}$ ms^{-1}, P_t = $275\,kN\,m^{-2}$, dP_t/dt = $9.15\,kN\,m^{-2}\,ms^{-1}$, TPT = 55 ms, $\frac{1}{2}RT$ = 60 ms.
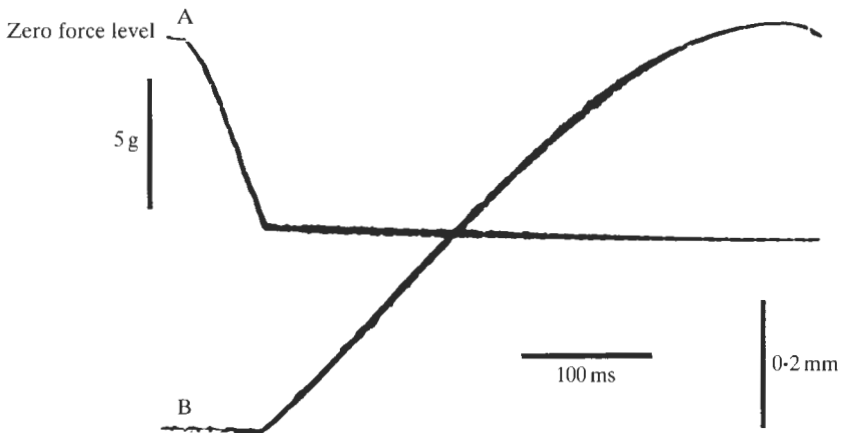
Fig. 2. Isotonic contraction at 10°C of the M. extensor iliotibialis pars anterior of *Ambystoma tigrinum nebulosum*. Force development (A) is set by ergometer ($7·25\,g = 50\%\,P_0$); muscle shortens (B) at $2·22\,mm\,s^{-1}$ ($= 0·32\,lengths\,s^{-1}$). Downward deflection of the force record represents increasing force. Upward deflection of the velocity record represents a decrease in muscle length.

the thermal range of muscle performance. All values were $\log_{10}$ transformed for statistical analysis, a procedure that increased linearity and normalized variance over the range of body masses used. Repeated-measures analysis of variance was performed using the MANOVA procedure of SPSS statistical programs. The analysis was performed with a between-subjects factor (acclimation temperature), a repeated-measures factor (measurement temperature) and a constant covariate (body mass). Body masses used were those measured independently for locomotor performance and muscle properties.

RESULTS

Effect of acclimation temperature

Responses to thermal acclimation can have two forms, capacity and resistance adaptation. Capacity adaptations are those that permit relative constancy of biological activity over a normally experienced temperature range. Resistance adaptations are those that permit survival at environmental extremes (Prosser, 1973). To test for capacity adaptations, all locomotor and muscle properties were analysed at 10° and 20°C (Table 1). Resistance adaptation in isometric muscle properties was examined at 0° and 35°C (Table 2). In no case were any significant differences found between the two acclimation groups for any measurement at any temperature examined (Tables 1, 2). No significant interaction terms were found between acclimation and measurement temperatures in any tests. The two acclimation groups were subsequently combined for the analysis of thermal dependence of locomotor and muscle contractile properties.

Table 1. *The effect of acclimation temperature on locomotor performance and muscle contractile function*

