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Environmental Effects on the Biomechanics and Motor Physiology of Elastically Powered Movements in Chameleons

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Environmental Effects on the Biomechanics and Motor Physiology of Elastically Powered Movements in Chameleons

by

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A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy
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Keywords: Temperature Effects, Electromyography, Contractile Properties, Feeding, Chamaeleonidae

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DEDICATION

I dedicate this dissertation to my parents, Eric and Melissa Anderson. Throughout the years, they have provided unwavering financial and emotional support, as well as endless encouragement. Whenever I needed it, they have always been there, and for that, I am truly grateful!

I would also like to thank my grandfather, Rae V. Anderson, for inspiring my passion for wildlife. I will always remember him flipping through animal books with me as a child, taking me on hikes in the mountains behind his house to catch newts, bringing me up the hill to see his birds, and later traveling throughout the world with him to visit some incredible habitats. His love for animals and nature has always been contagious, and I wouldn’t have followed the path I have had it not been for him.

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I love you all very much and thank you all for everything you’ve done!
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In addition to these individuals, numerous others have helped with my research and their contributions are acknowledged at the end of each chapter of my dissertation.
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ABSTRACT

Environmental temperature exhibits profound effects on the activity and ecology of ectotherms through its impact on muscle contractile physiology. While the performance of locomotor behaviors powered by muscle contraction directly decreases by at least 33% over a 10°C drop in body temperature, chameleons are known to feed, presumably with high performance, at body temperatures where sympatric lizard species remain inactive. I propose that ballistic movements that are powered by the recoil of preloaded elastic and collagenous tissues are less thermally dependent than movements that rely on direct muscular power. Despite the reduced thermal sensitivity of the elastic-recoil powered movement, I propose that the muscles associated with preloading these elastic tissues are themselves thermally sensitive and at low temperature, will take longer to load the elastic tissues. Finally, I expect that because of the different effect of temperature on elastic-recoil-powered and muscle-powered movements, performance declines for elastic-recoil-powered tongue projection at low temperature will not vary between species along an environmental temperature gradient (i.e., thermal effects will be the same for all species). Conversely, performance declines for muscle powered tongue retraction at low temperature will be lower in species from colder environments along an environmental temperature gradient. To test these predictions, I used high-speed videography, electromyography and in vitro muscle contractile experiment techniques in conjunction with temperature manipulations to test the mechanistic principles in
*Chamaeleo calyptratus*. I then used high-speed videography at different temperatures in three *Bradypodion* species from different habitats in South Africa to compare thermal effects on elastic-recoil and muscle-powered movements in different species. I found that the elastic-recoil mechanism of tongue projection in chameleons circumvents the constraints that low temperature imposes on muscle rate properties, thereby reducing the thermal dependence of tongue projection. In all species examined, tongue projection was relatively thermally robust, maintaining a high degree of maximal performance at temperatures as low as 15ºC. In contrast, the associated muscle-powered tongue retraction was strongly effected by temperature and experienced substantial performance declines over the same temperature range. While tongue projection performance was itself thermally robust, muscle contractile dynamics of the tongue projector muscle, which preloads the elastic elements responsible for powering projection, was strongly affected by temperature. Similarly, at cooler temperatures the tongue projector muscle became active earlier relative to the onset of tongue projection, due to the reduced rate of tension buildup and the resulting increase in time required to load the elastic elements of the tongue with the required force to subsequently power tongue projection. Further, the effect of temperature on both tongue projection performance and tongue retraction performance was found to vary between species living in different thermal environments. This suggests that despite differences in how temperature affects the performance of these different movement types, both elastic-recoil-powered movements and muscle-powered movements may experience selective pressure to optimize their performance to their environments. Based on these findings, I suggest that the relative thermal independence of tongue projection in chameleons is a more general characteristic of
elastic-recoil-powered mechanisms and organisms that use elastic recoil mechanisms for ecologically important movements, such as feeding and locomotion, may benefit from an expanded thermal niche. Further, given the prevalence of elastic power-amplification mechanisms in ectotherms, the benefit of reduced thermal sensitivity may promote the evolution of these mechanisms in other ectothermic animals. Finally, I propose that temperature manipulations may be a useful methodological approach to testing for the presence or prevalence of elastic recoil in powering other biomechanical systems. While these studies examined thermal effects on ballistic tongue projection and tongue retraction in chameleons at different mechanistic levels and within the framework of how these thermal relationships may be affected by their local environment, many of the results apply more broadly to similar systems in other ectotherms. Comparison of these findings to similar elastically powered systems may help solidify the generality of these findings among other taxa.
Due to its profound effect on physiological rate processes (Cossins & Bowler, 1987), temperature change is one of the most significant environmental challenges faced by ectothermic animals. Temperature exhibits a strong effect on organismal performance as a result of its effect on muscle contractile physiology, which has a clear impact on the ability of an organism to move, escape predators and engage in foraging behavior (Marsh & Bennett, 1985; van Berkum, 1986; Huey & Bennett, 1987; John-Alder et al., 1989; Lutz & Rome, 1996a; Lutz & Rome, 1996b; Peplowski & Marsh, 1997; Wintzer & Motta, 2004; Herrel et al., 2007). The systems that have been examined with regard to their response to temperature changes have generally focused on cyclical and locomotor movements (i.e. swimming and running) with relatively few examples of other widespread but more explosive or episodic dynamic movements, such as feeding and jumping. The effects of temperature on highly dynamic spring loaded systems, however, remained to be studied.

Ballistic tongue projection in chameleons represents an explosively dynamic feeding mechanism powered by elastic recoil (de Groot & van Leeuwen, 2004). This highly specialized feeding mechanism exhibits extreme performance, which is critical to the organisms’ survival (Herrel et al., 2009) and offers the opportunity to expand our understanding of the effect of temperature on different types of movements.
As generally slow moving predators, chameleons (Family Chamaeleonidae) rely largely on crypsis and the element of surprise to capture their food. In the absence of a more active hunting strategy, maximizing the ability to capture prey throughout the day is important (Herrel et al, 2009). One way chameleons cope with prey capture limitations resulting from locomotor muscle with contractile rate properties 2-10x slower than *Agama* locomotor muscle (Abu-Ghalyun et al., 1988) is their ballistic tongue projection mechanism, which is capable of projecting the tongue up to and even more than twice the animal’s own body length (Herrel et al, 2009; Anderson et al., 2012). This enables chameleons to capture prey items from a distance without physically chasing down their prey and while minimizing the chance of the prey item noticing their presence and attempting to flee (Herrel et al, 2009).

With over 200 currently recognized species and subspecies from eleven genera, chameleons live in habitats ranging from desert sand dunes where body temperature ($T_b$) exceeds 39ºC (Burrage, 1973) to alpine zones above 3500m with ambient temperatures below freezing (Reilly, 1982). Curiously, some chameleon species are known to feed at incredibly low $T_b$ (Burrage, 1973; Reilly, 1982; Hebrard et al, 1982; Bennett, 2004; Andrews, 2008). Of particular note, *Bradypodion pumilum* has been reported feeding at $T_b$ of 3.5ºC (Burrage, 1973), and *Trioceros hoehnelii* has been observed feeding at $T_b$ of 7ºC (Hebrard et al, 1982), and *Trioceros jacksonii* has been recorded feeding in the lab at $T_b$ of 10ºC (Bennett, 2004). While the biomechanics and physiological basis of chameleons feeding at low $T_b$ had not previously been explained, their ability to do so likely allows them to exploit an early morning peak in alpine insect activity (Reilly, 1982) when effective behavioral thermoregulation is not possible. This ability enables
them to be active earlier and over a wider thermal range than other sympatric lizard species (Hebrard et al, 1982).

In this dissertation I report three studies that help to expand our understanding of the effect of temperature on a highly dynamic spring-loaded system (ballistic tongue projection in chameleons) as compared to its effect on an associated muscle-powered system (non-ballistic tongue retraction). In the process, these studies also provide useful methodological approaches and insights into the biology and performance capabilities of chameleons, some of which may more broadly apply to other ectotherms using similar spring-loaded mechanisms.

First, I examined the question of whether the ability of chameleons to feed at low $T_b$ is the result of a reduced effect of temperature on their feeding movements in general or just their tongue projection. I tested this using high-speed videography of feeding events across a range of temperatures, hypothesizing that the performance of elastic-recoil-powered tongue projection would exhibit weak thermal dependence relative to the performance of muscle-powered tongue retraction.

Next, I used high-speed video of feedings synchronized with electromyographic recordings, and in vitro muscle contractile experiments to examine how motor control patterns and muscle contractile properties change with temperature to determine if the predicted weak thermal dependence of tongue projection could be explained by specializations to the muscle contractile physiology. I expected that muscles associated with tongue projection would not themselves be liberated from typical effects of temperature on muscle rate properties, but would instead require longer durations to
achieve the tensions required to load elastic elements in the tongue responsible for
powering tongue projection.

Then, I explored how different thermal environments affect patterns of thermal
specialization and performance curves in movements with different levels of thermal
sensitivity by looking at the feeding performance at different temperatures of three
chameleon species living over a 2000m elevation gradient in South Africa. I predicted
that performance declines for elastic-recoil-powered tongue projection at low temperature
would not vary between species along an environmental temperature gradient (i.e.,
thermal effects would be the same for all species). Conversely, I predicted that
performance declines for muscle powered tongue retraction at low temperature would be
lower in species from colder environments along an environmental temperature gradient.

Finally, these results are discussed within a more general framework, emphasizing
how these results not only provide considerable insight into the biology and performance
of chameleons, but also how they apply more broadly to similar systems in other
ectotherms. In the process of summarizing the major findings of this dissertation, I
discuss broad implications and general conclusions, concluding with remarks on future
directions and questions with which this research could continue.

References Cited
physiological basis of slow locomotion in chameleons. *Journal of Experimental


CHAPTER TWO: BALLISTIC TONGUE PROJECTION IN CHAMELEONS
MAINTAINS HIGH PERFORMANCE AT LOW TEMPERATURE

Abstract

Environmental temperature impacts the physical activity and ecology of ectothermic animals through its effects on muscle contractile physiology. Sprinting, swimming, and jumping performance of ectotherms decreases by at least 33% over a 10°C drop, accompanied by a similar decline in muscle power. We propose that ballistic movements that are powered by recoil of elastic tissues are less thermally dependent than movements that rely on direct muscular power. We found that an elastically powered movement, ballistic tongue projection in chameleons, maintains high performance over a 20°C range. Peak velocity and power decline by only 10%–19% with a 10°C drop, compared to >42% for nonelastic, muscle-powered tongue retraction. These results indicate that the elastic recoil mechanism circumvents the constraints that low temperature imposes on muscle rate properties and thereby reduces the thermal dependence of tongue projection. We propose that organisms that use elastic recoil mechanisms for ecologically important movements such as feeding and locomotion may benefit from an expanded thermal niche.

1 Portions of these results have been previously published (Anderson and Deban, 2010) and are utilized with permission of the publisher. Christopher V. Anderson and Stephen M. Deban designed the research and contributed analytic tools. The research was performed and analyzed by Christopher V. Anderson.
Introduction

Temperature influences diverse physiological processes, including metabolic rate, muscle dynamics, and nerve conduction velocity, which in turn can affect whole-organism performance. Ectothermic animals are particularly vulnerable to the effects of low ambient temperatures, because their body temperature \((T_b)\) is dictated by environmental conditions. The effect of \(T_b\) on muscle physiology has a clear impact on an organism’s ability to move, escape predators, and engage in foraging behavior (Huey and Stevenson, 1979; Bennett, 1985; Huey and Bennett, 1987; Rome, 1990; Lutz and Rome, 1996; Herrel et al., 2007); for example, a 10°C drop in \(T_b\) reduces sprint speed in lizards, swimming speed in fish, and jumping distance in frogs by at least 33% (Huey and Bennett, 1987; Rome, 1990). We find that, unlike these other dynamic movements, ballistic tongue projection in chameleons maintains extremely high performance over a \(T_b\) range of 20°C.

