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RESEARCH ARTICLE

Thermal effects on motor control and *in vitro* muscle dynamics of the ballistic tongue apparatus in chameleons

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SUMMARY

Temperature strongly affects whole-organism performance through its effect on muscle contractile rate properties, but movements powered by elastic recoil are liberated from much of the performance decline experienced by muscle-powered movements at low temperature. We examined the motor control and muscle contractile physiology underlying an elastically powered movement – tongue projection in chameleons – and the associated muscle powered retraction to test the premise that the thermal dependence of muscle contractile dynamics is conserved. We further tested the associated hypothesis that motor control patterns and muscle contractile dynamics must change as body temperature varies, despite the thermal robustness of tongue-projection performance. We found that, over 14–26°C, the latency between the onset of the tongue projector muscle activity and tongue projection was significantly affected by temperature (Q_{10} of 2.56), as were dynamic contractile properties of the tongue projector and retractor muscles (Q_{10} of 1.48–5.72), supporting our hypothesis that contractile rates slow with decreasing temperature and, as a result, activity durations of the projector muscle increase at low temperatures. Over 24–36°C, thermal effects on motor control and muscle contractile properties declined, indicating that temperature effects are more extreme across lower temperature ranges. Over the entire 14–36°C range, intensity of muscle activity for the tongue muscles was not affected by temperature, indicating that recruitment of motor units in neither muscle increases with decreasing temperature to compensate for declining contractile rates. These results reveal that specializations in morphology and motor control, not muscle contractile physiology, are responsible for the thermal robustness of tongue projection in chameleons.

Key words: feeding, tongue projection, *Chamaeleo calyptratus*, electromyography, contractile properties, temperature effects, elastic.

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INTRODUCTION

The effect of temperature on diverse physiological and biochemical processes is a significant challenge to organisms living in variable environments. Ectothermic animals are particularly vulnerable because environmental conditions directly affect their body temperature, and thus physiological rate processes. The decline of these rates, including muscle contractile velocity, with body temperature can ultimately affect whole-organism performance and, in the process, limit an organism's ability to perform critical behaviors, such as predator avoidance and feeding (Huey and Stevenson, 1979; Bennett, 1985; Huey and Bennett, 1987; Rome, 1990; Lutz and Rome, 1996; Herrel et al., 2007). In contrast to muscle-powered movements, movements that rely on elastic recoil can overcome rate limits imposed by contractile rates by decoupling muscle contraction from movement. Compared with muscle-powered movements, however, the thermal dependence of elastically powered movements has not received much attention. Here, we examine the motor control and muscle contractile physiology underlying an elastically powered movement, tongue projection in chameleons, to better understand the thermal robustness of this integrated system.

The effect of temperature on muscle contractile properties, and their consequent effect on whole-organism performance, has been examined in numerous systems, thereby establishing characteristic performance responses to changes in temperature. Muscle rate properties – such as peak contractile velocity, the rate of tension development, and power output – tend to drop by at least half with each 10°C drop [i.e. temperature coefficient (Q_{10})$\geq$2]. This decline in muscle rate property performance is echoed by similar declines in the performance of dynamic behaviors, such as sprint speed, swimming velocity and jumping distance, which experience a marked performance decline of more than 33% with a 10°C drop in body temperature (i.e. $Q_{10}\geq1.5$) (Huey and Bennett, 1987; Rome, 1990). In contrast, static contractile properties – such as tetanic tension and peak isometric twitch – experience considerably lower thermal dependence, with Q_{10} values typically remaining below 1.2 (Bennett, 1984; Rome, 1990). These more thermally robust static muscle properties in turn result in the maintenance of performance for behaviors that rely on them, such as biting with maximum force, with Q_{10} values typically remaining below 1.25 (Herrel et al., 2007).

Elastic-recoil-powered movements have been shown to be less thermally dependent than associated muscle-powered movements; tongue projection in chameleons and salamanders, and ballistic mouth opening in toads and frogs exhibit Q_{10} values from 1.0 to 1.4 for dynamic variables (Anderson and Deban, 2010; Deban and Lappin, 2011; Deban and Richardson, 2011; Sandusky and Deban, 2012). Chameleon tongue projection velocity, acceleration and power decline less than 20% with a 10°C drop in body temperature compared with over 42% in tongue retraction performance (Anderson and Deban, 2010). The thermal robustness exhibited by these ballistic tongue-projection movements is thought to be the result of the relative thermal independence of the elastic-recoil mechanism that powers projection. Elastic tissues show low thermal dependence to complete independence of mechanical properties with Q_{10} values in the 1.0–1.2 range (Rigby et al., 1959; Alexander, 1966; Denny and Miller, 2006). This study will test the premise that the thermal dependence of muscle contractile dynamics is conserved and the associated hypotheses that motor control patterns and muscle contractile dynamics have to change as body temperature varies, despite the thermal robustness of tongue-projection performance.

The morphology of the chameleon hyobranchial apparatus (e.g. Houston, 1828; Gnanamuthu, 1930; Bell, 1989; Schwenk, 2000; Herrel et al., 2001b; de Groot and van Leeuwen, 2004; Anderson et al., 2012) and the hypothesized mechanisms of tongue projection (Wainwright and Bennett, 1992b; de Groot and van Leeuwen, 2004) and retraction (Wainwright and Bennett, 1992a; Herrel et al., 2009) are relevant to this study, and are described in detail elsewhere. Briefly, the entoglossal process of the hyobranchial apparatus is parallel-sided with a tapered rostral tip and acts as a rigid structure for the tongue projector muscle, the m. accelerator linguae, to act against. The m. accelerator linguae is cylindrically shaped along its posterior three-quarters with a central lumen encompassing the entoglossal process while at rest. This tubular portion of the m. accelerator linguae stretches, and thus scores elastic energy in, collagen sheaths located between the m. accelerator linguae and entoglossal process as it contracts around and lengthens along the entoglossal process. As the m. accelerator linguae extends over the tapered tip of the entoglossal process, radial forces exerted by the m. accelerator linguae and the collagen sheaths on the parallel sides of the entoglossal process are converted into longitudinal forces on the tapered tip and projection of the tongue is triggered. As the intralingual sheaths recoil to their resting length, they release stored elastic energy and power the majority of tongue projection. After projection, the paired tongue retractor muscle, the m. hyoglossus, which originates on the ceratobranchials of the hyobranchial apparatus and inserts on the m. accelerator linguae, is directly responsible for retracting the tongue onto the entoglossal process.

We examined thermal effects on the motor control and muscle dynamics of the ballistic tongue apparatus in chameleons to better understand the thermal robustness of this integrated mechanism. We hypothesized that the m. accelerator linguae and the m. hyoglossus would both exhibit increased durations between activity onset and associated kinematic events with decreasing temperature ($Q_{10}{\approx}2$), despite the differences in the thermal effects on performance of the movements they power, because of the slowing of the rate at which the muscle builds tension and shortens. Similarly, we hypothesized that dynamic contractile properties of both muscles would exhibit a strong performance loss with declining temperature ($Q_{10}{\approx}2$) whereas static contractile properties would exhibit weaker declines in performance ($Q_{10}{\approx}1.2$). We also expected that the intensity of muscle activity for both the m. accelerator linguae and m. hyoglossus would not vary with temperature under the assumption that muscle recruitment is maximized at all temperatures. Finally, we hypothesized that thermal effects on both motor control and muscle dynamic variables would be higher at lower temperature than at higher temperature. In accordance with the premise that the thermal physiology of muscle is evolutionarily conservative, we hypothesized that the muscles associated with this elastic-recoil-powered mechanism exhibit typical thermal dependence of their contractile physiology. To test these hypotheses, we performed analyses of electromyographic (EMG) recordings with corresponding high-speed image sequences from feeding events and *in vitro* muscle contractile experiments across a range of temperatures (14–36°C).

MATERIALS AND METHODS

Specimens

Chamaeleo calyptratus Duméril & Duméril 1851 was chosen because they are willing to feed in the presence of observers and they naturally experience temperatures of 6 to 34°C (Schmidt, 2001; Nečas, 2004), making them well suited to the experimental temperature range. Individuals were obtained from feral populations in Florida or from animal suppliers and were housed individually in mesh-sided enclosures with live plants. Ambient temperatures were maintained between 20 and 22°C with a basking spot of approximately 35°C. Hydration was maintained *via* bi-daily misting and specimens were fed a diet of gut-loaded crickets.

Five individuals (128–153 mm snout–vent length) that fed readily under observation were selected for EMG recordings. An additional 16 individuals (67–136 mm snout–vent length) were used for *in vitro* muscle dynamics experiments. All procedures were approved by the Institutional Animal Care and Use Committee of the University of South Florida.

EMG

Bipolar hook electrodes were constructed from 125 cm strands of Formvar-coated, 0.05 mm diameter nichrome wire (A-M Systems #762000, Carlsborg, WA, USA). Electrodes were made of two strands of wire glued together at their ends with veterinary-grade cyanoacrylate. The wires were then threaded through a 27 gauge hypodermic needle, ~1 mm of insulation from the glued tips of the wires was removed, and the strands were bent away from each other at their ends.

Prior to electrode implantation, anesthesia was induced by isoflurane (IsoThesia, Butler Animal Health Supply, Dublin, OH, USA). Isoflurane was applied to cloth gauze inside a conduction chamber of known volume, at a concentration of 0.15–0.25 $\mathrm{ml\,l^{-1}}$ to produce a 3–5% concentration of vaporized isoflurane, which was administered to the chameleons for 15–45 min. For surgery, each chameleon was positioned on its left side on a stage next to a dissecting microscope (Wild Heerbrugg M5 or Leica MZ6 Stereomicroscope, Leica Microsystems, Wetzlar, Germany). The chameleon's mouth was then held open with a fitted silicone bit, and its tongue was extended from the mouth onto moist paper towels on the stage of the dissecting microscope. Electrodes were implanted into the right side of the m. accelerator linguae, ~1 cm from its posterior end, and into the right side of the m. hyoglossus, ~2 cm posterior to the m. accelerator linguae. Electrode placement was verified visually prior to feeding experiments.