Acclimation group	Units	Measurement temperature 10°C 10°C-acclimated	20°C-acclimated	20°C 10°C-acclimated	20°C-acclimated	Significance of acclimation temperature F value	P value
Locomotor performance							
Running burst speed	$cm\,s^{-1}$	14.0 ± 1.9	13.7 ± 1.2	20.2 ± 3.0	17.1 ± 1.0	0.21	0.65
Swimming burst speed	$cm\,s^{-1}$	24.8 ± 1.6	24.7 ± 1.3	24.4 ± 1.2	24.8 ± 1.6	0.01	0.96
Leg cycling frequency	Hz	2.24 ± 0.17	2.30 ± 0.11	2.95 ± 0.22	3.04 ± 0.15	0.47	0.50
Tail cycling frequency	Hz	4.74 ± 0.26	4.42 ± 0.33	5.24 ± 0.29	4.98 ± 0.40	0.74	0.40
Running endurance	m	42.8 ± 17.7	40.2 ± 5.7	73.1 ± 30.9	56.8 ± 10.7	0.36	0.56
Swimming endurance	m	73.4 ± 9.0	93.9 ± 14.4	137 ± 17	139 ± 23	0.31	0.59
Muscle contractile properties							
Isometric							
P_0	$kN\,m^{-2}$	305 ± 25	304 ± 38	339 ± 28	338 ± 42	0.03	0.87
P_t	$kN\,m^{-2}$	204 ± 24	218 ± 28	164 ± 22	203 ± 26	1.17	0.30
dP_0/dt	$kN\,m^{-2}\,ms^{-1}$	3.56 ± 0.06	3.78 ± 0.59	6.86 ± 1.12	6.90 ± 1.00	0.11	0.74
dP_t/dt	$kN\,m^{-2}\,ms^{-1}$	3.30 ± 0.43	3.58 ± 0.52	6.49 ± 1.16	6.85 ± 1.01	0.58	0.46
TPT	ms	133 ± 9	138 ± 4	55.8 ± 3.4	66.1 ± 1.8	0.92	0.35
$\frac{1}{2}$RT	ms	181 ± 13	200 ± 15	73.8 ± 6.0	78.9 ± 5.5	0.82	0.38
Isotonic							
V_{max}	$L\,s^{-1}$	1.59 ± 0.06	1.39 ± 0.01	3.08 ± 0.15	2.92 ± 0.21	2.03	0.17
$\dot{W}_{max}$	$W\,kg^{-1}$	59.9 ± 4.9	45.3 ± 8.1	132 ± 11	113 ± 17	2.75	0.12

Locomotor performance, 10°C-acclimated group, $N = 11$; 20°C-acclimated group, $N = 10$.
Muscle contractile properties, 10°C-acclimated group, $N = 11$; 20°C-acclimated group, $N = 9$.
Mean values ± S.E.M.
P_0, maximal isometric tension; P_t, peak twitch force; dP_0/dt, maximal rate of force development during a tetanus; dP_t/dt, maximal rate of force development during a twitch; TPT, time to peak twitch force; $\frac{1}{2}$RT, time from peak twitch force to half-relaxation; V_{max}, maximal velocity of shortening; $\dot{W}_{max}$, maximal power output; L, muscle length.

Table 2. *The effect of acclimation temperature on isometric muscle contractile properties at extremes of the thermal range*

| Muscle contractile properties | Measurement temperature 0°C | | Measurement temperature 35°C | | Significance of acclimation temperature | |
	10°C-acclimated	20°C-acclimated	10°C-acclimated	20°C-acclimated	F value	P value
P_0 ($kN\,m^{-2}$)	244 ± 21	239 ± 29	233 ± 30	239 ± 39	0·01	0·94
P_t ($kN\,m^{-2}$)	202 ± 20	206 ± 27	110 ± 17	108 ± 19	0·02	0·90
dP_0/dt ($kN\,m^{-2}\,ms^{-1}$)	$1·26 \pm 0·15$	$1·22 \pm 0·17$	$8·27 \pm 1·35$	$8·13 \pm 1·62$	0·01	0·93
dP_t/dt ($kN\,m^{-2}\,ms^{-1}$)	$1·20 \pm 0·15$	$1·20 \pm 0·17$	$8·46 \pm 1·23$	$8·31 \pm 1·67$	0·02	0·90
TPT (ms)	402 ± 32	432 ± 17	$25·4 \pm 1·4$	$25·5 \pm 0·7$	0·54	0·48
$\frac{1}{2}$RT (ms)	636 ± 47	751 ± 37	$30·1 \pm 2·3$	$35·0 \pm 3·7$	3·21	0·09

Measurement temperature 0°C, 10°C-acclimated group, $N = 11$; 20°C-acclimated group, $N = 9$.