The mechanism of chameleon prey capture is unique among lizards, relying on ballistic projection of the tongue up to twice the length of the body in as little as 0.07 second (Herrel et al., 2001; de Groot and van Leeuwen, 2004). This feeding mechanism is common to all chameleons and gives these slow, cryptic, sit-and-wait predators the element of surprise. Chameleons feed over a wider range of \(T_b\) than other lizards, using ballistic tongue projection in habitats ranging from deserts, where \(T_b\) exceeds 39°C (Burrage, 1973), to alpine zones above 3,500 m with temperatures below freezing (Reilly, 1982). Some chameleon species feed at a \(T_b\) of 3.5°C (Burrage, 1973), exploiting an early morning peak in alpine insect activity (Reilly, 1982) before sympatric lizard species become active (Hebrard et al., 1982). This ability to feed at low \(T_b\) has not been
explained; we propose that the elastic-recoil mechanism of tongue projection confers this temperature insensitivity.

Ballistic tongue projection in chameleons achieves its extreme performance by rapid elastic recoil of collagen tissue within the tongue—tissue that is first stretched by slow contraction of the tongue accelerator muscle (de Groot and van Leeuwen, 2004). This “bow and arrow” mechanism decouples muscle contraction temporally from tongue launch and thereby allows kinetic energy to be imparted to the tongue at a rate far exceeding that possible via direct muscle contraction (de Groot and van Leeuwen, 2004). Once launched—at accelerations exceeding 400 ms\(^{-2}\) (41 g)—the tongue travels to the target on its momentum alone and then adheres to the prey. Tongue retraction relies on neither ballistic launch nor elastic recoil to bring prey to the mouth, but rather is driven by continuous contraction of the lengthy hyoglossus muscle (Herrel et al., 2001).

The differing mechanisms of tongue projection and retraction in chameleons provide an opportunity to evaluate the hypothesis that the elastic-recoil mechanism confers low thermal dependence to tongue projection. We tested whether elastically powered tongue projection has a lower thermal dependence than nonelastic tongue retraction by examining the effects of temperature on performance parameters of these two movements. In addition, we propose that our findings can be generalized to explosive ballistic movements in other ectotherms, and that elastic-recoil mechanisms may serve to expand the thermal niche of ectotherms that use them for critical movements.
Materials and Methods

Five *Chamaeleo calyptratus* (12.5–14.0 cm snout–vent length) were imaged at 3 kHz at a $T_b$ of 15°C, 25°C, and 35°C while feeding on crickets at a range of distances, using a Photron Fastcam high-speed digital camera. Crickets were placed on a square of insect screen suspended vertically from above by thread. This “cricket trapeze” allowed the chameleon’s tongue to complete its trajectory naturally without being stopped by an immovable target, and thus permitted examination of performance and physiological parameters at a range of actual tongue projection distances.

To control $T_b$, after an acclimation period of at least 1 h, imaging trials were conducted in an environmental chamber set to the experimental $T_b$. Supplemental lighting was switched on immediately before tongue projection and turned off immediately after tongue retraction to prevent elevation of body temperature through light source radiation. During the prey reduction phase, immediately after tongue retraction, $T_b$ was verified orally using a calibrated Sixth Sense LT300 infrared thermometer (± 1°C accuracy). Only feeding sequences with a postfeeding $T_b$ of the target experimental temperature ± 1°C were included in the analysis.

Ten feeding sequences were collected from each of four individuals at each experimental $T_b$, for a total of 120 feedings. Five feeding sequences from a fifth individual were collected at each experimental $T_b$ before this animal was removed from the experiment due to illness. Between one and five feeding events were collected per individual at each feeding session. The sequence of experimental $T_b$ for each individual was selected randomly, and no two animals were exposed to an identical $T_b$ sequence. To account for natural variation in the distance between the prey and the chameleon’s snout
because of the distance that the chameleon leaned its body forward off the perch for any given feeding event, distance to the “cricket trapeze” was varied within a normal range of projection distances. Thus, feedings were collected over an 8-20 cm range of tongue projection distances. Distance to the prey was adjusted to elicit maximal tongue projection length for each individual at each experimental $T_h$. Effects of temperature on preprojection distance to the target and overshoot distance of the tongue beyond the target were examined using repeated-measures ANOVA.

An inverse-dynamics approach was used to compute the instantaneous velocity, acceleration, and power of tongue projection and retraction. Using National Institutes of Health Image J software (http://reb.info.nih.gov/ij), the distance of tongue projection for each scale-calibrated feeding sequence was recorded. Image J software was used to record the $x,y$ coordinates of the tip of the tongue on each frame throughout the tongue projection sequence. Using a custom script for the P-Spline package of R statistical software (R Project for Statistical Computing), a quintic spline was fitted to the position trace of the tongue and smoothed to remove secondary oscillation artifacts from the first and second derivatives of position. From these smoothed position data, instantaneous velocity (ms$^{-1}$) and acceleration (ms$^{-2}$) (i.e., first and second derivatives of the position) were calculated. For tongue retraction, coordinates of four positions along the length of the retractor muscle were recorded on each frame of the retraction sequence. These coordinate data were used to quantify the length of the retractor muscle in each frame, and this length was then used to compute the length change through the retraction sequence. These length data were then smoothed and subjected to the same inverse dynamics analysis as the tongue projection position data. Mass-specific power (in Wkg$^{-1}$)
was calculated as the product of velocity and acceleration (de Groot and van Leeuwen, 2004) and corrected for the mass of the active muscle in each phase. As in other species (de Groot and van Leeuwen, 2004), dissection and mass measurements of the tongue apparatus of seven C. calyptratus (12.0–15.5 cm snout–vent length) determined that the circular portion of the accelerator muscle accounts for ~50% (mean, 48.2% ± 2.9%) of the mass of the accelerator muscle complex and tongue pad, whereas the retractor muscle accounts for ~25% (mean, 25.8% ± 1.7%) of the mass of the accelerator muscle complex, tongue pad, and retractor muscle. Thus, mass-specific power is multiplied by a factor of 2 for projection and by a factor of 4 for retraction (de Groot and van Leeuwen, 2004).

To examine the effects of temperature on performance, tongue projection distance, peak velocity (ms$^{-1}$), peak acceleration (ms$^{-2}$) and peak mass specific power (Wkg$^{-1}$) for both tongue projection and retraction were computed for each feeding sequence. Performance was log-transformed and examined for effects of temperature (fixed effect), phase of feeding (fixed effect), and individual (random effect) using repeated-measures ANCOVA with projection distance as a covariate. The temperature × phase interaction term of the model allowed us to examine whether tongue projection and tongue retraction responded differently to temperature changes. In addition, the influence of experimental temperature sequence on performance was assessed using repeated-measures ANOVA to test for an effect of previous temperature on the projection distance residuals of each performance parameter.

Least squares regression of performance parameters during both projection and retraction, with projection distance as the independent variable, was performed for each individual at each temperature. The interpolated value of each performance parameter at
the overall average projection distance (12.5 cm) was calculated for each individual and used to calculate temperature coefficient ($Q_{10}$) values using the equation

$$Q_{10} = \left(\frac{R_2}{R_1}\right)^{\frac{10}{t_2-t_1}},$$

where $R_1$ and $R_2$ are the interpolated performance values at temperatures $t_1$ and $t_2$, respectively, and $t_2$ is greater than $t_1$. The $Q_{10}$ values for each individual were then used to calculate an average $Q_{10}$ value with SE.

**Results**

Veiled chameleons (*Chamaeleo calyptratus*) were able to project the tongue and capture prey across the same range of distances regardless of temperature (15°C–35°C). Overall, projection distances ranged from 6.6 cm to 19.6 cm. Individual average projection distances ranged from 10.4 cm to 14.2 cm, with an overall average of 12.5 cm. No significant effect of temperature on prey distance, tongue projection distance, or tongue overshoot distance was found.

Inverse dynamic analysis of tongue movements revealed that as temperature increased, performance increased significantly (Table 2.1) for both tongue projection and retraction. Nonetheless, peak performance measures of ballistic tongue projection were maintained at a high level at all temperatures (Table 2.2). At the low end of our experimental $T_b$ range (15°C), peak projection velocity averaged 3.4 ms$^{-1}$, peak acceleration averaged 357 ms$^{-2}$, and peak power averaged 1,892 Wkg$^{-1}$. At 35°C, values were somewhat higher: peak velocity averaged 4.4 ms$^{-1}$, peak acceleration averaged 433 ms$^{-2}$, and peak power averaged 2,900 Wkg$^{-1}$. In contrast, performance parameters of retraction increased markedly at higher temperature. At 15°C, peak velocity averaged 0.8
Table 2.1. Results from repeated-measures ANCOVA examining the performance parameters peak velocity, peak acceleration and peak power for effects of temperature, individual, feeding phase (projection vs. retraction), and projection distance (covariate).

<table>
<thead>
<tr>
<th></th>
<th>Peak Velocity</th>
<th></th>
<th></th>
<th>Peak Acceleration</th>
<th></th>
<th></th>
<th>Peak Power</th>
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<tr>
<td></td>
<td>df</td>
<td>F-value</td>
<td>P-value</td>
<td>df</td>
<td>F-value</td>
<td>P-value</td>
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<td>P-value</td>
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<td>15°C vs 25°C</td>
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<td>4</td>
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<td>1</td>
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<td>1</td>
<td>244.5</td>
<td>&lt;.0001</td>
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<td>0.4438</td>
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<td>0.0493</td>
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<td>Individual x Projection Distance</td>
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<td>0.1609</td>
<td>4</td>
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<td>0.5952</td>
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<td>Temperature x Projection Distance</td>
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<td>0.0157</td>
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<td>0.0004</td>
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<td>0.0023</td>
</tr>
<tr>
<td>Temperature x Phase</td>
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<td>250.5</td>
<td>&lt;.0001</td>
<td>1</td>
<td>73.86</td>
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<td>25°C vs 35°C</td>
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<tr>
<td>Individual</td>
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<td>1</td>
<td>45.15</td>
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<tr>
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<td>&lt;.0001</td>
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<td>0.207</td>
<td>0.6494</td>
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<td>16.50</td>
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<td>26.25</td>
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<td>&lt;.0001</td>
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<td>0.3523</td>
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<td>Individual x Projection Distance</td>
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<td>1.636</td>
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<td>1.528</td>
<td>0.1966</td>
</tr>
<tr>
<td>Temperature x Projection Distance</td>
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<td>0.0334</td>
<td>1</td>
<td>3.252</td>
<td>0.0732</td>
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<td>0.025</td>
<td>0.8759</td>
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<tr>
<td>Individual x Phase</td>
<td>4</td>
<td>5.597</td>
<td>0.0003</td>
<td>4</td>
<td>2.652</td>
<td>0.0353</td>
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<td>2.093</td>
<td>0.0843</td>
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<td>Temperature x Phase</td>
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<td>78.38</td>
<td>&lt;.0001</td>
<td>1</td>
<td>21.21</td>
<td>&lt;.0001</td>
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<td>54.06</td>
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<tr>
<td>Projection Distance x Phase</td>
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<td>37.25</td>
<td>&lt;.0001</td>
<td>1</td>
<td>14.22</td>
<td>0.0002</td>
<td>1</td>
<td>1.536</td>
<td>0.217</td>
</tr>
</tbody>
</table>

Note the significant temperature x phase interaction effects, which indicate that tongue projection and tongue retraction are affected differently by changes in temperature.

*Significant difference in ANCOVA at Bonferroni-corrected α=0.017, indicating significant effect.
ms⁻¹, peak acceleration averaged 170.3 ms⁻², and peak power averaged 34.4 Wkg⁻¹, whereas at 35°C, peak velocity averaged 1.9 ms⁻¹, peak acceleration averaged 478 ms⁻², and peak power averaged 453 Wkg⁻¹ (Table 2.2). The average power of projection also was maintained at a high level, averaging 1,092 ± 78 Wkg⁻¹ at 15°C (mean ± SE) and 1,911 ± 156 Wkg⁻¹ at 35°C. The order of experimental temperatures experienced by an individual had no significant effect on projection or retraction performance.

Table 2.2. Kinematic performance variables during projection and retraction at 15°C, 25°C, and 35°C.