Following electrode implantation, hypodermic needles were withdrawn, leaving the electrodes held in place by the hooks of their tips. A small dab of veterinary-grade cyanoacrylate was applied to the implantation site to aid in securing the electrodes in place. The electrode wires from both recording sites were then glued together with modeling cement ~5.0 cm from their implantation site along their remaining length. The ends of the wires were stripped and soldered to a plug that mated with a socket on the amplifier probe.

EMG signals were amplified 1000 or 5000 times using a differential amplifier (A-M Systems 3500) and filtered to remove 60 Hz line noise. Amplification level was maintained at a constant level within an individual's set of feedings with any particular electrode pair to enable within-individual comparisons of signal amplitude. Conditioned signals were sampled at 4 kHz with a PowerLab 16/30 analog-to-digital converter coupled with LabChart software version 7 (ADInstruments, Bella Vista, New South Wales, Australia) running on an Apple MacBook Pro (Apple, Cupertino, CA, USA). EMG recordings were synchronized with digital images *via* a trigger shared with the camera.

Feeding experiments

After recovery from surgery (2–6 h), chameleons were imaged at a 3 kHz frame rate and 1/12,000 s shutter speed with a Fastcam 1024 PCI camera (Photron USA, San Diego, CA, USA) as they fed on crickets. All feeding trials and recordings were conducted within 16 h of surgery. Chameleons were placed on a wooden dowel of known diameter oriented parallel to the image plane of the camera. Crickets were placed on a square of fiberglass insect screen suspended by thread in front of the dowel; this arrangement allowed the chameleon's tongue to complete its trajectory unimpeded (Anderson and Deban, 2010).

Feeding trials were conducted across a range of ambient temperatures (15–35°C) at 10°C increments within an environmental chamber (Environmental Growth Chambers, Chagrin Falls, OH, USA). Feeding trials for each individual were conducted in the sequence 15–25–35°C because feeding was harder to elicit at lower temperatures and because electrodes were more likely to be dislodged at higher temperatures. The order of experimental temperatures experienced by an individual has been shown to have no significant effect on projection or retraction performance (Anderson and Deban, 2010). Chameleons were allowed to acclimate to the experimental temperature for a period of at least 1 h prior to feeding trials. To prevent elevation of body temperatures through light-source radiation, supplemental lighting was switched on immediately before tongue projection and turned off immediately after tongue retraction. Body temperature was verified orally using a calibrated infrared thermometer (Sixth Sense LT300, Williston, VT, USA; ±1°C accuracy) following every feeding event. Only feeding sequences with a post-feeding body temperature of the target experimental temperature ±1°C were included in the analysis. One to three feedings were collected from each animal at each temperature. Feeding events were gathered until an equal number of feedings per experimental temperature were gathered or until either implanted electrode was pulled out.

Muscle contraction experiments

For all muscle contractile experiments, muscles were attached to a dual servo-motor force lever (Model 305C-LR, Aurora Scientific, Aurora, ON, Canada) by Spiderwire microfilament (Pure Fishing, Spirit Lake, IA, USA), for which previous viscoelastic property examination found no observable oscillations during rapid force reduction (Lappin et al., 2006). The muscle was located between the platinum-coated electrodes of a bi-polar pulse stimulator (Model 701B, Aurora Scientific) in the inner chamber of a tissue-organ bath (Model 805A, Aurora Scientific) filled with oxygenated reptilian Ringers solution. The tissue–organ bath was maintained at a set temperature with a temperature-controlled water circulator (IsoTemp 1013S, Fisher Scientific, Waltham, MA, USA). Force and position from the lever and stimulation pulses from the stimulator were recorded with an analog-to-digital interface (Model 604A, Aurora Scientific) connected to an Apple Power Mac G4 running a custom LabVIEW 8.2 instrument with a PCI-6221 data acquisition card (National Instruments, Austin, TX, USA) sampling at 1000 Hz.

Prior to muscle excision for contractile experiments, chameleons were killed by pithing. The chameleon's tongue was extended out of the mouth to approximate maximum tongue projection. A 1.5–2.5 cm length of the extended paired m. hyoglossus was tied off with Spiderwire, and its extended length was measured using digital calipers (Mitutoyo 700-126, Kawasaki-shi, Kanagawa, Japan; ±0.1mm accuracy) prior to being cut away from the remainder of the m. hyoglossus. The excised portion of m. hyoglossus was wrapped in paper towel moistened with reptilian Ringers solution and allowed to rest at 5°C for use immediately following contractile data collection from the m. accelerator linguae of the same chameleon. The remainder of the m. hyoglossus proximal to the m. accelerator linguae was removed and the dorsal and ventral anterior projections of the m. accelerator linguae (Gnanamuthu, 1930; Bell, 1989; Herrel et al., 2001b), along with the tongue pad, were cut away from the tubular portion of the m. accelerator linguae.

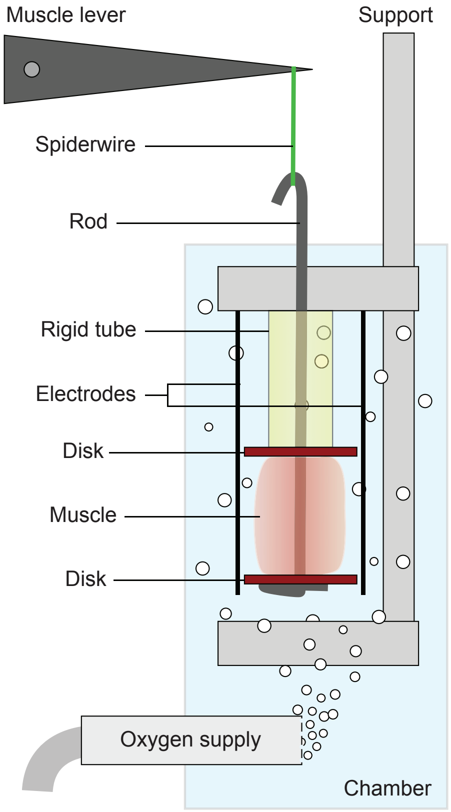

Fig. 1. Force-lever setup for measurement of properties of m. accelerator linguae elongation during contraction around a surrogate entoglossal process (rod). Note that the secure, fixed connection between the rigid tube and support causes elongation of the m. accelerator linguae as it contracts around the surrogate entoglossal process (rod) to push the lower disk downward, exerting a downward force on the rod and subsequently the muscle lever *via* the Spiderwire connection.

The tubular portion of the m. accelerator linguae was placed on a surrogate entoglossal process constructed from a 0.8 mm diameter, parallel-sided aluminum rod (Fig. 1). Flat plastic disks were placed on the surrogate entoglossal process on both ends of the m. accelerator linguae and one end of the aluminum rod was wound into a spiral to hold the plastic disk and m. accelerator linguae at one end. The other end of the surrogate entoglossal process was bent into a hook; this end was fed through the center of a hard plastic tube that was anchored in the chamber and attached with Spiderwire to the force lever. The distance between the force lever and stimulator was then adjusted until the m. accelerator linguae and the plastic disks filled the space between the spiraled end of the rod and the plastic tube. Because the tube was secured in place, elongation of the m. accelerator linguae during stimulation pushed the surrogate entoglossal process away from the force lever, placing tension in the Spiderwire and pulling on the lever. This arrangement thus directly measured the force of m. accelerator linguae elongation along the entoglossal process – the force responsible for stretching the intralingual sheaths of the tongue to store elastic energy that powers tongue projection.

Isometric contractions from the m. accelerator linguae were elicited with 80 V supramaximal stimulations at a frequency of 80 pulses s^{-1} and a current of 500 mA to achieve fused tetanus. The m. accelerator linguae was stimulated twice at each experimental temperature (15, 25 and 35°C) with a 10 min rest period between stimulations at the same temperature and a 20 min acclimation period to each experimental temperature. Because of rapid fatigue at 35°C, this temperature was the last experimental temperature for all individuals. Half of the muscles were subjected to 15°C first and the other half to 25°C first.

The excised m. hyoglossus sample was removed from the refrigerator for trials no longer than 2.5 h following excision. The Spiderwire on one end of sample was anchored to the bottom of the stimulation chamber and the Spiderwire on the other end was attached to the end of the force lever. The position of the stimulator was then adjusted until the sample was extended to the length measured prior to its excision.

The m. hyoglossus was stimulated at 80 V at 100 pulses s^{-1} and 500 mA. Rest periods between stimulations, temperature acclimation periods and temperature order were performed as in m. accelerator linguae experiments. At each temperature, an initial isometric contraction was performed. Following the initial isometric contraction, a series of afterloaded contractions was recorded by dictating a force at which the force lever will allow the muscle to shorten. These subsequent contractions were performed at forces below the recorded isometric force and collected at ~0.1 V (0.094 N) increments until the force was below 0.1 V.