Measurement temperature 35°C, 10°C-acclimated group, $N = 10$; 20°C-acclimated group, $N = 9$.

Values are mean ± S.E.M.

For abbreviations see Table 1.

Effect of measurement temperature

In contrast to the lack of any acclimation effect, every variable measured except swimming burst speed showed a significant thermal dependence (Table 3). Locomotor measurements between 10° and 20°C in general showed a low thermal dependence: the thermal coefficients for maximal burst speed and cycling frequencies on land and in water were low (Q_{10} values of 0·99–1·36). Running and swimming endurance had slightly higher thermal ratios (R_{10}, a number analogous to Q_{10} for non-rate variables, Bennett, 1984) of 1·58 and 1·66, respectively.

Muscle contractile properties displayed a variety of thermal dependencies (Fig. 3). Between 10° and 20°C, force production was relatively temperature-independent: P_t and P_0 had R_{10} values of 0·86 and 1·10, respectively. Rates of force development for twitch and tetanus (dP_t/dt and dP_0/dt), maximal velocity of shortening and maximal power output (V_{max} and $\dot{W}_{max}$, Fig. 4) had higher thermal dependencies, with Q_{10} values of 1·89–2·33. Time to peak twitch and half-relaxation time were the most thermally dependent factors with Q_{10} values of 2·32 and 2·49, respectively.

Muscle contractile performance was maintained over the range of 0° to 37·5°C (Fig. 3). Contractile failure occurred at 40°C in both acclimation groups. Tetanic force was maximal at 20°C, the preferred body temperature of this salamander (Brattstrom, 1963), and over 80% of maximal tetanic force was maintained between 5° and 32·5°C. Rates of contraction were positively thermally dependent up to 35°C (Fig. 3; Table 4).

DISCUSSION

Acclimation effects on locomotor ability and muscle properties

In the present study no changes in maximal burst or endurance capacities were found on land or in water as a result of different acclimation temperatures. In anurans, a variety of acclimatory effects has been reported. Warm-acclimated *Rana pipiens* jumped further at high temperatures but no differences were found in the jumping capacities of warm- and cold-acclimated *Bufo marinus* at different temperatures (Renaud & Stevens, 1983a). The distance travelled before exhaustion exhibited no acclimation effects in *Rana pipiens* or *Bufo boreas* in another study (Putnam & Bennett, 1981). In several fish species, an ability to maintain higher swimming speeds as acclimation temperature increased has been reported (Fry & Hart, 1948; Larimore & Duever, 1968; Griffiths & Alderdice, 1972; Rome *et al.* 1985).

Muscle contractile properties in the present study were also found to be independent of acclimation temperature. These results are congruent with five recent comprehensive studies on anuran muscle (Renaud & Stevens, 1981a,b, 1983b, 1984;

Table 3. *The effect of temperature on locomotor performance and muscle contractile function*