<table>
<thead>
<tr>
<th></th>
<th>Peak Velocity Mean ± SEM</th>
<th>Peak Acceleration Mean ± SEM</th>
<th>Peak Power Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Projection</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15°C</td>
<td>3.4 ± 0.1</td>
<td>357 ± 20</td>
<td>1892 ± 123</td>
</tr>
<tr>
<td>25°C</td>
<td>3.8 ± 0.1</td>
<td>406 ± 27</td>
<td>2336 ± 239</td>
</tr>
<tr>
<td>35°C</td>
<td>4.4 ± 0.1</td>
<td>433 ± 27</td>
<td>2900 ± 235</td>
</tr>
<tr>
<td><strong>Retraction</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15°C</td>
<td>0.8 ± 0.03</td>
<td>170 ± 21</td>
<td>69 ± 12</td>
</tr>
<tr>
<td>25°C</td>
<td>1.4 ± 0.04</td>
<td>293 ± 43</td>
<td>184 ± 27</td>
</tr>
<tr>
<td>35°C</td>
<td>1.9 ± 0.1</td>
<td>478 ± 14</td>
<td>453 ± 32</td>
</tr>
</tbody>
</table>

Values were calculated as the mean ± SE of each individual’s predicted performance at a projection distance of 12.5 cm based on each individual’s performance regressed against projection distance.

Although tongue projection and retraction both showed effects of temperature, retraction showed a significantly stronger effect. For each 10°C increment in temperature between 15°C and 35°C, a significant interaction effect of temperature (Tb) and phase (i.e., projection vs. retraction) on performance was found (Table 2.1). Q₁₀ values and percent decrease of average performance reveal that tongue projection maintained performance with decreasing temperature to a greater extent than did tongue retraction (Figs. 2.1 and 2.2). Performance parameters declined by only 10%–19% over the 15°C–25°C interval at a projection distance of 12.5 cm (Fig. 2.2). Temperature coefficients
Figure 2.1. Mean temperature coefficients ($Q_{10}$) with SE bars for tongue projection (green) and retraction (gold), indicating the factor by which each performance parameter changes over 10°C. Note the consistently lower values for projection versus retraction. $Q_{10}$ was calculated as the average of each individual’s $Q_{10}$ value for that parameter; individual $Q_{10}$ values were calculated from interpolated performance values at an average projection distance of 12.5 cm (from performance values regressed against projection distance).

($Q_{10}$) for projection parameters never exceeded 1.3 (Fig. 2.2) and varied by no more than 0.04 across all distances. In contrast, tongue retraction was strongly affected by temperature; it slowed visibly at 15°C, and its performance variables showed $Q_{10}$ values of 1.7–2.9 and declined by 42%–63% over 10°C (Figs. 2.1 and 2.2).

Discussion

Remarkably, *C. calyptratus* achieved extremely high-performance tongue projection even when cold. At a $T_b$ of 15°C, time-averaged muscle-mass–specific power output averaged 1,092 Wkg$^{-1}$, and peak instantaneous muscle-mass–specific power output during projection averaged 1,892 Wkg$^{-1}$. This peak value is well in excess of peak power output of muscle tissue during active contraction as measured or estimated in other vertebrates operating at higher $T_b$, including flying quail during vertical takeoff (1,121 Wkg$^{-1}$) (Askew and Marsh, 2001), sprinting lizards (952 Wkg$^{-1}$) (Curtin et al., 2005), and jumping frogs (373 Wkg$^{-1}$) (Lutz and Rome, 1996). High power outputs for rapid movements using the elastic-recoil mechanism, including jumping in bushbabies (Aerts,
Figure 2.2. Performance parameters (mean ± SE) as a percent of maximum for tongue projection and retraction, showing low thermal dependence of projection (green) compared with retraction (gold). Absolute values of means are shown in native units. Values were calculated as the average of each individual’s value for that parameter; individual values were interpolated at an average projection distance of 12.5 cm (from performance values regressed against projection distance).

1998) and insects (Bennet-Clark, 1975; Burrows, 2003), predatory strikes of mantis shrimp (Patek et al., 2004), and tongue projection in salamanders (Deban et al., 2007) and chameleons (de Groot and van Leeuwen, 2004), have been documented in numerous kinematic studies; little focus has been given to the maintenance of performance at low $T_b$, however.

The $Q_{10}$ values for tongue projection (1.1–1.3; Fig. 2.1) are well below the $Q_{10}$ values of contractile rate properties of isolated muscles and of other dynamic behaviors, which generally exceed 1.5 (Huey and Stevenson, 1979; Bennett, 1985; Huey and Bennett, 1987; Rome, 1990; Lutz and Rome, 1996; Herrel et al., 2007). This degree of temperature independence is similar to that of static contractile muscle properties, such as maximum isometric tetanic tension (Bennett, 1985; Lutz and Rome, 1996), and of static
behaviors, such as exertion of peak bite force (Herrel et al., 2007); however, the extent of
temperature dependence on tongue retraction ($Q_{10} = 1.7–2.9$; Figs. 2.1 and 2.2) resembles
that of contractile rate properties of isolated muscles and of dynamic behaviors, such as
sprinting (Huey and Stevenson, 1979; Bennett, 1985; Huey and Bennett, 1987; Rome,
1990; Lutz and Rome, 1996; Herrel et al., 2007). Jump distance in frogs, for example,
exhibits a $Q_{10}$ value of 1.6 over 14°C–25°C, and the power generated by the muscles
activated during jumping has a $Q_{10}$ value of 2.7 (Rome, 1990). Similarly, sprint speed in
lizards has an average $Q_{10}$ value of 1.5 at temperatures below the estimated optimal
temperature (Huey and Bennett, 1987).

The contrasting thermal dependence of tongue projection and retraction (Figs. 2.3
and 2.4) supports the hypothesis that the low thermal dependence of tongue projection in
chameleons is due to the elastic-recoil mechanism, in which temperature-dependent
muscle shortening occurs during the loading phase before tongue launch, and is
temporally decoupled from the temperature-independent elastic recoil of connective
tissue that powers ballistic tongue projection. This mechanism not only endows
chameleons with spectacular performance, but also liberates projection from the
constraints on muscle rate properties imposed by low temperature. Thus, the thermal
dependence of the contractile rate properties of the tongue accelerator muscle need not be
unusually low to maintain high performance at low temperature. In contrast, tongue
retraction declines at low temperature, because it relies on direct muscle power output,
which is thermally dependent. Projection performance depends instead on peak muscle
tension and the elastic modulus of collagen, both of which show low thermal dependence
or complete thermal independence (Rigby et al., 1959; Bennett, 1985; Tome, 1990; Lutz
Figure 2.3. Kinematic and dynamic profiles from two representative feedings of similar projection distance showing similar peak values for projection at 15°C and 35°C, compared with dissimilar values for retraction at the two temperatures. Retraction is analyzed only until the tongue reaches the entoglossal process. Profiles are overlaid at the time of maximum projection distance (dashed line). Power values are not corrected for muscle mass (2x for projection and 4x for retraction).
Figure 2.4. Image sequences of one individual of *Chamaeleo calyptratus* feeding at 15°C and 35°C showing little difference in the duration of tongue projection (P indicates peak projection) yet pronounced differences in the duration of tongue retraction (ending at the frame marked R). The end of tongue retraction for the 15°C feeding occurs after the final frame shown. Sequences begin at the start of tongue projection at time 0 in the top of the left column. The sequences progress downward from the top of the left column and continue at the top of the right column. The time step in the left column is 5.67 msec and 32 msec in the right column.

and Rome, 1996). Peak isometric muscle tension typically exhibits $Q_{10}$ values of 1.0–1.2 (Rome, 1990), and the load–strain relationship of collagenous tendon exhibits a $Q_{10}$ of 1 across the large physiological temperature range of 0°C–37°C (Rigby et al., 1959).

Studies of other animal systems that use elastic structures to power movements lend additional support to the conclusion that elastic-recoil mechanisms confer relative
thermal independence compared with movements that rely on muscle rate properties. Among ballistic systems, jumping in frogs is powered partially by recoil of in-series elastic elements that supplements muscle power output (Roberts and Marsh, 2003). Frog jumping appears to show a reduced effect of temperature on performance (Rome, 1990), but it is not liberated to the same extent as tongue projection in chameleons, probably because elastic recoil and muscle contraction overlap temporally (Roberts and Marsh, 2003). Among cyclical systems, wingbeat frequency of beetles shows very low temperature sensitivity, apparently because frequency is determined by the resonant frequency of the flight system, which is dictated by its physical properties rather than by its muscle rate properties (Oertli, 1989).

Because the mechanical properties of elastic tissues are known to have low thermal sensitivity (Rigby et al., 1959; Alexander, 1966; Denny and Miller, 2006), temperature manipulation may be a valuable methodological approach to test for the presence or prevalence of elastic recoil in powering movements. Elastic recoil is implicated if performance of a movement is maintained at a high level over a wide range of body temperatures. Our findings on chameleons thus serve as independent validation for the presence of an elastic-recoil mechanism in tongue projection.

Finally, chameleons have increased the thermal breadth of their feeding mechanism by decreasing the temperature effects on performance of ballistic tongue projection and thus are able to feed at very low $T_b$ (Burrage, 1973; Hebrard et al., 1982; Reilly, 1982; Bennett, 2004; Andrews, 2008). This ability likely grants them an expanded thermal niche, allowing them to feed early in the morning when effective thermoregulation is not possible (Reilly, 1982) and enabling them to be active over a
wider temperature range than other sympatric lizard species (Hebrard et al., 1982). The ability of chameleons to forage at low temperatures also may reduce thermoregulatory behavior and its ecological costs (Huey, 1974). Other ectothermic organisms that use explosive, ballistic movements for prey capture or locomotion across a range of temperatures may similarly benefit from the relative thermal independence of elastic recoil mechanisms.

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References Cited


CHAPTER THREE: THERMAL EFFECTS ON MOTOR CONTROL AND IN VITRO MUSCLE DYNAMICS OF THE BALLISTIC TONGUE APPARATUS IN CHAMELEONS

Abstract

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

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2 Portions of these results have been previously published (Anderson and Deban, 2012) and are utilized with permission of the publisher. Christopher V. Anderson and Stephen M. Deban designed the research and contributed analytic tools. The research was performed and analyzed by Christopher V. Anderson.
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.

**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. \( Q_{10} \geq 1.5 \)) (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 stores elastic energy in, collagen sheaths located between the m. accelerator linguae and entoglossal process as it contracts around and lengths 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.
Electromyography

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 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.
Figure 3.1. 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. 3.1). 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.

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
Figure 3.2. 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.

Kinematic events were measured (Fig. 3.2). 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 linguæ 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 linguæ, 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:

$$Power\ ratio = \frac{W_{max}}{(V_{max} \times P_o)}.$$ 

**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 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:

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

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 3.1). 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
Table 3.1. Minimum and maximum values of kinematic, electromyographic and contractile variables in *Chamaeleo calyptratus*.