Kinematic analysis

The timing and amplitude of movements of the tongue during prey capture, with respect to the dentary as a fixed reference, were quantified from the digital image sequences. Tongue projection distance was computed using ImageJ software (National Institutes of Health, Bethesda, MD, USA) running on an Apple iMac computer, using the diameter of the wooden dowel to calibrate distances in each feeding, as the distance from the tongue tip to the dentary tip. The time of the start of ballistic tongue projection, time of maximal tongue projection and time of completion of tongue retraction were measured relative to the start of ballistic tongue projection at time zero. To determine the timing of the start of ballistic tongue projection, ImageJ was used to record the *x*,*y* coordinates of the tip of the tongue on each frame throughout the tongue projection sequence and a quintic spline was fitted to the resultant position trace of the tongue using a custom script for the P-spline package of R statistical software (www.r-project.org) to yield instantaneous velocity ($m\,s^{-1}$) and acceleration ($m\,s^{-2}$) (i.e. first and second derivatives of the position). This spline was smoothed to remove secondary oscillation artifacts from the acceleration trace.

The start of the ballistic phase of tongue projection, in which the tongue is moving only under its own momentum, was recorded as the time of peak velocity and zero acceleration. The time of maximal tongue projection and the time of completion of tongue retraction were measured in ImageJ as the time of maximum dentary tip to tongue tip distance and the time at which the tongue tip reaches the gape plane, or the line between the tips of the maxilla and dentary, respectively. Durations of movements were calculated from these timing variables.

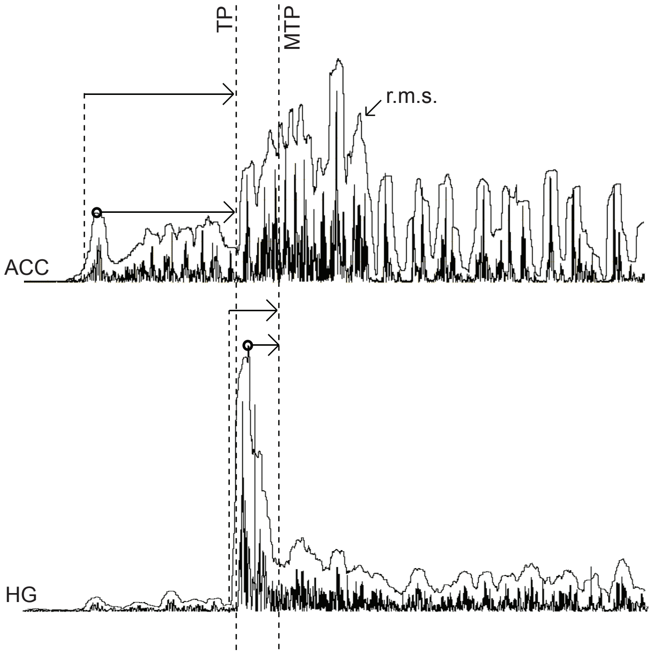

Fig. 2. Motor control timing variables examined as shown in a representative feeding event. The onset of tongue projection (TP) and the time of maximal tongue projection distance (MTP) are indicated by vertical dotted lines extending through both traces. The end of the feeding event, when the tongue was fully retracted, is not depicted in these traces. Traces illustrate rectified electromyographic (EMG) signals and the r.m.s. of the signals for the m. accelerator linguae (ACC) and m. hyoglossus (HG). Open circles indicate peak r.m.s. amplitudes. Horizontal arrows indicate latencies between the onset of ACC activity and the onset of tongue projection, peak ACC amplitude and the onset of tongue projection, the onset of HG activity and maximum tongue projection, and peak HG amplitude and maximum tongue projection. Additional variables are described in the Materials and methods.

Analysis of electromyograms

The amplitudes of activity of the m. accelerator linguae and m. hyoglossus and their timing of activity relative to kinematic events were quantified from the rectified EMG signals using LabChart software. Distinct primary (pre-projection) and secondary (post-projection) activity bursts of the m. accelerator linguae (Wainwright and Bennett, 1992a) were not discernible in most feedings. Further, activity of the m. accelerator linguae and m. hyoglossus extended beyond tongue retraction and mouth closure. Activity durations of the m. accelerator linguae and m. hyoglossus were not measured, because activity of the m. accelerator linguae following tongue projection and activity of the m. hyoglossus following mouth closure are not involved in powering the movements of interest (tongue projection and retraction), and because distinct, independent activity bursts following these movements were difficult to discern. Instead, latencies from the onset of activity and peak activity [peak of root mean square (r.m.s.)] to associated kinematic events were measured (Fig. 2). Onset of activity was defined as the time after which the EMG amplitude reached twice the background noise level for at least 10 ms. The latencies from the onset of m. accelerator linguae activity and peak of m. accelerator linguae activity to the start of ballistic tongue projection and the latencies of the onset of m. hyoglossus activity and peak of m. hyoglossus activity to maximal tongue projection were quantified.

Amplitude and intensity variables were measured between the onset of muscle activity and the time of associated kinematic events to quantify the intensity of muscle activity during loading (m. accelerator linguae) and tongue slowing (m. hyoglossus). Integrated area was measured as the sum of the values of the rectified signal between the onset of activity and the time of kinematic events. Intensity of the EMG bursts was measured as (1) the r.m.s. within these time periods and (2) the integrated area divided by the duration of these time periods. The peak amplitude of muscle activity was measured as the maximum r.m.s. value using a 20 ms time constant (i.e. the moving 20 ms time window over which the r.m.s. was calculated). Peak amplitude and intensity were measured for the m. accelerator linguae from the onset of m. accelerator linguae activity to the onset of tongue projection. Peak amplitude and intensity were measured for the m. hyoglossus from the onset of m. hyoglossus activity to the maximum tongue projection.

Ratios between EMG variables were calculated to examine the potential differential effect of temperature on the m. accelerator linguae and m. hyoglossus muscles. The r.m.s. of m. accelerator linguae activity from m. accelerator linguae onset to projection onset was divided by the r.m.s. of m. hyoglossus activity from m. hyoglossus onset to maximum projection to yield a ratio expressing the differential effects on intensity. Similarly, the latency of the onset of m. accelerator linguae activity to the onset of tongue projection was divided by the latency of the onset of m. hyoglossus activity to maximal tongue projection to examine the differential effects on latencies.

Analysis of muscle contractile data

Electromechanical delay and static and dynamic contractile characteristics of isometric contractions of the m. accelerator linguae were quantified from raw stimulation, force and length outputs using Microsoft Excel 2004 for Mac OS X running on an Apple MacBook Pro. Peak isometric force (P_0) was quantified as the maximum force recorded from each trace and 90% P_0 was calculated based on that value. The time of the start of force production from the m. accelerator linguae was quantified as the first time following the onset of stimulation where force over the following 6 ms increased consecutively. Subsequent timing events were measured relative to the start of force production at time zero. The time of the start of stimulation was quantified as the first spike in voltage from the recorded stimulation trace. The time of 90% P_0 was quantified as the time when the force trace first equaled or surpassed the calculated 90% P_0 value. Based on these timing variables, the electromechanical delay (or latency between the onset of stimulation and the start of force production) and the time to 90% P_0 (or the latency between the start of force production and the time of 90% P_0) were calculated for each contraction. The rate of force development was then calculated as the 90% P_0 value divided by the time to 90% P_0.

As with the m. accelerator linguae, the electromechanical delay, P_0, the time to 90% P_0 and the rate of force development were quantified for isometric contractions of the m. hyoglossus. For isotonic contractions of the m. hyoglossus at constant forces, contraction velocity as a function of muscle length was calculated from length change over a 50 ms period of relatively constant velocity. Hill's equation [the 'characteristic equation' (Hill, 1938)] was then fitted to these force–velocity data for each muscle at each temperature using the Curve Fit function of Microsoft Excel 2004 for Mac OS X running on an Apple MacBook Pro. The resultant equations were then used to calculate peak contraction velocity ($V_{\max}$, i.e. contraction velocity with a zero force) and instantaneous power output of the muscle, as the product of force and contractile velocity. Peak power ($W_{\max}$) was recorded from these power traces and the power ratio (Marsh and Bennett, 1986) was then calculated for each muscle at each temperature as:

$$\text{Power ratio} = W_{\max} / (V_{\max} \times P_0)\,. \qquad (1)$$

Statistical analyses

All EMG and contractile data were $\log_{10}$-transformed prior to statistical analysis because EMG and contractile variables were expected to have an exponential relationship with temperature. The EMG and contractile data sets were divided into two overlapping subsets based on the temperature at which the data were gathered, 14–26°C and 24–36°C, to examine whether the thermal relationship varied across the temperature range. Based on the published results of chameleons (Anderson and Deban, 2010) and other ectotherms (van Berkum, 1986; Huey and Kingsolver, 1989; Huey and Kingsolver, 1993; Bauwens et al., 1995; Deban and Lappin, 2011), the lower temperature range was expected to exhibit stronger thermal effects than the upper range. An analysis of covariance (ANCOVA) was conducted separately on each subset of the data on an Apple iMac computer using JMP 5.1 software (SAS Institute, Cary, NC, USA). To control for false discovery rate in multiple comparisons, the Benjamini–Hochberg method (Benjamini and Hochberg, 1995) was used to adjust significance levels.

Prior to statistical analysis, EMG amplitude variables were examined for an effect of electrode, because signal strength is known to vary between electrodes. To appropriately account for a potential effect of different electrodes within an individual, amplitude data from all individuals were restricted to data from the same electrode with feedings at multiple temperatures. Data from one individual with feedings from more than one electrode at multiple temperatures were tested for an effect of electrode in a model including temperature. When an effect of electrode was not significant for given amplitude variables, data from this individual were included in subsequent tests, whereas data from this individual were excluded when an effect of electrode was significant. Because timing data should not vary between electrodes for a single individual, timing data from all feedings were used to calculate EMG timing variables.