		Measurement temperature		Q_{10} or R_{10}	Significance of measurement temperature	
	Units	10°C	20°C		F value	P value
Locomotor performance						
Running burst speed	$\mathrm{cm\,s^{-1}}$	13.8 ± 1.1	18.7 ± 1.6	1.36	14.2	0.001
Swimming burst speed	$\mathrm{cm\,s^{-1}}$	24.8 ± 1.0	24.6 ± 0.9	0.99	0.01	0.936
Leg cycling frequency	Hz	2.27 ± 0.10	3.00 ± 0.13	1.32	15.6	<0.001
Tail cycling frequency	Hz	4.59 ± 0.21	5.12 ± 0.24	1.12	5.59	0.029
Running endurance	m	41.5 ± 9.5	65.4 ± 16.7	1.58	30.9	<0.001
Swimming endurance	m	83.2 ± 8.4	138 ± 13	1.66	36.3	<0.001
Muscle contractile properties						
Isometric						
P_0	$\mathrm{kN\,m^{-2}}$	304 ± 21	338 ± 24	1.10	47.0	<0.001
P_t	$\mathrm{kN\,m^{-2}}$	210 ± 18	182 ± 17	0.86	10.9	0.004
dP_0/dt	$\mathrm{kN\,m^{-2}\,ms^{-1}}$	3.66 ± 0.34	6.88 ± 0.75	1.89	136	<0.001
dP_t/dt	$\mathrm{kN\,m^{-2}\,ms^{-1}}$	3.42 ± 0.33	6.65 ± 0.76	1.95	132	<0.001
TPT	ms	135 ± 5	58.2 ± 2.1	2.32	2065	<0.001
$\frac{1}{2}$RT	ms	189 ± 10	76.1 ± 4.1	2.49	465	<0.001
Isotonic						
$\dot{V}_{max}$	$\mathrm{L\,s^{-1}}$	1.50 ± 0.06	3.01 ± 0.12	2.01	234	<0.001
$\dot{W}_{max}$	$\mathrm{W\,kg^{-1}}$	53.3 ± 4.7	124 ± 9	2.33	349	<0.001

Locomotor performance, $N = 21$; muscle contractile properties, $N = 20$.
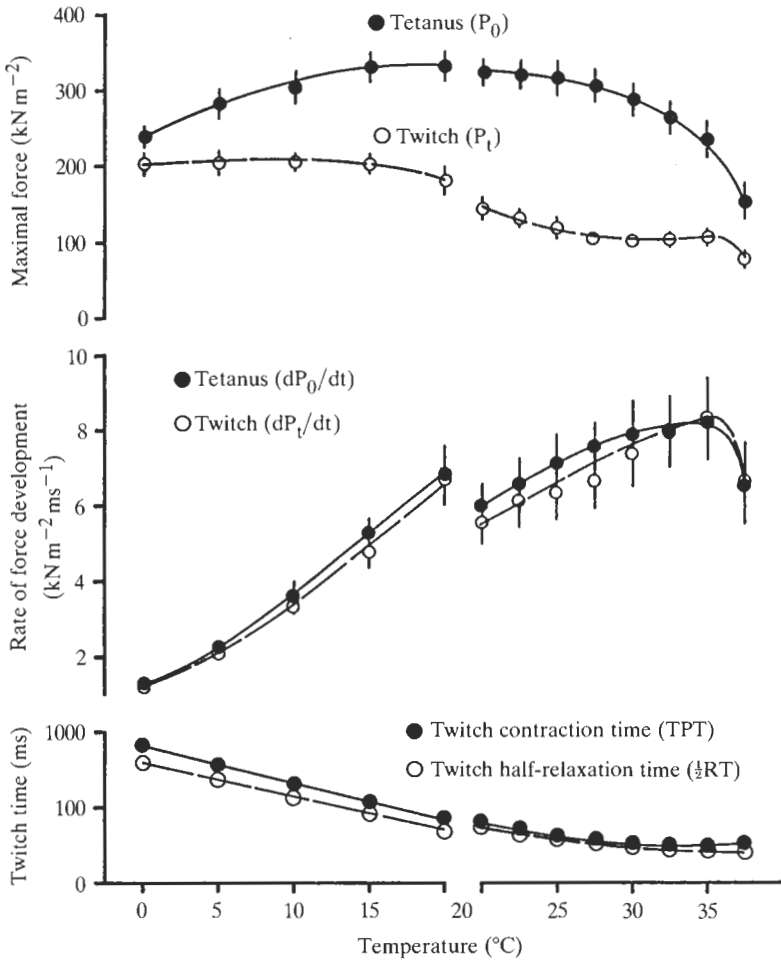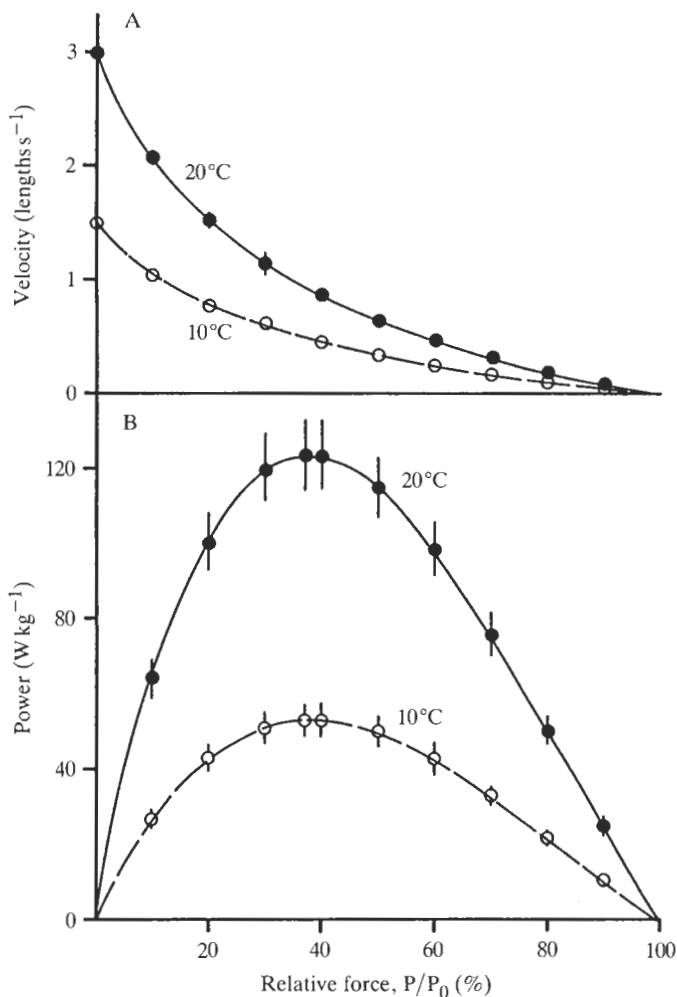Values are mean $\pm$ S.E.M.
For abbreviations see Table 1.