<table>
<thead>
<tr>
<th>Variables</th>
<th>15 ± 1°C</th>
<th>25 ± 1°C</th>
<th>35 ± 1°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kinematic Characteristics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Projection Distance (cm)</td>
<td>Min/Max 5.04/15.62</td>
<td>Min/Max 5.19/18.56</td>
<td>Min/Max 6.25/13.00</td>
</tr>
<tr>
<td>Kinematic Timing (s)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Max. projection time relative to projection onset</td>
<td>0.013/0.048 7 (5; 1-2)</td>
<td>0.010/0.051 15 (5; 1-4)</td>
<td>0.011/0.034 5 (4; 1-2)</td>
</tr>
<tr>
<td>Tongue Retracted time relative to projection onset</td>
<td>0.676/1.013 7 (5; 1-2)</td>
<td>0.255/1.090 15 (5; 1-4)</td>
<td>0.214/0.283 5 (4; 1-2)</td>
</tr>
<tr>
<td>Muscle Activity (s)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACC onset to tongue projection onset duration</td>
<td>0.162/0.372 7 (5; 1-2)</td>
<td>0.111/0.267 14 (5; 1-4)</td>
<td>0.015/0.101 5 (4; 1-2)</td>
</tr>
<tr>
<td>ACC max. amplitude to tongue projection duration</td>
<td>0.000/0.086 7 (5; 1-2)</td>
<td>0.000/0.103 14 (5; 1-4)</td>
<td>0.000/0.051 5 (4; 1-2)</td>
</tr>
<tr>
<td>HG onset to tongue projection onset duration</td>
<td>0.010/0.046 5 (4; 1-2)</td>
<td>-0.006/0.225 7 (5; 1-2)</td>
<td>0.019/0.060 4 (4; 1-1)</td>
</tr>
<tr>
<td>HG onset to max. tongue projection duration</td>
<td>0.032/0.093 5 (4; 1-2)</td>
<td>0.036/0.261 7 (5; 1-2)</td>
<td>0.049/0.091 4 (4; 1-1)</td>
</tr>
<tr>
<td>HG max. amplitude to max. projection duration</td>
<td>0.015/0.048 5 (4; 1-2)</td>
<td>0.001/0.076 7 (5; 1-2)</td>
<td>0.012/0.041 4 (4; 1-1)</td>
</tr>
<tr>
<td>ACC Contractile Variables</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak Isometric Force - $P_0$ (N)</td>
<td>0.66/4.84 33 (16; 2-3)</td>
<td>1.36/6.36 33 (16; 2-3)</td>
<td>0.37/6.01 32 (16; 1-3)</td>
</tr>
<tr>
<td>Time to 90% $P_0$ (s)</td>
<td>0.115/0.211 33 (16; 2-3)</td>
<td>0.056/0.156 33 (16; 2-3)</td>
<td>0.031/0.256 32 (16; 1-3)</td>
</tr>
<tr>
<td>Rate of Force Development (N s$^{-1}$)</td>
<td>5.16/31.79 33 (16; 2-3)</td>
<td>10.83/80.56 33 (16; 2-3)</td>
<td>2.87/108.17 32 (16; 1-3)</td>
</tr>
<tr>
<td>Electromechanical Delay (s)</td>
<td>0.007/0.018 33 (16; 2-3)</td>
<td>0.003/0.008 33 (16; 2-3)</td>
<td>0.002/0.012 32 (16; 1-3)</td>
</tr>
<tr>
<td>HG Contractile Variables</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak Isometric Force - $P_0$ (N)</td>
<td>0.03/0.31 12</td>
<td>0.03/0.53 12</td>
<td>0.02/0.50 11</td>
</tr>
<tr>
<td>Time to 90% $P_0$ (s)</td>
<td>0.132/0.426 11</td>
<td>0.099/0.282 11</td>
<td>0.081/0.332 10</td>
</tr>
<tr>
<td>Rate of Force Development (N s$^{-1}$)</td>
<td>0.17/1.45 11</td>
<td>0.33/2.36 11</td>
<td>0.23/4.19 10</td>
</tr>
<tr>
<td>Electromechanical Delay (s)</td>
<td>0.016/0.047 11</td>
<td>0.010/0.024 11</td>
<td>0.005/0.019 10</td>
</tr>
<tr>
<td>Specific Tension (N cm$^{-2}$)</td>
<td>1.76/7.56 12</td>
<td>1.74/10.67 12</td>
<td>1.08/10.09 11</td>
</tr>
<tr>
<td>Peak Contractile Velocity ($L_0$ s$^{-1}$)</td>
<td>0.21/3.25 12</td>
<td>1.42/4.74 12</td>
<td>0.44/3.72 10</td>
</tr>
<tr>
<td>Peak Contractile Velocity (m s$^{-1}$)</td>
<td>0.005/0.067 12</td>
<td>0.036/0.128 12</td>
<td>0.011/0.101 10</td>
</tr>
<tr>
<td>Peak Power (W)</td>
<td>4.93x10$^3$/ 12</td>
<td>1.30x10$^3$/ 12</td>
<td>2.95x10$^3$/ 10</td>
</tr>
<tr>
<td>Peak Power (W kg$^{-1}$ HG segment mass)</td>
<td>1.36x10$^3$/ 12</td>
<td>5.04x10$^3$/ 12</td>
<td>2.67x10$^3$/ 10</td>
</tr>
<tr>
<td>Power Ratio</td>
<td>0.11/18.08 12</td>
<td>2.90/55.69 12</td>
<td>0.66/35.55 10</td>
</tr>
<tr>
<td>ACC, m. accelerator linguae; HG, m. hyoglossus; $L_0$, muscle length.</td>
<td></td>
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</tbody>
</table>

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.
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 3.1), 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 3.2, Figs 3.3, 3.4): latency between the onset of activity for the m. accelerator linguae and the onset of tongue projection (1/Q₁₀ = 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 3.3, Fig. 3.4). Further, across the 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 3.2, 3.3): the EMG
Table 3.2. Results of ANCOVA examining effects on electromyographic amplitude and duration variables over the 14-26°C range in Chamaeleo calyptratus.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Individual P-value</th>
<th>Temp. P-value</th>
<th>Projection Distance P-value</th>
<th>Temperature Slope</th>
<th>Q&lt;sub&gt;10&lt;/sub&gt;</th>
<th>1/Q&lt;sub&gt;10&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACC (onset to projection) r.m.s.</td>
<td>0.0162</td>
<td>0.0337</td>
<td>0.5126</td>
<td>0.0741</td>
<td>5.51</td>
<td>0.18</td>
</tr>
<tr>
<td>ACC (onset to projection) integrated area/duration</td>
<td>0.0100</td>
<td>0.0542</td>
<td>0.3714</td>
<td>0.0643</td>
<td>4.40</td>
<td>0.23</td>
</tr>
<tr>
<td>ACC (onset to projection) r.m.s. max. amplitude</td>
<td>0.0104</td>
<td>0.1248</td>
<td>0.4986</td>
<td>0.0530</td>
<td>3.39</td>
<td>0.30</td>
</tr>
<tr>
<td>ACC onset to tongue projection onset duration</td>
<td>0.0016</td>
<td><strong>0.0005</strong></td>
<td>0.2391</td>
<td>-0.0429</td>
<td>0.37</td>
<td><strong>2.69</strong></td>
</tr>
<tr>
<td>ACC max. amplitude to tongue projection onset duration</td>
<td>0.8487</td>
<td>0.2181</td>
<td>0.6862</td>
<td>0.0763</td>
<td>5.79</td>
<td>0.17</td>
</tr>
<tr>
<td>HG (onset to max. projection) r.m.s.</td>
<td>0.1277</td>
<td>0.1398</td>
<td>0.1023</td>
<td>0.0709</td>
<td>5.12</td>
<td>0.20</td>
</tr>
<tr>
<td>HG (onset to max. projection) integrated area/duration</td>
<td>0.0922</td>
<td>0.1233</td>
<td>0.1115</td>
<td>0.0721</td>
<td>5.26</td>
<td>0.19</td>
</tr>
<tr>
<td>HG (onset to max. projection) r.m.s. max. amplitude</td>
<td>0.1449</td>
<td>0.1212</td>
<td>0.0904</td>
<td>0.0745</td>
<td>5.56</td>
<td>0.18</td>
</tr>
<tr>
<td>HG onset to max. tongue projection duration</td>
<td>0.3362</td>
<td>0.7306</td>
<td>0.8221</td>
<td>0.0062</td>
<td>1.15</td>
<td>0.87</td>
</tr>
<tr>
<td>HG max. amplitude to max. tongue projection duration</td>
<td>0.4330</td>
<td>0.3291</td>
<td>0.7438</td>
<td>-0.0402</td>
<td>0.40</td>
<td>2.52</td>
</tr>
<tr>
<td>ACC (onset to projection) r.m.s. / HG (onset to max. projection) r.m.s.</td>
<td>0.0923</td>
<td>0.3788</td>
<td>0.0766</td>
<td>-0.0409</td>
<td>2.56</td>
<td>0.39</td>
</tr>
<tr>
<td>ACC onset to tongue projection onset / HG onset to max. tongue projection duration</td>
<td>0.3741</td>
<td>0.0325</td>
<td>0.4403</td>
<td>-0.0611</td>
<td>0.24</td>
<td>4.08</td>
</tr>
</tbody>
</table>

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<sub>10</sub> 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 Hochberg, 1995). Bold Q<sub>10</sub> values indicate significant temperature effects.
Figure 3.3. 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.

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. 3.4), 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
Figure 3.4. 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 the m. accelerator linguae onset to projection duration across the 14-26°C range depicts a significant effect of temperature (see Tables 3.2, 3.3 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.

higher temperatures (Fig. 3.4). Neither EMG ratio in the lower range exhibited a significant effect of temperature.
Table 3.3. Results of ANCOVA examining effects on electromyographic amplitude and duration variables over the 24-36°C range in *Chamaeleo calyptratus*.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Individual P-value</th>
<th>Temp. P-value</th>
<th>Projection Distance P-value</th>
<th>Temperature Slope</th>
<th>Q₁₀</th>
<th>1/Q₁₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACC (onset to projection) r.m.s.</td>
<td>0.1067</td>
<td>0.5776</td>
<td>0.2111</td>
<td>-0.0250</td>
<td>0.56</td>
<td>1.78</td>
</tr>
<tr>
<td>ACC (onset to projection) integrated area/duration</td>
<td>0.0636</td>
<td>0.4767</td>
<td>0.0972</td>
<td>-0.0325</td>
<td>0.47</td>
<td>2.11</td>
</tr>
<tr>
<td>ACC (onset to projection) r.m.s. max. amplitude</td>
<td>0.0708</td>
<td>0.4991</td>
<td>0.2384</td>
<td>-0.0293</td>
<td>0.51</td>
<td>1.96</td>
</tr>
<tr>
<td>ACC onset to tongue projection onset duration</td>
<td><strong>0.0007</strong></td>
<td></td>
<td>0.2852</td>
<td>-0.0334</td>
<td>0.46</td>
<td>2.16</td>
</tr>
<tr>
<td>ACC max. amplitude to tongue projection onset duration</td>
<td>0.2972</td>
<td>0.4837</td>
<td>0.1741</td>
<td>-0.0474</td>
<td>0.34</td>
<td>2.98</td>
</tr>
<tr>
<td>HG (onset to max. projection) r.m.s.</td>
<td>0.2402</td>
<td>0.1514</td>
<td>0.2794</td>
<td>-0.0736</td>
<td>0.18</td>
<td>5.45</td>
</tr>
<tr>
<td>HG (onset to max. projection) integrated area/duration</td>
<td>0.1878</td>
<td>0.1019</td>
<td>0.2594</td>
<td>-0.0821</td>
<td>0.15</td>
<td>6.62</td>
</tr>
<tr>
<td>HG (onset to max. projection) r.m.s. max. amplitude</td>
<td>0.2751</td>
<td>0.1751</td>
<td>0.3182</td>
<td>-0.0682</td>
<td>0.21</td>
<td>4.81</td>
</tr>
<tr>
<td>HG onset to max. tongue projection duration</td>
<td>0.0389</td>
<td>0.3928</td>
<td>0.1973</td>
<td>0.0112</td>
<td>1.29</td>
<td>0.77</td>
</tr>
<tr>
<td>HG max. amplitude to max. tongue projection duration</td>
<td>0.1606</td>
<td>0.1991</td>
<td>0.7331</td>
<td>0.0456</td>
<td>2.86</td>
<td>0.35</td>
</tr>
<tr>
<td>ACC (onset to projection) r.m.s. / HG (onset to max. projection) r.m.s.</td>
<td><strong>0.0002</strong></td>
<td><strong>0.0031</strong></td>
<td><strong>0.0014</strong></td>
<td>0.0373</td>
<td><strong>2.36</strong></td>
<td><strong>0.42</strong></td>
</tr>
<tr>
<td>ACC onset to tongue projection onset / HG onset to max. tongue projection duration</td>
<td><strong>0.0012</strong></td>
<td><strong>0.0039</strong></td>
<td>0.2265</td>
<td>-0.0367</td>
<td>0.43</td>
<td><strong>2.33</strong></td>
</tr>
</tbody>
</table>

*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 *Q₁₀* 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 *Q₁₀* values indicate significant temperature effects.
Over the entire 15–35°C range, the m. accelerator linguae produced a $P_0$ of elongation of 0.37–6.36 N (Table 3.1) 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 3.1) 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$^{-1}$. $V_{\text{max}}$ 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$^{-1}$. $W_{\text{max}}$ 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 3.4, Figs 3.5–3.8): $P_0$ of the m. accelerator linguae, time to 90% $P_0$ of the m. accelerator linguae, rate of force development of the m. accelerator linguae, electromechanical delay of the m. accelerator linguae, $P_0$ of the m. hyoglossus, time to 90% $P_0$ of the m. hyoglossus, rate of force development of the m. hyoglossus, electromechanical delay of the m. hyoglossus, $V_{\text{max}}$ of the m. hyoglossus, $W_{\text{max}}$ 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 3.4, Fig. 3.5): $P_0$ of the m. accelerator linguae, time to 90% $P_0$ of the m. accelerator linguae, electromechanical delay of the m. accelerator linguae,
Table 3.4. Results of ANCOVA examining effects on muscle contractile variables in *Chamaeleo calyptratus*.