Electromyographic data were then tested for three effects: (1) temperature, (2) individual and (3) projection distance. Temperature effects were included as a continuous variable to examine how the motor control of elastically powered and non-elastic movements responded to changes in body temperature. To account for body size and other random individual differences, a random individual effect was included. Because projection distance has been found to influence some prey-capture kinematics (Anderson and Deban, 2010), projection distance was included to account for potential effects on motor control patterns; it was dropped from the model when non-significant for a given variable to increase sample size and statistical power.

Muscle contraction data were tested for two effects on the variables: (1) temperature and (2) individual. As with the EMG data, temperature effects were included as a continuous variable to examine how contractile properties are affected by changes in body temperature. Similarly, an individual effect was included to account for muscle size and other random individual differences.

Temperature coefficients (Q_{10}) were computed across each temperature range (14–26°C and 24–36°C) for each muscle variable from the partial regression coefficients (PRCs) of the temperature effect in the ANCOVAs. The ANCOVA models include effects of individual (and projection distance for EMG data) that influence

Table 1. Minimum and maximum values of kinematic, electromyographic and contractile variables in *Chamaeleo calyptratus*

	15±1°C		25±1°C		35±1°C	
Variable	Min./Max.	Sample size	Min./Max.	Sample size	Min./Max.	Sample size
Kinematic characteristics						
Projection distance (cm)	5.04/15.62	7 (5; 1–2)	5.19/18.56	15 (5; 1–4)	6.25/13.00	5 (4; 1–2)
Kinematic timing (s)						
Max. projection time relative to projection onset	0.013/0.048	7 (5; 1–2)	0.010/0.051	15 (5; 1–4)	0.011/0.034	5 (4; 1–2)
Tongue retracted time relative to projection onset	0.676/1.013	7 (5; 1–2)	0.255/1.090	15 (5; 1–4)	0.214/0.283	5 (4; 1–2)
Muscle activity (s)						
ACC onset to tongue projection onset duration	0.162/0.372	7 (5; 1–2)	0.011/0.267	14 (5; 1–4)	0.015/0.101	5 (4; 1–2)
ACC max. amplitude to tongue projection duration	0.000/0.086	7 (5; 1–2)	0.000/0.103	14 (5; 1–4)	0.000/0.051	5 (4; 1–2)
HG onset to tongue projection onset duration	0.010/0.046	5 (4; 1–2)	–0.006/0.225	7 (5; 1–2)	0.019/0.060	4 (4; 1–1)
HG onset to max. tongue projection duration	0.032/0.093	5 (4; 1–2)	0.036/0.261	7 (5; 1–2)	0.049/0.091	4 (4; 1–1)
HG max. amplitude to max. projection duration	0.015/0.048	5 (4; 1–2)	0.001/0.076	7 (5; 1–2)	0.012/0.041	4 (4; 1–1)
ACC contractile variables						
Peak isometric force, P_0 (N)	0.66/4.84	33 (16; 2–3)	1.36/6.36	33 (16; 2–3)	0.37/6.01	32 (16; 1–3)
Time to 90% P_0 (s)	0.115/0.211	33 (16; 2–3)	0.056/0.156	33 (16; 2–3)	0.031/0.256	32 (16; 1–3)
Rate of force development (N s^{-1})	5.16/31.79	33 (16; 2–3)	10.83/80.56	33 (16; 2–3)	2.87/108.17	32 (16; 1–3)
Electromechanical delay (s)	0.007/0.018	33 (16; 2–3)	0.003/0.008	33 (16; 2–3)	0.002/0.012	32 (16; 1–3)
HG contractile variables						
Peak isometric force, P_0 (N)	0.03/0.31	12	0.03/0.53	12	0.02/0.50	11
Time to 90% P_0 (s)	0.132/0.426	11	0.099/0.282	11	0.081/0.332	10
Rate of force development (N s^{-1})	0.17/1.45	11	0.33/2.36	11	0.23/4.19	10
Electromechanical delay (s)	0.016/0.047	11	0.010/0.024	11	0.005/0.019	10
Specific tension (N cm^{-2})	1.76/7.56	12	1.74/10.67	12	1.08/10.09	11
Peak contractile velocity (L_0 s^{-1})	0.21/3.25	12	1.42/4.74	12	0.44/3.72	10
Peak contractile velocity (m s^{-1})	0.005/0.067	12	0.036/0.128	12	0.011/0.101	10
Peak power (W)	4.93×10^{-6}/ 1.36×10^{-3}	12	1.30×10^{-4}/ 5.04×10^{-3}	12	2.95×10^{-5}/ 2.67×10^{-3}	10
Peak power (W kg^{-1} HG segment mass)	0.11/18.08	12	2.90/55.69	12	0.66/35.55	10
Power ratio	0.032/0.233	12	0.082/0.245	12	0.122/0.250	10

ACC, m. accelerator linguae; HG, m. hyoglossus; L_0, muscle length.

The total number of feedings (for kinematic and electromyographic variables) or contractions (for ACC contractile variables) is presented for each variable as well as the number of individuals data was gathered from and the range of feedings for each individual (in parentheses separated by a semicolon) in the sample size columns. For HG contractile variables, the number of individuals for each variable is presented because only a single contraction from each individual was collected for each variable.

the estimate of the relationship between the variable and temperature, so calculation of Q_{10} values from the PRC accounts for these effects as well. The Q_{10} values were calculated as the base 10 antilogarithm of the PRC multiplied by 10:

$$Q_{10} = 10^{(\mathrm{PRC} \times 10)}. \quad (2)$$

To express them as rates, the temperature coefficients for duration variables are reported as inverse Q_{10} values (i.e. $1/Q_{10}$).

RESULTS

Motor control of prey capture

A total of 27 feedings with associated EMG recordings were collected from five individuals across a 15.5–35.2°C temperature range (Table 1). EMG recordings from the m. accelerator linguae were gathered in all feedings with the exception of one at 25±1°C. A total of 16 feedings provided EMG recordings from the m. hyoglossus.

For amplitude variables, only feedings with electrodes that were used at multiple temperatures could be used so that an effect of electrode could be ruled out across the temperature ranges. As a result, the number of EMG recordings used for amplitude variables was fewer than those gathered and used for timing variables. Six feedings (one to two feedings, five individuals) at 15±1°C, 10 feedings (one to four feedings, five individuals) at 25±1°C, and five feedings (one to two feedings, four individuals) at 35±1°C were collected for the m. accelerator linguae. For the m. hyoglossus, five feedings (one to two feedings, four individuals) at 15±1°C, five feedings (one to two feedings, four individuals) at 25±1°C, and four feedings (one feeding, four individuals) at 35±1°C were collected. Only a single individual for which feedings from multiple electrodes were collected was included. No amplitude variables for this individual indicated a significant effect of temperature; the feedings from this individual were thus included in the statistical analysis for all amplitude variables.

In feedings across the entire 15–35°C temperature range (Table 1), the m. accelerator linguae became active 11–372 ms prior to the onset of tongue projection. The peak of activity of the m. accelerator linguae occurred from 0 to 103 ms before the onset of tongue projection. The m. hyoglossus became active 32–261 ms before the tongue reached its maximum projection length and its pre-maximum tongue projection peak activity occurred from 1 to 76 ms before maximum projection. The m. hyoglossus was then active in pulses between the time of maximum tongue projection and when the tongue was completely retracted, which ranged from 18 to 1049 ms.

Temperature significantly affected a single motor control variable across the 14–26°C range (Table 2, Figs 3, 4): latency between the onset of activity for the m. accelerator linguae and the onset of tongue projection ($1/Q_{10}$=2.69, P=0.0005). The remaining three timing variables and all six amplitude variables showed no significant effect of temperature across the 14–26°C range. No significant effect of tongue projection distance was found for any motor control variable across the 14–26°C range. Across the 24–36°C range, temperature did not significantly affect any motor control variables (Table 3, Fig. 4). Further, across the

Table 2. Results of ANCOVA examining effects on electromyographic amplitude and duration variables over the 14–26ºC range in *Chamaeleo calyptratus*

Variable	Individual *P*-value	Temperature *P*-value	Projection distance *P*-value	Temperature Slope	Temperature Q_{10}	Temperature $1/Q_{10}$
ACC (onset to projection) r.m.s.	**0.0162**	0.0337	0.5126	0.0741	5.51	0.18
ACC (onset to projection) integrated area/duration	**0.0100**	0.0542	0.3714	0.0643	4.40	0.23
ACC (onset to projection) r.m.s. max. amplitude	**0.0104**	0.1248	0.4986	0.0530	3.39	0.30
ACC onset to tongue projection onset duration	**0.0016**	**0.0005**	0.2391	–0.0429	0.37	**2.69**
ACC max. amplitude to tongue projection onset duration	0.8487	0.2181	0.6862	0.0763	5.79	0.17
HG (onset to max. projection) r.m.s.	0.1277	0.1398	0.1023	0.0709	5.12	0.20
HG (onset to max. projection) integrated area/duration	0.0922	0.1233	0.1115	0.0721	5.26	0.19
HG (onset to max. projection) r.m.s. max. amplitude	0.1449	0.1212	0.0904	0.0745	5.56	0.18
HG onset to max. tongue projection duration	0.3362	0.7306	0.8221	0.0062	1.15	0.87
HG max. amplitude to max. tongue projection duration	0.4330	0.3291	0.7438	–0.0402	0.40	2.52
ACC (onset to projection) r.m.s./HG (onset to max. projection) r.m.s.	0.0923	0.3788	0.0766	–0.0409	2.56	0.39
ACC onset to tongue projection onset/HG onset to max. tongue projection duration	0.3741	0.0325	0.4403	–0.0611	0.24	4.08

ACC, m. accelerator linguae; HG, m. hyoglossus.