Fig. 3. The effect of temperature on the isometric properties of M. extensor iliotibialis pars anterior from *Ambystoma tigrinum nebulosum* between 0° and 37·5°C. From top to bottom: tetanic force (P_0), twitch force (P_t), rate of force development for a tetanus (dP_0/dt) and twitch (dP_t/dt), time to peak twitch (TPT) and twitch half-relaxation time ($\frac{1}{2}$RT). Bars are ±S.E.M. ($N = 20$).

Rome, 1983). These studies found no major effects of thermal acclimation on muscle performance; the minor differences reported were considered to have no apparent biological significance. One of these studies (Rome, 1983) reported no changes in shortening velocity, power output or isometric force for frog muscle even after 8 months of acclimation. Only in cyprinid fish have significant acclimation effects on muscle contractile properties been demonstrated (Altringham & Johnston, 1985; Johnston, Sidell & Driedzic, 1985).

Thermal effects on locomotor performance and muscle properties

Temperature had no effect on swimming burst speed and only a small effect on running burst speed of tiger salamanders. A low thermal dependence ($Q_{10} =$

1·1–1·6) for running burst speed has previously been found in lizards (e.g. Bennett, 1980; Hertz, Huey & Nevo, 1983). In contrast, garter snakes show high thermal dependence for maximal crawling velocity ($Q_{10} = 2·3$) but low thermal dependence for maximal swimming velocity ($Q_{10} = 1·4$ between 10° and 30°C; Stevenson, Peterson & Tsuji, 1985). The difference in thermal dependence between crawling and swimming in garter snakes is similar to the trend found for tiger salamanders between maximal land and water burst capacities. Anurans have a low thermal