<table>
<thead>
<tr>
<th></th>
<th>Individual P-value</th>
<th>Temperature P-value</th>
<th>Temperature Slope</th>
<th>Temperature Q&lt;sub&gt;10&lt;/sub&gt;</th>
<th>1/Q&lt;sub&gt;10&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>14-26°C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak Isometric Force (P&lt;sub&gt;0&lt;/sub&gt;)</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.0128</td>
<td>1.34</td>
<td>0.75</td>
</tr>
<tr>
<td>Time to 90% P&lt;sub&gt;0&lt;/sub&gt;</td>
<td>0.0011</td>
<td>&lt;0.0001</td>
<td>-0.0264</td>
<td>0.55</td>
<td>1.83</td>
</tr>
<tr>
<td>Rate of Force Development</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.0391</td>
<td>2.46</td>
<td>0.41</td>
</tr>
<tr>
<td>Electromechanical Delay</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>-0.0265</td>
<td>0.54</td>
<td>1.84</td>
</tr>
<tr>
<td>HG</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak Isometric Force (P&lt;sub&gt;0&lt;/sub&gt;)</td>
<td>&lt;0.0001</td>
<td>0.0007</td>
<td>0.0107</td>
<td>1.28</td>
<td>0.78</td>
</tr>
<tr>
<td>Time to 90% P&lt;sub&gt;0&lt;/sub&gt;</td>
<td>0.0753</td>
<td>0.0051</td>
<td>-0.0169</td>
<td>0.68</td>
<td>1.48</td>
</tr>
<tr>
<td>Rate of Force Development</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.0287</td>
<td>1.94</td>
<td>0.52</td>
</tr>
<tr>
<td>Electromechanical Delay</td>
<td>0.0006</td>
<td>&lt;0.0001</td>
<td>-0.0253</td>
<td>0.56</td>
<td>1.79</td>
</tr>
<tr>
<td>Peak Contractile Velocity (V&lt;sub&gt;max&lt;/sub&gt;)</td>
<td>0.0561</td>
<td>0.0052</td>
<td>0.0310</td>
<td>2.04</td>
<td>0.49</td>
</tr>
<tr>
<td>Peak Power (W&lt;sub&gt;max&lt;/sub&gt;)</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.0758</td>
<td>5.72</td>
<td>0.17</td>
</tr>
<tr>
<td>Power Ratio [W&lt;sub&gt;max&lt;/sub&gt;/V&lt;sub&gt;max&lt;/sub&gt;x P&lt;sub&gt;0&lt;/sub&gt;]</td>
<td>0.0572</td>
<td>0.0004</td>
<td>0.0341</td>
<td>2.19</td>
<td>0.46</td>
</tr>
<tr>
<td>24-36°C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak Isometric Force (P&lt;sub&gt;0&lt;/sub&gt;)</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>-0.0150</td>
<td>0.71</td>
<td>1.41</td>
</tr>
<tr>
<td>Time to 90% P&lt;sub&gt;0&lt;/sub&gt;</td>
<td>&lt;0.0001</td>
<td>0.0091</td>
<td>-0.0057</td>
<td>0.88</td>
<td>1.14</td>
</tr>
<tr>
<td>Rate of Force Development</td>
<td>&lt;0.0001</td>
<td>0.0442</td>
<td>-0.0093</td>
<td>0.81</td>
<td>1.24</td>
</tr>
<tr>
<td>Electromechanical Delay</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>-0.0146</td>
<td>0.71</td>
<td>1.40</td>
</tr>
<tr>
<td>HG</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak Isometric Force (P&lt;sub&gt;0&lt;/sub&gt;)</td>
<td>&lt;0.0001</td>
<td>0.0008</td>
<td>-0.0123</td>
<td>0.75</td>
<td>1.33</td>
</tr>
<tr>
<td>Time to 90% P&lt;sub&gt;0&lt;/sub&gt;</td>
<td>0.0786</td>
<td>0.0139</td>
<td>-0.0172</td>
<td>0.67</td>
<td>1.48</td>
</tr>
<tr>
<td>Rate of Force Development</td>
<td>0.0072</td>
<td>0.4362</td>
<td>0.0057</td>
<td>1.14</td>
<td>0.88</td>
</tr>
<tr>
<td>Electromechanical Delay</td>
<td>0.0094</td>
<td>0.0147</td>
<td>-0.0119</td>
<td>0.76</td>
<td>1.32</td>
</tr>
<tr>
<td>Peak Contractile Velocity (V&lt;sub&gt;max&lt;/sub&gt;)</td>
<td>0.0382</td>
<td>0.0711</td>
<td>-0.0138</td>
<td>0.73</td>
<td>1.37</td>
</tr>
<tr>
<td>Peak Power (W&lt;sub&gt;max&lt;/sub&gt;)</td>
<td>0.0002</td>
<td>0.0663</td>
<td>-0.0156</td>
<td>0.70</td>
<td>1.43</td>
</tr>
<tr>
<td>Power Ratio [W&lt;sub&gt;max&lt;/sub&gt;/V&lt;sub&gt;max&lt;/sub&gt;x P&lt;sub&gt;0&lt;/sub&gt;]</td>
<td>0.0285</td>
<td>0.0251</td>
<td>0.0115</td>
<td>1.30</td>
<td>0.77</td>
</tr>
</tbody>
</table>

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<sub>10</sub> values were calculated. Bold P-values indicate significance levels adjusted for false discovery rate (Benjamini and Hochberg, 1995). Bold Q<sub>10</sub> values indicate significant temperature effects.

linguae, P<sub>0</sub> of the m. hyoglossus, time to 90% P<sub>0</sub> 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 3.4, Figs 3.5, 3.6, 3.8).
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. 3.3) (Wainwright and Bennett, 1992a;
Figure 3.6. 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. 3.4, 3.5.

Herrel et al., 2000), during which time the m. accelerator linguae loads elastic structures with strain energy (de Groot and 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
Figure 3.7. Original data from isometric and isotonic contraction of the m. hyoglossus at 15ºC (left) and 25ºC (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 25ºC compared with 15ºC. Further, note the increased excursion rates at 25ºC compared with 15ºC for similar force contractions. All traces are shown on the same time scale.

the onset of tongue projection (Table 3.1) 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).
Figure 3.8. 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 Hills equation fitted to these data points (Hill, 1938) (see Materials and Methods and Fig. 3.7 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.

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. 3.3), 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 3.1).

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 linguæ or m. hyoglossus showed a significant effect of temperature.
across either temperature range (Tables 3.2, 3.3). 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. 3.4). 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 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 3.2, 3.3, Fig. 3.4). 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 3.3, Fig. 3.4), as compared with the 14–26°C range (Table 3.2, Fig. 3.4), 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 3.2, 3.3, Fig. 3.4), 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 N s$^{-1}$. Force–velocity relationships for the m. hyoglossus calculated an average $V_{\text{max}}$ of 2.56 $L_0$ s$^{-1}$ (0.06 m s$^{-1}$) and an average mass-specific $W_{\text{max}}$ of 13.17 W kg$^{-1}$. Further, an average power ratio for the m. hyoglossus of
0.13 was calculated. These values are 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 \(V_{\text{max}}\) 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 \(V_{\text{max}}\) 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 P₀ 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 Vₘₐₓ of *C. calyptratus* m. hyoglossus is similar to that of *C. senegalensis* m. iliofibularis [2.5 L₀ s⁻¹ (Abu-Ghalyun et al., 1988)]. The Vₘₐₓ 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 3.1), 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 3.4, Fig. 3.5). Similarly, all dynamic contractile properties of the m. hyoglossus were influenced by temperature in the 15–25°C range (Table 3.4, Figs. 3.5–3.8), whereas the rate of force development, Vₘₐₓ and Wₘₐₓ, 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 3.4, Fig. 3.5). 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 3.4). The curvature of the relationship was reduced at higher temperatures, resulting in higher peak power (Fig. 3.8) and power ratios (0.082–0.250; Table 3.1). 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, 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.

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.
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CHAPTER FOUR: THERMAL EFFECTS ON THE PERFORMANCE OF BALLISTIC PREY CAPTURE IN SOUTH AFRICAN DWARF CHAMELEONS (*BRADYPODION*) LIVING ALONG AN ELEVATION AND TEMPERATURE GRADIENT

Abstract

The effect of temperature on physiological rate properties is a significant challenge to organisms living in variable environments. Whole-organism performance is strongly affected by temperature through its effect on muscle contractile dynamics, but movements powered by the recoil of preloaded elastic elements are liberated from much of the performance decline experienced by muscle-powered movements at low temperature. Strong thermal effects in muscle-powered movements at low temperature, however, can be mitigated by adaptation and acclimation to low muscle temperature. We compared the effect of temperature on an elastically powered movement – tongue projection in chameleons – to that on a muscle-powered movement – tongue retraction in chameleons – in species living along a strong elevation and temperature gradient. We tested the hypothesis that movements that benefit from lower thermal dependence would vary less between different habitats than movements that experience strong thermal effects. We found that tongue projection performance of our three species far exceeded that previously reported in other studies, due to scaling effects and a negative relationship of both acceleration and power output with body size. Further, tongue projection performance was maintained across temperatures to a higher degree than tongue
retraction performance in all three species, similar to previous studies on other species. However, despite the thermal robustness of tongue projection performance, variation between the species in both tongue projection and tongue retraction performance was observed at different experimental temperatures. While this variation was not tied to altitudinal or mean temperature differences between the habitats of each species, it illustrates that despite differences in how temperature affects the performance of these different movements, both elastic-recoil-powered movements and muscle-powered movements exhibit differences in their thermal performance curves among populations.

**Introduction**

The ability for an organism to perform optimally in their environment is advantageous as suboptimal performance can have negative fitness consequences (Arnold, 1983). Selective pressures may then be expected to drive behaviors and physiological processes that are affected by environmental conditions to fit an organism’s local environment (Angilletta et al., 2002). The degree to which these behaviors and physiological processes are affected by environmental conditions, however, may result in differences in the intensity of the selective pressures acting on them. Here, we examine two movement types—elastically powered tongue projection and muscle-powered tongue retraction in chameleons—that are affected by environmental temperature to differing degrees across a range of local environments for patterns that may suggest differences in the selective pressures acting on these movement types.

Variable and changing environmental conditions impose substantial challenges on organisms through their effects on diverse physiological and biochemical processes,
which in turn can affect whole-organism performance. For instance, because an ectotherm’s body temperature is directly affected by environmental conditions, the effect of temperature on muscle contractile dynamics in ectotherms can have a significant effect on their ability to perform critical locomotor and feeding behaviors (Huey and Stevenson, 1979; Bennett, 1985; Huey and Bennett, 1987; Rome, 1990; Lutz and Rome, 1996; Herrel et al., 2007). Performance of dynamic behaviors – such as sprint speed, swimming velocity and jumping distance – declines by more than 33% with a 10°C drop in temperature [i.e. temperature coefficient ($Q_{10}$) ≥ 1.5] (Huey and Bennett, 1987; Rome, 1990). This decline is the result of strong thermal effects on muscle rate properties, such as peak contractile velocity, the rate of tension development and power output, which tend to drop by at least half with each 10°C drop (i.e. $Q_{10}$ ≥ 2) (Bennett, 1984; Bennett, 1985). Adaptation on evolutionary timescales and acclimation on organismal timescales, however, are known to partially, but not completely, mitigate strong thermal effects on muscle-powered movements at low temperature. For instance, nocturnal geckos, which unlike diurnal lizards do not benefit from basking opportunities and experience lower ambient temperatures, are known to outperform diurnal lizards during low temperature locomotion trials (Autumn et al., 1994). Similarly, northern tree frog populations outperform southern tree frogs in low temperature jumping performance (John-Alder et al., 1988). Finally, swimming performance in carp (Rome et al., 1985; Rome, 1990) and swimming burst speed in plethodontid salamanders (Marvin, 2003 a,b) at low temperature increases following acclimation to the lower temperatures.