P-values are shown for individual and temperature, as is the partial regression coefficient for the temperature effect (i.e. slope) from the model from which Q_{10} values were calculated. Projection distance was included as a covariate only when it showed a significant effect for that variable.

Bold *P*-values indicate significance levels adjusted to correct for false discovery rate (Benjamini and Hockberg, 1995). Bold Q_{10} values indicate significant temperature effects.

24–36°C range, tongue projection distance showed no significant effect on any motor control variable.

Two ratios relating EMG variables of the m. accelerator linguae and m. hyoglossus exhibited a significant effect of temperature (Tables 2, 3): the EMG intensity ratio (i.e. m. accelerator linguae r.m.s./m. hyoglossus r.m.s., as defined above) across the 24–36°C range (Q_{10}=2.36, P=0.0031), indicating a relatively greater reduction in m. hyoglossus recruitment at the highest temperatures (Fig. 4), and the EMG timing ratio (i.e. m. accelerator linguae onset to projection/m. hyoglossus onset to maximum projection) across the 24–36°C range ($1/Q_{10}$=2.33, P=0.0039), indicating a relatively larger increase in m. hyoglossus activity duration prior to maximum tongue projection at higher temperatures (Fig. 4). Neither EMG ratio in the lower range exhibited a significant effect of temperature.

Muscle contractile dynamics

Over the entire 15–35°C range, the m. accelerator linguae produced a P_0 of elongation of 0.37–6.36 N (Table 1) from tubular muscle segments of 0.09–0.70 g. These contractions reached 90% P_0 in 31–256 ms at a rate of 2.87–108 N s^{-1}. Force production across this temperature range occurred after a 2–18 ms electromechanical delay.

The m. hyoglossus reached a P_0 of 0.02–0.53 N over the entire 15–35°C range (Table 1) from paired linear muscle segments of 0.05–0.25 g, with a specific tension of 1.08–10.67 N cm^{-2}. Following an electromechanical delay of 5–47 ms, these contractions reached 90% P_0 in 81–426 ms at a rate of 0.17-4.19 N s^{-1}. V_{max} values were estimated at 0.21–4.74 L_0 s^{-1} (where L_0 is muscle length), or 0.005–0.128 m s^{-1}. W_{max} was calculated to range from 4.93×10^{-6}–5.04×10^{-3} W, with a mass-specific peak power range of 0.11–55.69 W kg^{-1}. These values produce power ratios ranging from 0.032 to 0.250.

In the 15–25°C range, temperature significantly affected all 11 contractile property variables with Q_{10} values of 1.28–5.72 (Table 4, Figs 5–8): P_0 of the m. accelerator linguae, time to 90% P_0 of the m. accelerator linguae, rate of force development of the m. accelerator lingaue, electromechanical delay of the m. accelerator linguae, P_0 of the m. hyoglossus, time to 90% P_0 of the m.

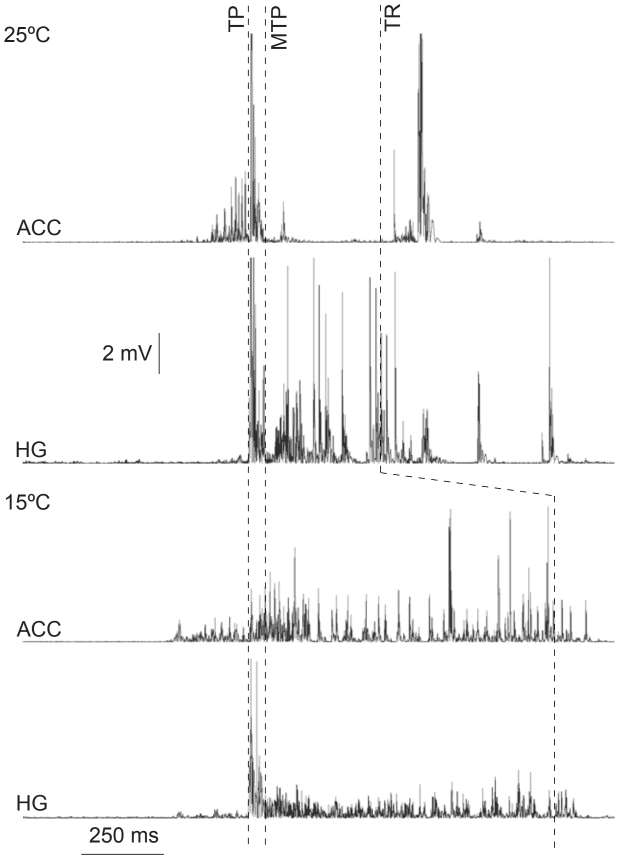

Fig. 3. Rectified EMG signals from the m. accelerator linguae (ACC) and m. hyoglossus (HG) in the same individual of *Chamaeleo calyptratus* feeding at 25°C (top) and 15°C (bottom). Traces are aligned at the onset of tongue projection (TP; first dashed line) and major kinematic events are shown: TP, maximum tongue projection (MTP) and tongue fully retracted (TR). Note the activation of the m. accelerator linguae prior to tongue projection and the extended activation of the m. accelerator linguae prior to tongue projection at 15°C compared with 25°C. All signals are shown on the same scale.

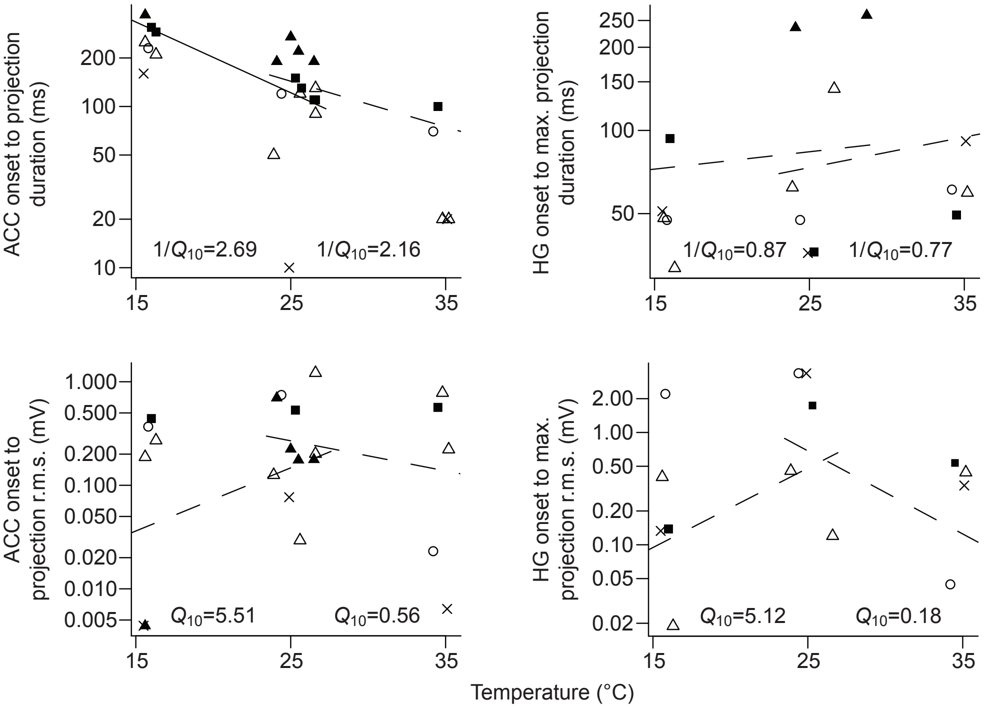

Fig. 4. Scatterplots of EMG variables from all feedings analyzed for each variable *versus* temperature. Left panels depict variables for the m. accelerator linguae (ACC) and right panels depict variables for the m. hyoglossus (HG). Regressions representing Q_{10} values are derived from the partial regression coefficients of the temperature effect in the ANCOVA (see Materials and methods for details), which are shown as lines overlaid on the data points across the 14–26°C and the 24–36°C ranges. Only m. accelerator linguae onset to projection duration across the 14–26°C range depicts a significant effect of temperature (see Tables 2, 3 for details). A significant temperature effect is depicted as a solid regression line, whereas non-significant temperature effects are depicted as dashed regression lines. Individual chameleons are shown as different symbols.

hyoglossus, rate of force development of the m. hyoglossus, electromechanical delay of the m. hyoglossus, V_{max} of the m. hyoglossus, W_{max} of the m. hyoglossus and power ratio of the m. hyoglossus. Temperature significantly affected six contractile variables in the 25–35°C range with Q_{10} values of 0.71–1.48 (Table 4, Fig. 5): P_0 of the m. accelerator linguae, time to 90% P_0 of the m. accelerator linguae, electromechanical delay of the m. accelerator linguae, P_0 of the m. hyoglossus, time to 90% P_0 of the m. hyoglossus and electromechanical delay of the m. hyoglossus. No significant effect of temperature was detected for the remaining five contractile variables across the 25–35°C temperature range (Table 4, Figs 5, 6, 8).