Fig. 4. The effect of temperature on the isotonic properties of M. extensor iliotibialis pars anterior from *Ambystoma tigrinum nebulosum* at 10° and 20°C. (A) Force–velocity curves at 10° and 20°C. Values were obtained from hyperbolic–linear equations describing the original data of each individual muscle. Power ratios ($\dot{W}_{max}/V_0P_0$, Marsh & Bennett, 1985, 1986a) are 0·122 and 0·121 and normalized *a* values (Hill, 1938) are 0·348 and 0·352 at 10° and 20°C, respectively. (B) Power output at 10° and 20°C. Values were obtained from cubic polynomial regressions fitted to the original data of each individual salamander. The power curves at 10° and 20°C were broad: 80 % of maximal power was maintained between 19 and 61 % of maximal force output. Bars are ±S.E.M. ($N = 20$).

Table 4. *The thermal coefficients and ratios for isometric muscle contractile properties*

Temperature (°C)	P_0	P_t	dP_0/dt	dP_t/dt	TPT	$\frac{1}{2}$RT
0–10	1·26	1·03*	2·95	2·90	3·08	3·65
10–20	1·10	0·86	1·89	1·95	2·32	2·49
20–30	0·89	0·72	1·32	1·32	1·84	2·01
30–35	0·66	1·12*	1·07*	1·31	1·22	1·01*

* Not significantly different from 1·00.
For abbreviations see Table 1.

dependence for maximal jumping distance ($Q_{10} = 1\cdot3$–$1\cdot6$ at $15°$–$25°C$) (Renaud & Stevens, 1983a; Hirano & Rome, 1984).

Temperature had a marked effect on endurance capacity of tiger salamanders. In fish, values of prolonged swimming speeds, a similar measurement to endurance capacity measured in our study, show thermal coefficients ranging from $1\cdot36$ to $1\cdot73$ for six species (summarized in Beamish, 1978). The maximal aerobic speed, another measure of endurance, shows a positive thermal dependence in several lizard species with Q_{10} values between $1\cdot11$ and $1\cdot68$ (Bennett & John-Alder, 1984). These values are similar to the thermal ratio of endurance found for tiger salamanders.

The thermal dependences of leg and tail cycling frequencies of tiger salamanders were similar to those for running and swimming burst speeds. The same low thermal dependence for cycling frequencies has also been found in lizards ($Q_{10} = 1\cdot3$, Marsh & Bennett, 1985, 1986b).

The thermal dependence of muscle contractile properties reported here is very typical of that of vertebrates in general: isometric forces have a low thermal dependence, isometric and isotonic rate processes have Q_{10} values of approximately $2\cdot0$ (Bennett, 1984, 1985). The rate of tension development and relaxation continues to increase with increasing temperature far beyond the normal range experienced by these salamanders. There is no apparent optimization of contractile speed at low body temperatures.

None of the experimental data collected in this study indicate any special adaptations that have reduced the acute thermal dependence of contractile rate processes in this salamander.

Physiological limitation of burst locomotor performance

What limits running burst performance in quadrupedal ectotherms? Marsh & Bennett (1985, 1986b) have suggested that the time course of an isometric twitch limits limb cycling frequency, and hence burst speed, at moderate and low body temperatures in lizards. Data on lizard (Marsh & Bennett, 1985) and frog muscle (Jewell & Wilkie, 1960) suggest that twitch time course (the sum of time to peak twitch, TPT, and half-relaxation time, $\frac{1}{2}$RT) approximates to the duration of the active state in skeletal muscle. Contraction of antagonistic muscles during this period

would encounter residual resistance in the original muscles. This antagonism would require work against the residual force with a consequent loss of efficiency.