In contrast to muscle-powered movements, the performance of movements powered by elastic recoil is thermally robust. Performance of tongue projection in
chameleons and salamanders, and ballistic mouth opening in toads and frogs, for instance, exhibit $Q_{10}$ values from 1.0 to 1.4 (Anderson and Deban, 2010; Deban and Lappin, 2011; Deban and Richardson, 2011; Sandusky and Deban, 2012). While muscles associated with these movements are themselves subject to typical thermal effects on muscle contractile dynamics (Deban and Lappin, 2011; Anderson and Deban, 2012), elastic tissues exhibit relative thermal independence of their mechanical properties, with $Q_{10}$ values in the 1.0-1.2 range (Rigby et al., 1959; Alexander, 1966; Denny and Miller, 2006).

Our understanding of how the performance of thermally robust elastic-recoil-powered movements at low temperature is affected by adaptation or acclimation, however, is not understood. As a result of the reduced thermal sensitivity, however, fitness consequences from elastic-recoil-powered movements not tightly fitting the local environment may not be as strong as in muscle-powered movements. This could result in reduced selection pressure on the thermal performance of elastic-recoil-powered movements and less variation between closely related populations living in different environments.

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), as well as the morphology of the chameleon hyobranchial apparatus (Houston, 1828; Gnanamuthu, 1930; Bell, 1989; Schwenk, 2000; Herrel et al., 2001; de Groot and van Leeuwen, 2004; Anderson et al., 2012) are described in detail elsewhere. Briefly, the recoil of preloaded elastic elements powers tongue projection (de Groot and van Leeuwen, 2004), while tongue retraction is powered via skeletal muscle contraction directly (Wainwright and Bennett, 1992a; Herrel et al., 2009). Collagen intralingual sheaths are located between the parallel-sided entoglossal process of the
hyobranchial apparatus and the tongue projector muscle, the m. accelerator linguae. The m. accelerator linguae is cylindrically shaped with a central lumen encompassing the entoglossal process while at rest. As the m. accelerator linguae contracts around and lengthens along the entoglossal process, it stretches, and thus stores elastic energy in the intralingual sheaths. As the m. accelerator linguae extends over the anterior tapered tip of the entoglossal process, the collagen fibers of the intralingual sheaths recoil rapidly to their resting lengths, powering the majority of tongue projection. Following projection, the paired tongue retractor muscles, the m. hyoglossus, is directly responsible for retracting the tongue onto the entoglossal process.

A variety of dwarf chameleon taxa (Bradypodion sp.) live across South Africa in a broad range of habitats (Tolley and Burger, 2007; Tilbury, 2010). Included in this diversity, three taxa, B. melanocephalum, B. thamnobates, and the so-called “Emerald Dwarf Chameleon” (sensu Tolley and Burger, 2007; “B. sp. Giants Castle” sensu Tolley et al., 2004; “B. sp. 8” sensu Tolley et al., 2008; henceforth B. sp. “emerald”), live over a nearly 2000m elevation range with a strong associated temperature gradient. Phylogenetic analyses have revealed that these three taxa represent a recent radiation within the genus and may be so recent that it lacks the genetic divergence in mitochondrial markers expected at the species level (Tolley et al., 2004; Tolley et al., 2008; da Silva and Tolley, unpublished data), making them ideal for interspecific comparisons without deep phylogenetic effects. Further, the local environments of these three taxa range from warm summer and moderate winter temperatures on the coast where B. melanocephalum occurs, to moderate-to-cool summer temperatures and cold winters with snow in the foothills of the Drakensberg Mountains where B. sp. “emerald”
occurs, thus providing an ample range of environmental temperatures for thermal specialization to occur.

We examined thermal effects on tongue projection and tongue retraction in *B. melanoccephalum*, *B. thamnobates*, and *B*. sp. “emerald” to better understand patterns of thermal specialization in movements powered by elastic recoil compared to those powered by muscle contraction directly. We hypothesized that the thermal robustness of elastic-recoil-powered mechanisms will cause performance of tongue projection at different temperatures to differ less than muscle-powered tongue retraction, when comparing species living in different thermal environments. Specifically, we predicted that performance declines for elastic-recoil-powered tongue projection at low temperature will not vary between species along an environmental temperature gradient (i.e., thermal effects will be the same for all species). Conversely, performance declines for muscle powered tongue retraction at low temperature are expected to be lower in chameleons from colder environments along an environmental temperature gradient. To test these hypotheses, we recorded high-speed image sequences from feeding events across a range of temperatures (14-36°C) in all three species.

**Materials and Methods**

*Specimens and Localities*

*Bradypodion melanoccephalum*, *B. thamnobates*, and *B*. sp. “emerald” were chosen for this study because they are closely related (Tolley et al., 2004; Tolley et al., 2008) and occur over a strong elevation gradient of approximately 2000m in elevation, corresponding with a robust temperature gradient (Fig. 4.1; Schulze, 1997). Ten
individuals each of B. melanocephalum, B. thamnobates and B. sp. “emerald” were collected by hand during nighttime surveys in KwaZulu-Natal Province. Collection took place over two consecutive nights in February 2012. During collection, GPS coordinates and elevation of the collection location were recorded for every specimen.

Bradypodion melanocephalum specimens were collected from Roosfontein Nature Reserve. This area is a largely open grassland hillside habitat east of Durban at an elevation of approximately 140m above sea level. The Durban area is characterized by warm summer and moderate winter temperatures, with average daily mean summer temperatures of approximately 24ºC and an average temperature range of 21-28ºC (in February), and average daily mean winter temperatures of approximately 17ºC and an average temperature range of 11-22ºC (in August) (Fig. 4.1; Schulze, 1997).

Bradypodion thamnobates specimens were collected from Howick in the Natal Midlands. This species is typically found in closed or thick canopy vegetation (Tolley and Burger, 2007), however these specimens were collected along the road in mature shrubs and trees at approximately 1040m in elevation. The Natal Midlands are characterized by a moderate summer and cold winter temperatures, with average daily mean summer temperatures of approximately 20ºC and an average temperature range of 16-26ºC (in February), and average daily mean winter temperatures of approximately 13ºC and an average temperature range of 6-20ºC (in August) (Fig. 4.1; Schulze, 1997).

Bradypodion sp. “emerald” specimens were collected from Kamberg Nature Reserve in the foothills of uKhahlamba-Drakensburg Park. Specimens were collected within and along the border between disturbed Afromontane forests and mixed ouhout (Leucosidae sericea) alpine veld habitat at approximately 1710m in elevation. The
Figure 4.1. Elevation (A), and August (B) and February (C) daily mean temperature GIS maps for KwaZulu-Natal Province, South Africa showing collection sites for *B. melanocephalum* (1), *B. thamnobates* (2) and *B. sp. “emerald”* (3) specimens. Circles on maps represent collection sites for each species, pictured at right. Temperature maps and climate data from Schulze (1997).

Drakensburg foothills are characterized by moderate-to-cool summer temperatures and cold winters with occasional snow or frost, with average daily mean summer temperatures of approximately 18°C and an average temperature range of 13-24°C (in
February), and average daily mean winter temperatures of approximately 11°C and an average temperature range of 3-14°C (in August) (Fig. 4.1; Schulze, 1997).

Following collection, specimens were flown from Durban to Cape Town in the Western Cape Province, where they were transported to the South African National Biodiversity Institute for housing and feeding trials. All specimens were kept in intraspecific pairs in mesh enclosures filled with fresh foliage. Enclosures were kept inside an environmental chamber where ambient temperatures were maintained between 20 and 24°C. Artificial lighting was provided on a 12 hour light cycle with lights creating a temperature gradient inside the enclosures. Hydration was maintained via bi-daily misting and specimens were fed a diet of gut-loaded crickets.

All feeding trials were collected within two weeks of initial collection. Following feeding trials, all specimens were flown back to KwaZulu-Natal Province and each specimen was released at the exact site of capture.

Feeding Experiments

All chameleons were imaged at 3 kHz with a Photron Fastcam 1024 PCI camera as they fed on crickets. Chameleons were placed on a wooden dowel oriented parallel to the image plane of the camera. Crickets were placed on a square of insect screen suspended by thread in front of the dowel to create a “cricket trapeze”, which allowed the chameleon’s tongue to complete its trajectory unimpeded (Anderson and Deban, 2010).

Feeding trials were conducted across a range of ambient temperatures within an environmental chamber. Feeding trials for each individual were conducted in a randomly assigned temperature sequence. Temperature sequences consisted of eight temperature
blocks with each experimental temperature (15°C, 25°C, 30°C and 35°C) being visited once in the first four blocks and once again in the second four (e.g., 25º-15º-30º-35º-15º-35º-25º-30º, 35º-15º-25º-30º-25º-35º-15º-30º, etc.). For each individual, five feeding events were recorded at each experimental temperature block prior to proceeding to the next temperature in their temperature sequence.

Chameleons were acclimated to the experimental temperature within the environmental chamber for a period of at least 1 h prior to feeding trials. To minimize elevation of body temperatures through light-source radiation, supplemental lighting was provided by a LED light panel composed of 36 1 watt white (5500 K color temperature) LEDs. Body temperature was verified orally using a calibrated Sixth Sense LT300 infrared thermometer (± 1°C accuracy) following every feeding event and exact body temperature for each feeding was recorded.

Kinematic Analysis

All feeding events were scale-calibrated using known distances between points in each feeding event. Tongue projection distance was computed using NIH ImageJ software as the distance from the tongue tip to the dentary tip. Tongue projection and retraction performance was quantified by calculating peak velocity (m s⁻¹), peak acceleration (m s⁻²) and peak mass-specific power (W kg⁻¹) for both tongue projection and retraction for each feeding sequence. The x,y coordinates of the tip of the tongue on each frame throughout the tongue projection sequence were recorded using ImageJ software. A quintic spline was fitted to the resultant position trace of the tongue using a custom script including the P-spline package of R statistical software and smoothed to
remove secondary oscillation artifacts from the first and second derivatives of position. From these smoothed position data, instantaneous velocity (m s$^{-1}$) and acceleration (m s$^{-2}$) traces (i.e., the first and second derivatives of the position) were calculated. For tongue retraction, coordinates of four positions along the length of the retractor muscle were recorded on each frame through the tongue retraction sequence. These coordinates were used to quantify the length of the retractor muscle in each frame. The first and second derivatives of the change in that length were quantified using the same methods as the tongue projection position data. Mass-specific power (W kg$^{-1}$) was calculated for both tongue projection and retraction as the product of velocity and acceleration and corrected for the mass of the active muscle in each phase. Given that the relative proportions of the musculoskeletal components of the chameleon feeding apparatus are conserved both within and among species (Anderson et al., 2012), we corrected for the mass of the active muscle in both tongue projection and retraction as in other studies (i.e., de Groot and van Leeuwen, 2004; Anderson and Deban, 2010), by multiplying mass-specific power by a factor of two for projection and by a factor of four for retraction to obtain power in units of W per kg of muscle mass.

Statistical Analysis

All performance data were log$_{10}$-transformed prior to statistical analysis because performance variables were expected to have an exponential relationship with temperature (Hill et al., 2012). The data were analyzed in three different models. In the first two models, the data were divided into three overlapping subsets based on the temperature at which the data were gathered (14-26°C, 24-31°C and 29-36°C) to examine
whether the thermal relationships for each species varied across different sections of the
temperature range. In the last model, the data was divided into four subsets based on the
four target experimental temperatures (15±1°C, 25±1°C, 30±1°C, 35±1°C) to examine
whether any individual species outperformed the others at a given body temperature. An
analysis of covariance (ANCOVA) was conducted separately on each temperature subset
of the data using JMP 5.1 software. To control for false discovery rate in multiple
comparisons, the Benjamini-Hochberg method (Benjamini and Hochberg, 1995) was
used to adjust significance levels.