DISCUSSION

Motor control of prey capture

The chameleons in this study captured prey by ballistic tongue projection, in which the tongue is projected out of the mouth as it is pushed off the entoglossal process, and then travels to the prey under its own momentum (Bell, 1989; Wainwright and Bennett, 1992b; Herrel et al., 2001b; de Groot and van Leeuwen, 2004). The activation patterns of the m. accelerator linguae are consistent with a pattern of activation prior to tongue projection found in previous studies (Fig. 3) (Wainwright and Bennett, 1992a; Herrel et al., 2000), during which time the m. accelerator linguae loads elastic structures with strain energy (de Groot and

Table 3. Results of ANCOVA examining effects on electromyographic amplitude and duration variables over the 24–36ºC range in *Chamaeleo calyptratus*

Variable	Individual *P*-value	Temperature *P*-value	Projection distance *P*-value	Temperature		
				Slope	Q_{10}	1/Q_{10}
ACC (onset to projection) r.m.s.	0.1067	0.5776	0.2111	–0.0250	0.56	1.78
ACC (onset to projection) integrated area/duration	0.0636	0.4767	0.0972	–0.0325	0.47	2.11
ACC (onset to projection) r.m.s. max. amplitude	0.0708	0.4991	0.2384	–0.0293	0.51	1.96
ACC onset to tongue projection onset duration	**0.0007**	0.0159	0.2852	–0.0334	0.46	2.16
ACC max. amplitude to tongue projection onset duration	0.2972	0.4837	0.1741	–0.0474	0.34	2.98
HG (onset to max. projection) r.m.s.	0.2402	0.1514	0.2794	–0.0736	0.18	5.45
HG (onset to max. projection) integrated area/duration	0.1878	0.1019	0.2594	–0.0821	0.15	6.62
HG (onset to max. projection) r.m.s. max. amplitude	0.2751	0.1751	0.3182	–0.0682	0.21	4.81
HG onset to max. tongue projection duration	0.0389	0.3928	0.1973	0.0112	1.29	0.77
HG max. amplitude to max. tongue projection duration	0.1606	0.1991	0.7331	0.0456	2.86	0.35
ACC (onset to projection) r.m.s./HG (onset to max. projection) r.m.s.	**0.0002**	**0.0031**	**0.0014**	0.0373	**2.36**	0.42
ACC onset to tongue projection onset/HG onset to max. tongue projection duration	**0.0012**	**0.0039**	0.2265	–0.0367	0.43	**2.33**

P-values are shown for individual and temperature, as is the partial regression coefficient for the temperature effect (i.e. slope) from the model from which Q_{10} values were calculated. Projection distance was included as a covariate only when it showed a significant effect for that variable.

Bold *P*-values indicate significance levels adjusted to correct for false discovery rate (Benjamini and Hockberg, 1995). Bold Q_{10} values indicate significant temperature effects.

Table 4. Results of ANCOVA examining effects on muscle contractile variables in *Chamaeleo calyptratus*

	Individual *P*-value	Temperature *P*-value	Temperature		
			Slope	Q_{10}	$1/Q_{10}$
14–26ºC					
ACC					
Peak isometric force (P_0)	**<0.0001**	**<0.0001**	0.0128	**1.34**	0.75
Time to 90% P_0	**0.0011**	**<0.0001**	–0.0264	0.55	**1.83**
Rate of force development	**<0.0001**	**<0.0001**	0.0391	**2.46**	0.41
Electromechanical delay	**<0.0001**	**<0.0001**	–0.0265	0.54	**1.84**
HG					
Peak isometric force (P_0)	**<0.0001**	**0.0007**	0.0107	**1.28**	0.78
Time to 90% P_0	0.0753	**0.0051**	–0.0169	0.68	**1.48**
Rate of force development	**<0.0001**	**<0.0001**	0.0287	**1.94**	0.52
Electromechanical delay	**0.0006**	**<0.0001**	–0.0253	0.56	**1.79**
Peak contractile velocity (V_{max})	0.0561	**0.0052**	0.0310	**2.04**	0.49
Peak power (W_{max})	**<0.0001**	**<0.0001**	0.0758	**5.72**	0.17
Power ratio [$W_{max}/(V_{max} \times P_0)$]	0.0572	**0.0004**	0.0341	**2.19**	0.46
24–36ºC					
ACC					
Peak isometric force (P_0)	**<0.0001**	**<0.0001**	–0.0150	**0.71**	1.41
Time to 90% P_0	**<0.0001**	**0.0091**	–0.0057	0.88	**1.14**
Rate of force development	**<0.0001**	0.0442	–0.0093	0.81	1.24
Electromechanical delay	**<0.0001**	**<0.0001**	–0.0146	0.71	**1.40**
HG					
Peak isometric force (P_0)	**<0.0001**	**0.0008**	–0.0123	**0.75**	1.33
Time to 90% P_0	0.0786	**0.0139**	–0.0172	0.67	**1.48**
Rate of force development	**0.0072**	0.4362	0.0057	1.14	0.88
Electromechanical delay	**0.0094**	**0.0147**	–0.0119	0.76	**1.32**
Peak contractile velocity (V_{max})	0.0382	0.0711	–0.0138	0.73	1.37
Peak power (W_{max})	**0.0002**	0.0663	–0.0156	0.70	1.43
Power ratio [$W_{max}/(V_{max} \times P_0)$]	**0.0285**	0.0251	0.0115	1.30	0.77

P-values are shown for individual and temperature, as is the partial regression coefficient for the temperature effect (i.e. slope) from the model from which Q_{10} values were calculated.

Bold *P*-values indicate significance levels adjusted to correct for false discovery rate (Benjamini and Hockberg, 1995). Bold Q_{10} values indicate significant temperature effects.

van Leeuwen, 2004). The onset of activity of the m. accelerator linguae occurred on average 146 ms prior to the onset of tongue projection, which was 4.5 times the average time for the tongue to reach maximum projection and sufficient time for the m. accelerator linguae to load elastic structures with strain energy. Although feeding events in this study did not always show a clear break in EMG activity prior to the onset of tongue projection [i.e. a distinct second burst of activity following the onset of tongue projection, as has been found in previous studies (e.g. Wainwright and Bennett, 1992a)], the activity of the m. accelerator linguae for up to 372 ms prior to the onset of tongue projection (Table 1) is consistent with a ‘bow and arrow’ mechanism of elastic recoil. Such activation of muscles well in advance of high-powered movements has been found or implicated not only in chameleons (Wainwright and Bennett, 1992a; Wainwright and Bennett, 1992b; de Groot and van Leeuwen, 2004; Anderson and Deban, 2010), but also in high-powered movements of many other vertebrate and invertebrate systems, including mantis shrimp and trap-jaw ants (Patek et al., 2004; Patek et al., 2006; Patek et al., 2007), various jumping insects (Burrows, 2006; Burrows, 2009), pipefish (Van Wassenbergh et al., 2008) and frogs (Deban and Lappin, 2011; Sandusky and Deban, 2012).

The m. hyoglossus showed activity consistent both with braking the tongue at the end of tongue projection and retracting the tongue into the mouth (Fig. 3), as found in other studies (Wainwright and Bennett, 1992a; Herrel et al., 2009). The m. hyoglossus exhibited a series of short bursts of varying duration across its activity period. Activity began on average 84 ms prior to the tongue reaching maximum projection and continued after the tongue was fully retracted into the mouth. The tongue took on average only 32 ms to reach maximum projection, with the m. hyoglossus becoming active prior to the onset of tongue projection in all but one feeding (Table 1).

Intensity measures of EMG recordings do not explain the reduced thermal sensitivity of tongue projection at low temperature. None of the measures of intensity for the m. accelerator linguae or m. hyoglossus showed a significant effect of temperature across either temperature range (Tables 2, 3). The lack of temperature effect indicates that muscles were not differentially recruited at different temperatures and thus that the chameleons did not recruit muscle fibers when cold that were held in reserve when warm. A significant temperature effect on the ratio of the m. accelerator linguae r.m.s. to the m. hyoglossus r.m.s. was found in the 24–36°C range; however, a Q_{10} value of 2.36 indicates that the intensity of the m. accelerator linguae declined less than the intensity of the m. hyoglossus from 24 to 36°C (Fig. 4). However, this reduced effect on the EMG intensity of the m. accelerator linguae at higher temperatures does not explain the reduced thermal sensitivity of tongue projection at low temperature. The thermal independence of EMG intensity for the tongue muscles in chameleons is in contrast to the results of studies on muscle-powered movements, such as swimming in fish, in which compensation for the loss of power at low temperatures occurs *via* the recruitment of more fibers at lower temperature for a given level of performance (Rome et al., 1984; Rome et al., 1990; Rome et al., 1992). However, the chameleons are similar to another elastic system: the m. depressor mandibulae

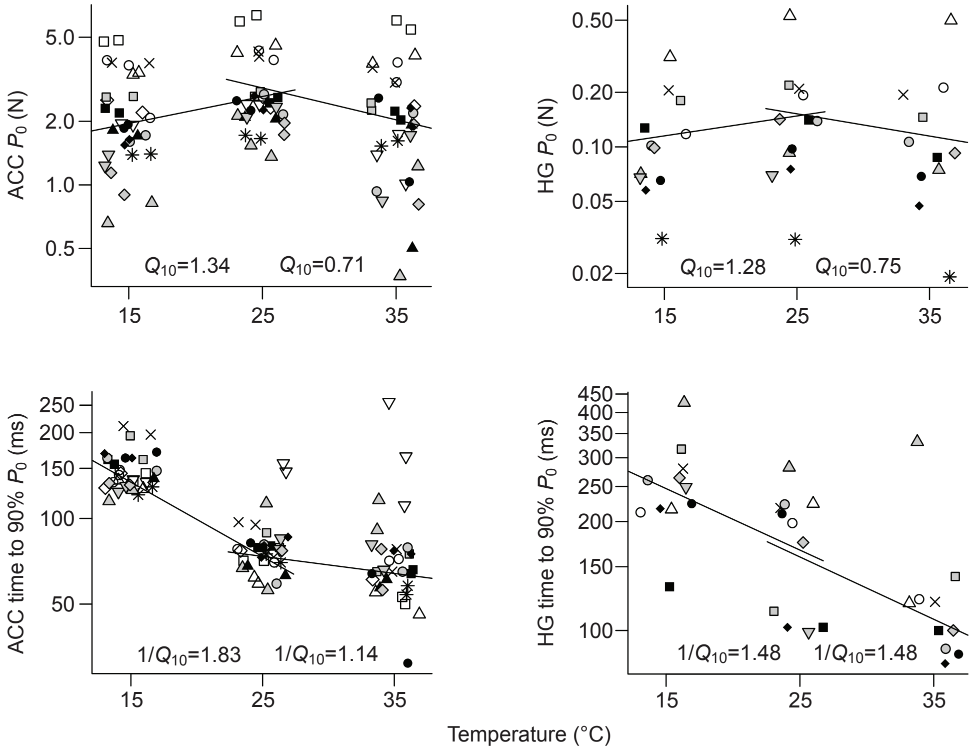

Fig. 5. Scatterplots of contractile properties *versus* temperature. Left panels depict variables for the m. accelerator linguae (ACC) and right panels depict variables for the m. hyoglossus (HG). Note that data are from experiments conducted at 15, 25 and 35±1°C, yet data points are depicted here with random 'jitter' on the temperature axis to allow individual points to be discerned. All variables shown across both temperature ranges and in both muscles experienced a significant effect of temperature. Indications as in Fig. 4.

in the elastic-recoil-powered ballistic feeding of toads shows no effect of temperature on EMG intensity (Deban and Lappin, 2011).