Is there a similar match between limb cycling frequency and twitch duration in the muscles of these salamanders? The extension of the limb during running bursts was determined by video analysis to be 45 % and 43 % of total limb cycle at 10° and 20°C, respectively. The extension phase of the limb thus requires 203 ms at 10°C and 143 ms at 20°C. The sum of the TPT and $\frac{1}{2}$RT is 324 ms at 10°C and 134 ms at 20°C. Thus at 20°C the match between twitch contraction time and limb cycling frequency (134 ms *vs* 143 ms) is quite good. At 10°C, however, the twitch time course exceeds that of the extension portion of the limb cycle by more than 100 ms (324 ms *vs* 203 ms). Apparently at low temperatures, muscles must begin activity against a significant resistance remaining in their antagonists during burst locomotion. These data reaffirm the view that the twitch response of the muscle can be a significant limitation to burst locomotor speed at low temperatures in quadrupedal ectothermic animals.

This species of salamander, therefore, shows no particular locomotor or muscle adjustments, other than thermal independence of burst swimming speed, to its variable and seasonally cold thermal regime. The factors measured do not acclimate and have similar acute thermal dependence to that reported for other vertebrates, including endotherms. Thus, behavioural performance is significantly impaired by low body temperatures, even those at which many biologically important activities, such as reproduction, occur. These data accord with observations on several species of anuran amphibians, indicating that adjustment of muscle function and locomotor activity to normally experienced temperature is virtually absent in all amphibians examined. From an adaptative perspective, these results are puzzling, considering the importance of these locomotor activities to survival and reproduction.

REFERENCES

ALTRINGHAM, J. D. & JOHNSTON, I. A. (1985). Changes in tension generation and ATPase activity in muscle fibers of the carp following temperature acclimation. *Pflügers Arch. ges. Physiol.* **403**, 449–451.

ARNOLD, S. J. (1976). Sexual behavior, sexual interference and sexual defense in the salamanders *Ambystoma maculatum*, *Ambystoma tigrinum* and *Plethodon jordani*. *Z. Tierpsychol.* **42**, 247–300.

BEAMISH, F. W. H. (1978). Swimming capacity. In *Fish Physiology*, vol. 7 (ed. W. S. Hoar & D. J. Randall), pp. 101–187. New York: Academic Press.

BENNETT, A. F. (1980). The thermal dependence of lizard behaviour. *Anim. Behav.* **28**, 752–762.

BENNETT, A. F. (1984). Thermal dependence of muscle function. *Am. J. Physiol.* **247**, R217–R229.

BENNETT, A. F. (1985). Temperature and muscle. *J. exp. Biol.* **115**, 333–344.

Bennett, A. F. & John-Alder, H. B. (1984). The effect of body temperature on the locomotory energetics of lizards. *J. comp. Physiol.* **155**, 21–27.

Brattstrom, B. H. (1963). A preliminary review of the thermal requirements of amphibians. *Ecology* **44**, 238–255.

Brattstrom, B. H. & Lawrence, P. (1962). The rate of thermal acclimation in anuran amphibians. *Physiol. Zool.* **35**, 148–156.

Collins, J. P. (1981). Distribution, habitats and life history variation in the tiger salamander, *Ambystoma tigrinum*, in east-central and southeast Arizona. *Copeia* **1981**, 666–675.

Feder, M. E. (1982). Environmental variability and thermal acclimation of metabolism in tropical anurans. *J. therm. Biol.* **7**, 23–28.

Francis, E. T. B. (1934). *Anatomy of the Salamander*. Oxford: Oxford University Press.

Fry, F. E. J. & Hart, J. S. (1948). Cruising speed of goldfish in relation to water temperature. *J. Fish. Res. Bd Can.* **7**, 169–175.

Griffiths, J. S. & Alderdice, D. F. (1972). Effects of acclimation and acute temperature experience on swimming speed of juvenile Coho salmon. *J. Fish. Res. Bd Can.* **29**, 251–264.

Hertz, P. E., Huey, R. B. & Nevo, E. (1983). Homage to Santa Anita: Thermal sensitivity of sprint speed in agamid lizards. *Evolution* **37**, 1075–1084.

Hill, A. V. (1938). The heat of shortening and the dynamic constants of muscle. *Proc. R. Soc.* B **126**, 136–195.