Data from each species in overlapping temperature range subsets was tested for
three main effects: (1) temperature, (2) individual and (3) projection distance.
Temperature effects were included as a continuous variable to examine how performance
of elastically powered and non-elastic movements within each species responded to
changes in body temperature. A random individual effect was included to account for
body size and other random individual differences. Projection distance was included to
account for potential effects on performance, as projection distance has been found to
influence some prey-capture kinematics (Anderson and Deban, 2010). Projection distance
was dropped from the model when non-significant for a given variable to increase sample
size and statistical power.

To test for differences among species, data from species pairs within each
temperature range was examined using a nested design. The combined data was tested for
five effects: (1) individual (nested within species), (2) species, (3) temperature, (4)
species x temperature interaction and (5) projection distance. Projection distance was
once again dropped from the model when non-significant for a given variable. The
species x temperature interaction effect in the ANCOVAs of each species pair indicated whether the effect of temperature across each temperature range differed between the species.

To test for differences among species at each temperature, data from species pairs within each target experimental temperature was examined using a nested design. The combined data was tested for three effects: (1) each individual’s snout-vent length (nested within species), (2) species and (3) projection distance. In order to account for scaling effects on performance variables, snout-vent length was included in the model in place of an individual term, as no two individuals in the study were the same size. As with the previous models, projection distance was dropped from the model when non-significant for a given variable. The species effect in the ANCOVAs of each species pair indicated whether either species outperformed the other at the given temperature.

For models where data was divided into overlapping temperature range subsets, temperature coefficients ($Q_{10}$) for each species were calculated across each temperature range (14-26°C, 24-31°C and 29-36°C) for each performance variable from the partial regression coefficients (PRCs) of the temperature effect in the within species ANCOVAs. Calculating the $Q_{10}$ values from the PRC accounts for effects of individual and projection distance that influence the estimate of the relationship between the performance variable and temperature because the ANCOVA models includes these effects as well. The $Q_{10}$ values were calculated as the base 10 antilogarithm of the PRC multiplied by 10:

$$Q_{10} = 10^{(PRC \times 10)}.$$
Table 4.1. Summary data of kinematic performance variables during projection and retraction.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Sample Size</th>
<th>Peak Velocity (m s(^{-1}))</th>
<th>Peak Acceleration (m s(^{-2}))</th>
<th>Peak Power (W kg(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean ± SEM</td>
<td>Min./Max.</td>
<td>Mean ± SEM</td>
</tr>
<tr>
<td><strong>B. melanocephalum</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Projection</td>
<td>15 ± 1°C</td>
<td>16 (4; 1-5)</td>
<td>6.37 ± 0.33</td>
<td>3.79/7.93</td>
</tr>
<tr>
<td></td>
<td>25 ± 1°C</td>
<td>18 (5; 1-5)</td>
<td>6.98 ± 0.43</td>
<td>3.54/8.83</td>
</tr>
<tr>
<td></td>
<td>30 ± 1°C</td>
<td>15 (3; 5-5)</td>
<td>7.65 ± 0.12</td>
<td>6.88/8.44</td>
</tr>
<tr>
<td></td>
<td>35 ± 1°C</td>
<td>15 (3; 5-5)</td>
<td>7.21 ± 0.45</td>
<td>3.93/8.56</td>
</tr>
<tr>
<td>Retraction</td>
<td>15 ± 1°C</td>
<td>16 (4; 1-5)</td>
<td>1.02 ± 0.10</td>
<td>0.46/1.67</td>
</tr>
<tr>
<td></td>
<td>25 ± 1°C</td>
<td>18 (5; 1-5)</td>
<td>1.48 ± 0.12</td>
<td>0.69/2.49</td>
</tr>
<tr>
<td></td>
<td>30 ± 1°C</td>
<td>15 (3; 5-5)</td>
<td>1.79 ± 0.13</td>
<td>0.64/2.45</td>
</tr>
<tr>
<td></td>
<td>35 ± 1°C</td>
<td>15 (3; 5-5)</td>
<td>2.27 ± 0.15</td>
<td>1.19/3.46</td>
</tr>
<tr>
<td><strong>B. thamnobates</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Projection</td>
<td>15 ± 1°C</td>
<td>35 (6; 5-10)</td>
<td>5.71 ± 0.22</td>
<td>3.07/7.26</td>
</tr>
<tr>
<td></td>
<td>25 ± 1°C</td>
<td>35 (6; 5-10)</td>
<td>6.84 ± 0.14</td>
<td>5.02/8.57</td>
</tr>
<tr>
<td></td>
<td>30 ± 1°C</td>
<td>30 (6; 5-5)</td>
<td>7.10 ± 0.15</td>
<td>5.49/8.53</td>
</tr>
<tr>
<td></td>
<td>35 ± 1°C</td>
<td>50 (8; 5-10)</td>
<td>6.81 ± 0.19</td>
<td>3.62/8.60</td>
</tr>
<tr>
<td>Retraction</td>
<td>15 ± 1°C</td>
<td>35 (6; 5-10)</td>
<td>1.28 ± 0.08</td>
<td>0.12/2.55</td>
</tr>
<tr>
<td></td>
<td>25 ± 1°C</td>
<td>35 (6; 5-10)</td>
<td>2.11 ± 0.07</td>
<td>1.35/3.01</td>
</tr>
<tr>
<td></td>
<td>30 ± 1°C</td>
<td>30 (6; 5-5)</td>
<td>2.32 ± 0.09</td>
<td>1.52/4.22</td>
</tr>
<tr>
<td></td>
<td>35 ± 1°C</td>
<td>50 (8; 5-10)</td>
<td>2.54 ± 0.08</td>
<td>1.30/3.86</td>
</tr>
<tr>
<td><strong>B. sp. “Emerald”</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Projection</td>
<td>15 ± 1°C</td>
<td>40 (5; 5-10)</td>
<td>6.61 ± 0.18</td>
<td>3.30/7.95</td>
</tr>
<tr>
<td></td>
<td>25 ± 1°C</td>
<td>35 (5; 10)</td>
<td>6.90 ± 0.25</td>
<td>4.06/8.87</td>
</tr>
<tr>
<td></td>
<td>30 ± 1°C</td>
<td>25 (5; 5-5)</td>
<td>7.69 ± 0.09</td>
<td>6.94/8.62</td>
</tr>
<tr>
<td></td>
<td>35 ± 1°C</td>
<td>45 (6; 5-10)</td>
<td>7.11 ± 0.24</td>
<td>3.93/9.13</td>
</tr>
<tr>
<td>Retraction</td>
<td>15 ± 1°C</td>
<td>40 (5; 5-10)</td>
<td>1.62 ± 0.08</td>
<td>0.81/2.75</td>
</tr>
<tr>
<td></td>
<td>25 ± 1°C</td>
<td>35 (5; 10)</td>
<td>2.09 ± 0.10</td>
<td>0.99/3.27</td>
</tr>
<tr>
<td></td>
<td>30 ± 1°C</td>
<td>25 (5; 5-5)</td>
<td>2.50 ± 0.11</td>
<td>1.11/3.32</td>
</tr>
<tr>
<td></td>
<td>35 ± 1°C</td>
<td>45 (6; 5-10)</td>
<td>2.67 ± 0.11</td>
<td>1.43/4.04</td>
</tr>
</tbody>
</table>

The total number of feedings is presented 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.
Results

In total, 359 feeding sequences were collected across a temperature range of 14.0-36.0°C (Table 4.1). These feedings consisted of 64 feedings from six *B. melanocephalum* (low-elevation taxon; 50.89-59.67 mm snout-vent length), 150 feedings from eight *B. thamnobates* (mid-elevation taxon; 53.15-95.72 mm snout-vent length), and 145 feedings from six *B. sp. “emerald”* (high-elevation taxon; 69.37-88.06 mm snout-vent length).

Tongue projection lengths ranged from 5.45 to 11.87 cm in *B. melanocephalum*, 5.41-15.09 cm in *B. thamnobates*, and 6.24-14.67 cm in *B. sp. “emerald”*.

Across the entire 14-36°C temperature range, peak tongue projection velocity ranged from 3.07 to 9.13 m s\(^{-1}\) (Table 4.1). Peak tongue projection acceleration ranged from 339 to 4520 m s\(^{-2}\) and peak muscle mass-specific power of tongue projection ranged from 1998 to 51800 W kg\(^{-1}\). Peak performance of tongue retraction, on the other hand, was much lower, with peak velocity ranging from 0.12 to 4.22 m s\(^{-1}\), peak acceleration ranging from 19 to 271 m s\(^{-2}\), and peak power ranging from 25 to 992 W kg\(^{-1}\).

Tongue projection performance was highest at 30°C for all species (Tables A1-A3, Figs. 4.2-4.5). Between 15 and 25°C, projection performance remained relatively constant for *B. sp. “emerald”* (\(Q_{10}\) values of 1.00-1.02; Table A3, Fig. 4.4-4.5), with performance for *B. melanocephalum* and *B. thamnobates* increasing as temperature increased (\(Q_{10}\) values of 1.12-1.61; Tables A1-A2, Figs. 4.2-4.3, 4.5). As temperature increased from 25 to 30°C, projection performance increased for all species, with the exception of peak acceleration in *B. thamnobates* (\(Q_{10}\) value of 1.00; Table A2, Figs. 4.3, 4.5), with \(Q_{10}\) values ranging from 1.09-2.44 (Tables A1-A3, Figs. 4.2-4.5). Projection performance declined for all species as temperature increased from 30 to 35°C, with \(Q_{10}\) values
Figure 4.2. Scatterplots of tongue projection (left) and retraction (right) performance versus temperature for *B. melanocephalum*. 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, 24-31°C and 29-36°C ranges. A significant temperature effect is depicted as a solid regression line and asterisk following the $Q_{10}$ value, whereas non-significant temperature effects are depicted as dashed regression lines. Individual chameleons are shown as different symbols.
Figure 4.3. Scatterplots of tongue projection (left) and retraction (right) performance versus temperature for *B. thamnobates*. Indications as in Fig. 4.2.

ranging from 0.49-0.86. Across the entire temperature range and for every performance parameter, the effect of temperature on projection performance of *B. melanocephalum*
Figure 4.4. Scatterplots of tongue projection (left) and retraction (right) performance versus temperature for *B*. sp. "emerald". Indications as in Figs. 4.2-4.3.

(low-elevation taxon) did not vary significantly from either *B. thamnobates* or *B*. sp. "emerald" (Table A4, Fig. 4.5). The effect of temperature on projection performance did

For tongue retraction, peak velocity was maximal at 35°C for all species (Tables A5-7, Figs. 4.2-4.4, 4.6). Peak acceleration was maximal at 30°C for *B. melanocephalum* and *B.* sp. “emerald” but maximal at 25°C for *B. thamnobates*. Peak power was maximal at 30°C for *B. thamnobates* and *B.* sp. “emerald” but maximal at 35°C for *B. melanocephalum*. For all species, retraction performance increased as temperature increased from 15 to 25°C with $Q_{10}$ values ranging from 1.26-3.09. With the exception of peak retraction acceleration in *B. thamnobates* ($Q_{10}$ value of 0.95; Table A6, Figs. 4.3, 4.6), all retraction performance increased as temperature increased from 25 to 30°C ($Q_{10}$ values of 1.24-2.78; Tables A5-7, Figs. 4.2-4.4, 4.6). As temperature increased from 30 to 35°C, peak retraction velocity increased for all species ($Q_{10}$ values of 1.10-1.74), peak retraction acceleration decreased for all species ($Q_{10}$ values of 0.67-0.73), and peak retraction power decreased for *B. thamnobates* and *B.* sp. “emerald” ($Q_{10}$ values of 0.74-0.85) but increased for *B. melanocephalum* ($Q_{10}$ value of 1.06). Across the entire temperature range and for each performance parameter, no significant difference between the effect of temperature on performance for *B. melanocephalum* (low-elevation taxon) and either *B. thamnobates* or *B.* sp. “emerald” was observed (Table A4, Fig. 4.6). The effect of temperature on performance for *B. thamnobates* (mid-elevation taxon), however, varied significantly from *B.* sp. “emerald” multiple times.