Temperature effects on the timing of activity of the m. accelerator linguae were significant only for the latency between the onset of activity to the onset of tongue projection in the 14–26°C range with a $1/Q_{10}$ value of 2.69 (Tables 2, 3, Fig. 4). As indicated by the ratio between the m. accelerator linguae activity to tongue projection onset and the m. hyoglossus activity to maximum tongue projection only being significant in the 24–36°C range ($1/Q_{10}$=2.33), the change in these activity durations for the m. accelerator linguae and m. hyoglossus are not significantly different from each other at the lower temperature range. These results indicate that the m. accelerator linguae took significantly longer to load the tongue projection mechanism between 14 and 26°C than between 24 and 36°C. The reduction in $1/Q_{10}$ values and the loss of significance for this variable in the 24–36°C range (Table 3, Fig. 4), as compared with the 14–26°C range (Table 2, Fig. 4), indicate, however, that temperature effects are greater in the lower temperature range. Similar patterns of significant temperature effects on muscle activity have been found in the jaw muscles of toads when feeding, with increased duration at low temperature and a plateau at higher temperature (Deban and Lappin, 2011).

The latency between the onset of m. hyoglossus activity and the time of maximum tongue projection was not significantly effected by temperature (Tables 2, 3, Fig. 4), likely because tongue projection is only weakly sensitive to temperature (Anderson and Deban, 2010) and increasing the amount of time prior to tongue projection onset that the m. hyoglossus is active could result in reduced tongue projection performance.

Muscle contractile dynamics

In vitro contractile experiments of the m. accelerator linguae were performed to examine thermal effects on biologically relevant contractile properties of the m. accelerator linguae. Previously, pressure within the central lumen of the m. accelerator linguae has been examined as a surrogate for force during *in vitro* contractile experiments (Wainwright and Bennett, 1992b); however, the forces behind the shape change that loads elastic elements with energy prior to tongue projection are more relevant to the mechanism, because recoil of these elastic elements is now known to produce much of the tongue's projection performance (de Groot and van Leeuwen, 2004). Measuring the force of elongation of the m. accelerator linguae around a surrogate entoglossal process estimates the forces storing strain energy in the collagenous intralingual sheaths of the tongue apparatus. With the limited length change possible in this experimental arrangement, construction of a complete force–velocity relationship was not possible. However, when active isometrically, the m. accelerator linguae reached an average P_0 of 2.44 N. The m. accelerator linguae reached 90% P_0 in an average of 102 ms following an average electromechanical delay of 6.9 ms. Given that the latency between the onset of activity of the m. accelerator linguae and the onset of tongue projection was on average 146 ms, this rate of force development is sufficient for the m. accelerator linguae to load elastic structures with strain energy. Our results are in line with those of previous contractile experiments in *Trioceros jacksonii* measuring the pressure within the central lumen of the m. accelerator linguae during contraction, which found an average electromechanical delay of 13.5 ms and time to 90% peak pressure of 110.4 ms (Wainwright and Bennett, 1992b).

Contractile experiments on the m. hyoglossus, in contrast, followed a more conventional experimental preparation that allowed for the calculation of complete force–velocity relationships for each individual muscle. Under isometric contraction, the m. hyoglossus reached an average P_0 of 0.14 N. Following, on average, a 20 ms electromechanical delay, the m. hyoglossus reached 90% P_0 in an average of 189 ms at an average rate of force development of $0.86\,\mathrm{N\,s^{-1}}$. Force–velocity relationships for the m. hyoglossus calculated an average $V_{\max}$ of $2.56\,L_0\,\mathrm{s^{-1}}$ ($0.06\,\mathrm{m\,s^{-1}}$) and an average mass-specific $W_{\max}$ of $13.17\,\mathrm{W\,kg^{-1}}$. Further, an average power ratio for the m. hyoglossus of 0.13 was calculated. These values are

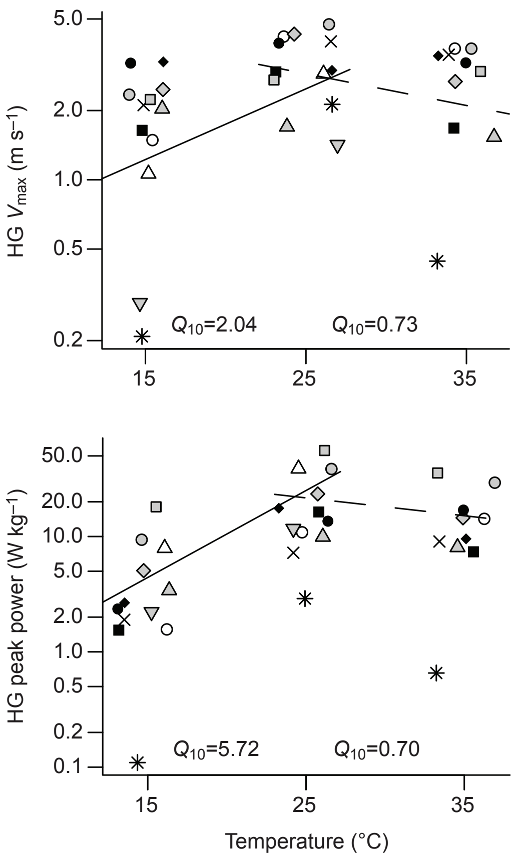

Fig. 6. Scatterplots of contractile properties *versus* temperature for the m. hyoglossus (HG). Both variables experienced a significant effect of temperature across the 14–26°C range, whereas neither experienced a significant temperature effect across the 24–36°C range. Indications as in Figs 4, 5.

consistent with previous contractile experiments for the m. hyoglossus in *C. calyptratus*, which found lower time to peak tension values than our study because of the muscle's length–tension relationship at resting length compared with maximum projection length, but a comparable rate of force development [0.64 N s^{-1} (Herrel et al., 2001a)]. Further, these values indicate that the m. hyoglossus of *C. calyptratus* is considerably slower than the m. iliofibularis from either *Sceloporus occidentalis*, which reaches peak tension in less than 80 ms with a V_{max} of more than 5 L_0 s^{-1} even at temperatures as low as 15°C (Marsh and Bennett, 1986), or *Agama agama*, which reaches peak tension in 58 ms and has a V_{max} of 5.8 L_0 s^{-1} on average (Abu-Ghalyun et al., 1988).

The time required to reach 90% P_0 indicates that even though the m. hyoglossus became active on average 52 ms prior to the onset of tongue projection, its tension should not have reached its peak, thus reducing the impact on tongue projection performance. In fact, considering the average time the m. hyoglossus became active prior to the onset of projection at each temperature, contractile data at similar temperatures indicate that by the onset of projection, the m. hyoglossus would reach on average 24% P_0 at 15°C and 43% P_0 at 35°C. The activity of m. hyoglossus prior to tongue projection, however, frequently exhibits low levels of activity until immediately prior to projection, suggesting that only a limited number of motor units may be activated at initial activity and tension developed by the onset of projection may be considerably lower. Similarly, given the average time to maximum projection distance, contractile data indicate that by the time of maximal tongue projection, the m. hyoglossus would reach on average 34% of P_0 at 15°C and 69% of P_0 at 35°C. Assuming that peak tension is not required to stop the forward motion of the tongue, reaching P_0 in the tongue retraction phase rather than prior to or at the time of peak projection would further serve to reduce the impact of m. hyoglossus activity on tongue projection performance.

A longer prey-transport cycle duration has been observed in *Chamaeleo* as compared with a generalized agamid lizard (*Pogona*) and has been attributed to the divergent morphology of the tongue apparatus in chameleons, or their supercontracting m. hyoglossus muscle fibers (Herrel et al., 2009); however, our data suggest that such performance differences may be the result of systemic characteristics of chameleon muscle contractile physiology. We found that the V_{max} of *C. calyptratus* m. hyoglossus is similar to that of *C. senegalensis* m. iliofibularis [2.5 L_0 s^{-1} (Abu-Ghalyun et al., 1988)]. The V_{max} of the m. iliofibularis in chameleons was half that of the m. iliofibularis of *A. agama* (Abu-Ghalyun et al., 1988). Further, the specific tension of *C. senegalensis* m. iliofibularis [7.3 N cm^{-2} (Abu-Ghalyun et al., 1988)] falls within the range of specific tension we found for *C. calyptratus* m. hyoglossus (Table 1), although the average value for the m. hyoglossus is slightly lower (4.1 N cm^{-2}). These results suggest numerous similarities in the contractile properties of skeletal muscles of chameleons.