Hirano, M. & Rome, L. C. (1984). Jumping performance of frogs (*Rana pipiens*) as a function of muscle temperature. *J. exp. Biol.* **108**, 429–439.

Jewell, B. R. & Wilkie, D. R. (1960). The mechanical properties of relaxing muscle. *J. Physiol., Lond.* **152**, 30–47.

Johnston, I. A., Sidell, B. D. & Driedzic, W. R. (1985). Force–velocity characteristics and metabolism of carp muscle fibres following temperature acclimation. *J. exp. Biol.* **119**, 239–249.

Larimore, R. W. & Duever, M. J. (1968). Effects of temperature acclimation on the swimming ability of small mouth bass fry. *Trans. Am. Fish. Soc.* **97**, 175–184.

Marsh, R. L. & Bennett, A. F. (1985). Thermal dependence of isotonic contractile properties of skeletal muscle and sprint performance of the lizard *Dipsosaurus dorsalis*. *J. comp. Physiol.* **155**, 541–551.

Marsh, R. L. & Bennett, A. F. (1986a). Thermal dependence of contractile properties of skeletal muscle from the lizard *Sceloporus occidentalis*, with comments on methods for fitting and comparing force–velocity curves. *J. exp. Biol.* **126**, 63–77.

Marsh, R. L. & Bennett, A. F. (1986b). Thermal dependence of sprint performance of the lizard *Sceloporus occidentalis*. *J. exp. Biol.* **126**, 79–87.

Prosser, C. L. (1973). *Comparative Animal Physiology*, 3rd edn. Philadelphia: W. B. Saunders Co.

Putnam, R. W. & Bennett, A. F. (1981). Thermal dependence of behavioural performance of anuran amphibians. *Anim. Behav.* **29**, 502–509.

Putnam, R. W. & Bennett, A. F. (1982). Thermal dependence of isometric contractile properties of lizard muscle. *J. comp. Physiol.* **147**, 11–20.

Rahmann, H., Schmidt, W. & Schmidt, B. (1980). Influence of long-term thermal acclimation on the conditionability of fish. *J. therm. Biol.* **5**, 11–16.

Renaud, J. M. & Stevens, E. D. (1981a). The interactive effects of temperature and pH on the isometric contraction of toad sartorius muscle. *J. comp. Physiol.* **145**, 67–71.

Renaud, J. M. & Stevens, E. D. (1981b). Effects of acclimation temperature and pH on contraction of frog sartorius muscle. *Am. J. Physiol.* **240**, R301–R309.

Renaud, J. M. & Stevens, E. D. (1983a). The extent of long-term temperature compensation for jumping distance in the frog, *Rana pipiens* and the toad, *Bufo americanus*. *Can. J. Zool.* **61**, 1284–1287.

Renaud, J. M. & Stevens, E. D. (1983b). Effects of step changes in pH on isometric tetanic tension of toad sartorius muscle. *Can. J. Physiol. Pharmac.* **61**, 830–835.

Renaud, J. M. & Stevens, E. D. (1983c). A comparison between field habits and contractile performance of frog and toad sartorius muscle. *J. comp. Physiol.* **151**, 127–131.

RENAUD, J. M. & STEVENS, E. D. (1984). The extent of short-term and long-term compensation to temperature shown by frog and toad sartorius muscle. *J. exp. Biol.* **108**, 57–75.

ROME, L. C. (1983). The effect of long-term exposure to different temperatures on the mechanical performance of frog muscle. *Physiol. Zool.* **56**, 33–40.

ROME, L. C., LOUGHNA, P. T. & GOLDSPINK, G. (1985). Temperature acclimation: Improved sustained swimming performance in carp at low temperatures. *Science* **228**, 194–196.

STEVENSON, R. D., PETERSON, C. R. & TSUJI, J. S. (1985). The thermal dependence of locomotion, tongue flicking, digestion, and oxygen consumption in the wandering garter snake. *Physiol. Zool.* **58**, 46–57.