All dynamic contractile properties of the m. accelerator linguae in the 15–25°C range showed a significant effect of temperature, whereas in the 25–35°C range, all except the rate of force development was influenced by temperature (Table 4, Fig. 5). Similarly, all dynamic contractile properties of the m. hyoglossus were influenced by temperature in the 15–25°C range (Table 4, Figs 5–8), whereas the rate of force development, V_{max} and W_{max} were not affected by temperature in the 25–35°C range. These results are consistent with the pattern of lower thermal dependence at higher temperatures found in muscles and muscle-powered movements of other organisms (Bennett, 1984; Bennett, 1985; Putnam and Bennett, 1982; Hirano and Rome, 1984; John-Alder et al., 1989; Swoap et al., 1993; Stevenson and Josephson, 1990).

The P_0 for both the m. hyoglossus and m. accelerator linguae exhibited temperature effects in both the 15–25°C and 25–35°C ranges; however, Q_{10} values were relatively low in all cases (Table 4, Fig. 5). In fact, in the 15–25°C range, Q_{10} values of this static property for both muscles was 1.34 or lower, while for the aforementioned dynamic properties, Q_{10} or $1/Q_{10}$ values were 1.48 or higher. This is consistent with previous research that found a lower thermal dependence for static contractile properties than for dynamic contractile properties, yet unusual in that static contractile properties still exhibited a significant effect of temperature (Bennett, 1985; Lutz and Rome, 1996).

The shape of the force–velocity curve, represented as the power ratio, of the m. hyoglossus was significantly affected by temperature in the 15–25°C range (Q_{10}=2.19; Table 4). The curvature of the relationship was reduced at higher temperatures, resulting in higher peak power (Fig. 8) and power ratios (0.082–0.250; Table 1). These values encompass the power ratio of the m. iliofibularis of *S. occidentalis* [0.107–0.119 (Marsh and Bennett, 1986)], which, in contrast to that of m. hyoglossus of *C. calyptratus*, shows no significant effect of temperature from 10 to 35°C. The decline in power at low temperatures for chameleon m. hyoglossus may be related to a reduced importance of that power once prey has been secured by the tongue because of the highly effective mechanism of prey prehension in chameleons (Herrel et al., 2000). Power may be maintained at low temperatures in *S. occidentalis*, however,

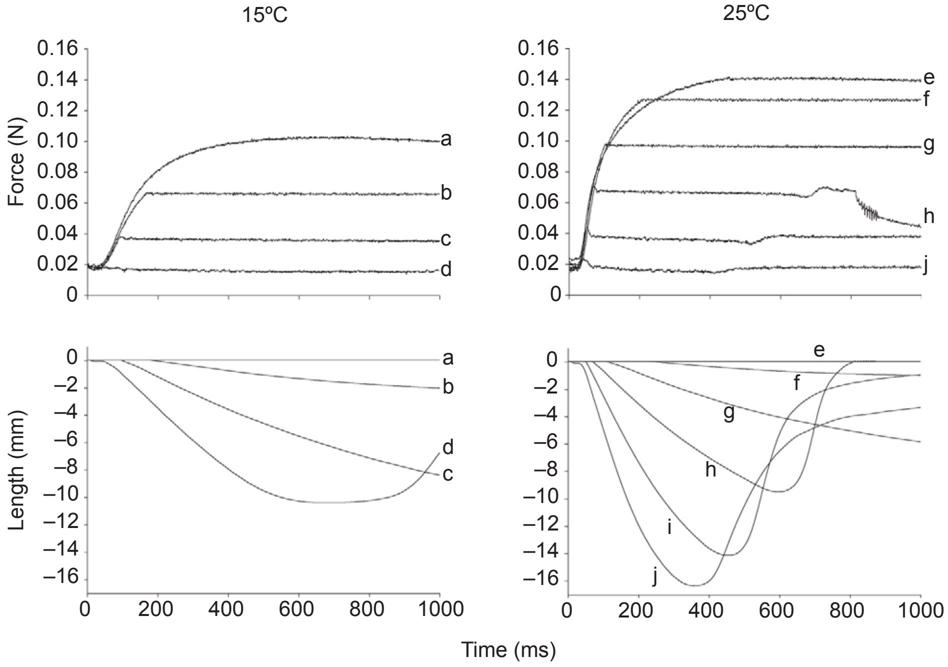

Fig. 7. Original data from isometric and isotonic contractions of the m. hyoglossus at 15°C (left) and 25°C (right). Upper panels show force development *versus* time and lower panels show length change *versus* time from the same trials (indicated by letters). Note the overall increase in force produced and rate at which force is produced at 25°C compared with 15°C. Further, note the increased excursion rates at 25°C compared with 15°C for similar force contractions. All traces are shown on the same time scale.

because power levels associated with locomotor performance are likely of high importance.

Conclusions

The performance of ballistic tongue projection in *C. calyptratus* exhibits significantly lower thermal dependence than tongue retraction (Anderson and Deban, 2010). This differential thermal response was proposed to be the result of the difference between the mechanism of tongue projection, which is powered by recoil of preloaded elastic elements (de Groot and van Leeuwen, 2004), and that of tongue retraction, which is powered by muscle contraction alone (Wainwright and Bennett, 1992a; Herrel et al., 2001b). Data presented here on the motor patterns of tongue projector and tongue retractor muscles reveal no thermal relationship in muscle recruitment that would explain the greater thermal robustness of tongue projection relative to tongue retraction (such as greater muscle recruitment at lower temperature). Increased activity duration of the m. accelerator linguae leading up to tongue projection at low temperature indicates a typical thermal response to slowing contractile properties. Further, the contractile properties of both muscles confirm that they have reduced dynamic contractile performance at low temperature.

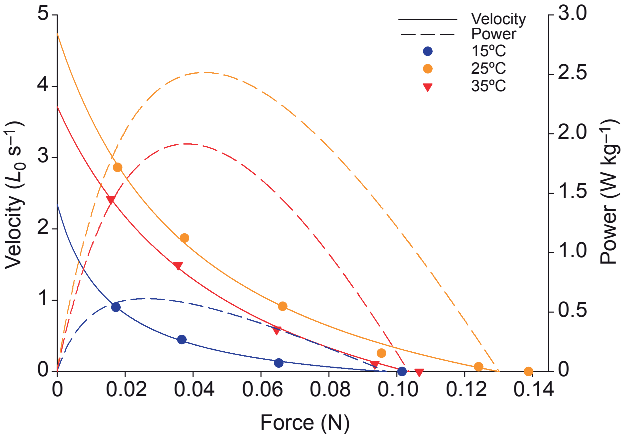

Fig. 8. Force–velocity curves (solid lines, left axis) with overlaid power curves (dashed lines, right axis) for the m. hyoglossus from the same muscle at 15, 25 and 35°C. Points indicate experimentally gathered force and corresponding velocity values, whereas force–velocity curves depict Hill's equation fitted to these data points (Hill, 1938) (see Materials and methods and Fig. 7 for details). Power curves are derived from the product of force and velocity points from the fitted force–velocity curves. Note the increased curvature of the force–velocity curve at 15°C indicating a decreased power ratio, and an outward shift in the trace from 15 to 25°C and an inward shift of the curve from 25 to 35°C.

Our results indicate that neither the tongue projector nor the tongue retractor muscle is able to circumvent typical thermal effects on muscle contractile properties, nor do they differentially activate at varying intensities at different temperatures to overcome these thermal constraints on their muscle physiology. The tongue projector muscle also shows no evidence of physiological specializations that would explain the reduced thermal dependence of tongue projection. Our results are thus consistent with a model of tongue projection in which the biomechanics and morphology of the tongue apparatus itself, combined with the timing of muscle activation, are responsible for the reduced thermal dependence of tongue projection in chameleons. Finally, these results suggest that evolutionary modifications of gross morphology and motor control, in the absence of changes in muscle contractile physiology, are sufficient to produce high-performance and thermal robustness.

Although a pattern of thermal robustness in independently evolved ballistic movements powered by elastic recoil – in chameleons, salamanders, toads and frogs – is becoming increasingly apparent (Anderson and Deban, 2010; Deban and Lappin, 2011; Deban and Richardson, 2011; Sandusky and Deban, 2012), the extent to which these mechanisms have converged on similar patterns of modifications to overcome strong thermal effects on muscle-powered movements remains unknown. The mechanisms may vary considerably in gross morphology; however, they may have converged upon similar patterns of interactions between components of the mechanisms and similar modifications to motor control patterns and muscle contractile physiology. Given similarities in the thermal effects on motor control patterns underlying elastic-recoil-powered movements in toads (Deban and Lappin, 2011) and chameleons, we expect that other elastic systems may exhibit evolutionary modifications of gross morphology and motor control without changes in associated muscle contractile physiology. Examination of thermal effects on kinematics, motor control and muscle contractile physiology of feeding movements across closely related lineages with varying morphologies and multiple independent evolutions of ballistic tongue projection may help shed light on how these similar mechanisms evolve.

LIST OF SYMBOLS AND ABBREVIATIONS

ACC	m. accelerator linguae
HG	m. hyoglossus
L_0	muscle length
MTP	maximal tongue projection
P_0	peak isometric force
PRC	partial regression coefficients of the temperature effect
Q_{10}	temperature coefficient
TP	onset of tongue projection
TR	tongue fully retracted
V_{max}	peak contractile velocity
W_{max}	peak power

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