Raw performance of tongue projection and retraction overlapped considerably between species at each temperature (Tables 4.1-4.2, Figs. 4.2-4.4). Because of the variability of performance observed within individuals at each temperature, in most cases
Figure 4.5. Performance parameters for tongue projection as a percent of maximum versus temperature for *B. melanoccephalum* (red), *B. thamnobates* (blue) and *B.* sp. “emerald” (green), and comparison of the effect of temperature for each species over the 14-26°C, 24-31°C and 29-36°C ranges. Letters A-F used to signify significant differences in the effect of temperature over each temperature range between species. The presence of the same letter indicates non-significant difference in the effect of temperature for those species, while lack of the same letter indicates significant difference.
Figure 4.6. Performance parameters for tongue retraction as a percent of maximum versus temperature for *B. melanocephalum* (red), *B. thamnobates* (blue) and *B. sp. “emerald”* (green), and comparison of the effect of temperature for each species over the 14-26°C, 24-31°C and 29-36°C ranges. Indications as in Fig. 4.5.
no species outperformed either other species at a given temperature when differences in body size were taken into account. At 25°C, however, *B. melanocephalum* (low-elevation taxon) experienced larger variability in their feeding performance and as a result tended to underperform both *B. thamnobates* and *B. sp.* “emerald” for their body size, particularly during tongue projection (Table 4.2). Further, at 30°C *B. sp.* “emerald” (high-elevation taxon) experienced much less variability in their feeding performance and tended to outperform *B. thamnobates* (mid-elevation taxon) during tongue projection as a result (Table 4.2). Generally, peak acceleration and peak power output of tongue projection in *B. thamnobates* and *B. sp.* “emerald” – individuals of which covered a larger body size range than *B. melanocephalum* – exhibited a negative relationship with body size. As a result, smaller *B. thamnobates* performed comparably to the smaller *B. melanocephalum*, while larger *B. thamnobates* performed comparably to the larger *B. sp.* “emerald” specimens.

**Discussion**

The chameleons in this study captured prey by ballistic tongue projection, in which peak performance of tongue projection largely exceeded that reported in previous studies of feeding in chameleons. Previous studies have reported peak tongue projection velocities of up to 5.8 m s\(^{-1}\) (Wainwright et al., 1991), peak tongue projection accelerations of up to 486 m s\(^{-2}\) (Wainwright et al., 1991) or 50 g (50 times the acceleration due to gravity), and peak mass-specific power outputs during tongue projection of up to 3168 W kg\(^{-1}\) (de Groot and van Leeuwen, 2004). In contrast, we found peak projection velocities of up to 9.1 m s\(^{-1}\), peak tongue projection accelerations of up to
Table 4.2. *P*-values of species effect in ANCOVAs for species pairs at four experimental temperatures testing for outperformance of one species over another at a given temperature.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Bmelan &amp; Btham</th>
<th>Bmelan &amp; Bsp</th>
<th>Btham &amp; Bsp</th>
</tr>
</thead>
<tbody>
<tr>
<td>15±1ºC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Projection Velocity</td>
<td>0.2228</td>
<td>0.3462</td>
<td>0.0122</td>
</tr>
<tr>
<td>Projection Acceleration</td>
<td>0.0398</td>
<td>0.0971</td>
<td>0.2110</td>
</tr>
<tr>
<td>Projection Power</td>
<td>0.0823</td>
<td>0.1774</td>
<td>0.0829</td>
</tr>
<tr>
<td>Retraction Velocity</td>
<td>0.5638</td>
<td>0.2523</td>
<td>0.0627</td>
</tr>
<tr>
<td>Retraction Acceleration</td>
<td>0.6239</td>
<td>0.3343</td>
<td>0.3740</td>
</tr>
<tr>
<td>Retraction Power</td>
<td>0.1751</td>
<td>0.1284</td>
<td>0.0739</td>
</tr>
<tr>
<td>25±1ºC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Projection Velocity</td>
<td>&lt;0.0001 ‡</td>
<td>0.0011 *</td>
<td>0.7305</td>
</tr>
<tr>
<td>Projection Acceleration</td>
<td>&lt;0.0001 ‡</td>
<td>0.0010 *</td>
<td>0.9711</td>
</tr>
<tr>
<td>Projection Power</td>
<td>&lt;0.0001 ‡</td>
<td>0.0008 *</td>
<td>0.9416</td>
</tr>
<tr>
<td>Retraction Velocity</td>
<td>0.0013 ‡</td>
<td>0.0075 *</td>
<td>0.3684</td>
</tr>
<tr>
<td>Retraction Acceleration</td>
<td>0.6700</td>
<td>0.9601</td>
<td>0.0261</td>
</tr>
<tr>
<td>Retraction Power</td>
<td>0.0035 ‡</td>
<td>0.0565</td>
<td>0.1091</td>
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<tr>
<td>30±1ºC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Projection Velocity</td>
<td>0.7615</td>
<td>0.0012 *</td>
<td>0.0037 *</td>
</tr>
<tr>
<td>Projection Acceleration</td>
<td>0.8297</td>
<td>0.2987</td>
<td>0.0154 *</td>
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<td>Projection Power</td>
<td>0.5683</td>
<td>0.0858</td>
<td>0.0016 *</td>
</tr>
<tr>
<td>Retraction Velocity</td>
<td>0.4929</td>
<td>0.7040</td>
<td>0.2481</td>
</tr>
<tr>
<td>Retraction Acceleration</td>
<td>0.3086</td>
<td>0.2802</td>
<td>0.9946</td>
</tr>
<tr>
<td>Retraction Power</td>
<td>0.8019</td>
<td>0.3645</td>
<td>0.5990</td>
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<tr>
<td>35±1ºC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Projection Velocity</td>
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<td>0.2300</td>
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<tr>
<td>Projection Power</td>
<td>0.4599</td>
<td>0.2552</td>
<td>0.4055</td>
</tr>
<tr>
<td>Retraction Velocity</td>
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<td>0.1689</td>
<td>0.2713</td>
</tr>
<tr>
<td>Retraction Acceleration</td>
<td>0.2799</td>
<td>0.3144</td>
<td>0.3305</td>
</tr>
<tr>
<td>Retraction Power</td>
<td>0.1378</td>
<td>0.2184</td>
<td>0.5184</td>
</tr>
</tbody>
</table>


*P*-values are shown for species effect from ANCOVA models run for species pairs with each individual’s snout-vent length (nested within species), species and projection distance included in the models as effects. 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). Symbol next to bold *P*-values indicates which species on average performed higher for their body size: ‡ denotes *B. thamnobates* and * denotes *B. sp. “emerald” performed higher.
4520 m s\(^2\) (461 g), and peak mass-specific power outputs during tongue projection up to 51800 W kg\(^{-1}\). The discrepancy between previously reported performance values and those of our study are likely the result of scaling effects, with previous studies focusing on large species of over 180 mm snout-vent length (Wainwright et al., 1991; de Groot and van Leeuwen, 2004). While exact scaling relationships between body size and tongue projection and tongue retraction performance are not yet known, scaling trends based on morphology would generally predict smaller individuals to have higher acceleration and power values than larger individuals, and velocity to be size independent (Hill, 1950; Pennycuick, 1992; Anderson et al., 2012). It should be noted, however, that the expectation of constant velocity was developed for muscle-powered movements and may not be applicable to spring-loaded systems. Our tongue projection performance data conforms to general scaling relationships based on morphology with regard to acceleration and power output (Hill 1950; Pennycuick 1992; Anderson et al. 2012) and overall, our heightened performance values can thus largely be explained by scaling effects, in addition to possible phylogenetic, motivational, or other differences.

Tongue projection and tongue retraction performance in this study overlapped considerably between species, both within and across body temperatures examined (Table 4.1, Figs. 4.2-4.4). In most cases, individual species did not outperform other species at any temperature (Table 4.2). Variation in performance within individuals tended to result in largely overlapping performance ranges, however increased variation for *B. melanocephalum* at 25\(^\circ\)C resulted in this species generally underperforming both *B. thamnobates* and *B. sp. “emerald”* (Tables 4.1-4.2; Figs. 4.2-4.4). Further, reduced variation for *B. sp. “emerald”* at 30\(^\circ\)C resulted in this species outperforming *B.
thamnobates in tongue projection (Tables 4.1-4.2; Figs. 4.3-4.4). In general, however, performance tended to overlap with other species when body size was accounted for.

Relative to muscle-powered tongue retraction, performance of elastic-recoil-powered tongue projection maintained a higher degree of performance at low temperature for each species in this study (Figs. 4.5-4.6). This is generally similar to other studies, which have found that movements powered by the recoil of elastic elements are more thermally robust than associated movements powered by muscle contraction directly (Anderson and Deban, 2010; Deban and Lappin, 2011; Deban and Richardson, 2011; Sandusky and Deban, 2012). For instance, previous examination of the effect of temperature on ballistic tongue projection and tongue retraction in Chamaeleo calyptratus found that at 15ºC projection velocity maintained 77%, projection acceleration maintained 82% and projection power output maintained 65% of peak performance (Anderson and Deban, 2010). Conversely, retraction velocity maintained 42%, retraction acceleration maintained 36%, and retraction power output maintained 15% of peak performance (Anderson and Deban, 2010). We found that for projection, velocity maintained at least 79%, acceleration maintained at least 76%, and power maintained at least 59% of peak performance, while for retraction, velocity maintained at most 56%, acceleration maintained at most 67%, and power maintained at most 37% of peak performance.

Comparison of the effect of temperature on tongue projection performance over each temperature range (i.e., 14-26ºC, 24-31ºC, and 29-36ºC) revealed that B. melanocephalum, the species from the lowest elevation along the coast, did not differ from either other species (Table A4, Fig. 4.5). Conversely, the effect of temperature on
tongue projection performance for *B. thamnobates* and *B*. sp. “emerald” frequently differed despite their close geographical, elevational, and climatic proximity. In large part, this appears to be caused by an apparent broadening or downward shift of the thermal performance breadth in *B. thamnobates*, where projection performance at 25°C is maintained to a higher degree than in either *B. melanocephalum* or *B*. sp. “emerald” (Fig. 4.5). Conversely, projection performance in *B*. sp. “emerald” declines sharply as temperature declines from 30 to 25°C but is almost invariant in the 15 to 25°C range (Figs. 4.4-4.5).

As with tongue projection performance, comparison of thermal effects on tongue retraction performance revealed no difference between *B. melanocephalum* and either other species over any temperature range (Table A4, Fig. 4.6). Similarly, *B. thamnobates* once again appears to exhibit a broadened or downward shifted thermal performance breadth resulting in multiple instances of significant differences with *B*. sp. “emerald” in its effect of temperature over different temperature ranges.

The prevalence of significant differences in thermal effects on both projection and retraction performance between *B. thamnobates* and *B*. sp. “emerald” could stem from multiple sources. First, it is possible that an elevational/thermal cline in how different species perform and respond to different temperatures may exist more broadly, but that one or more species in our study may not conform well to this general trend. For instance, *B*. sp. “emerald” may be more likely to go into winter torpor due to the consistently colder conditions where it lives (ca. 10 degree daily temperature shifts in August; Schulze, 1997), whereas *B. thamnobates* experiences a more heterogeneous diurnal thermal environment (ca. 15 degree daily temperature shifts in August; Schulze, 1997) and may
be able to remain active during some periods in winter, and therefore would need to have a wider tolerance to temperature than *B. sp. “emerald”*. Alternatively, an elevational/thermal cline may not be a good predictor of differences among species. While elevation and mean ambient temperature were used to characterize the differences in the local environment for each of the species in this study, other environmental factors may be responsible for driving the thermal relationship of tongue projection and retraction performance. As a result, our predictions of how the performance of each species at different temperatures compare to each other may not reflect the trend expected for other environmental characteristics. Further, our comparison may be limited by thermal resolution, in which our ability to detect subtle performance curve shifts or differences at 5-10°C increments may not be sufficient to elucidate and tie variations to environmental characteristics. Finally, sampling within the populations at different times of the year would also help differentiate effects of acclimation to the immediate climatic conditions from effects of adaptation to the local habitat. Additional and broader sampling could help rectify these uncertainties, however a more complete understanding of scaling effects and more accurate thermal control capabilities would be required to do so.

Despite the thermal robustness of tongue projection performance, however, we found variation in how temperature affects both tongue projection and tongue retraction performance. While ultimately maintaining a high degree of tongue projection performance at temperatures as low as 15°C and with minimal differences between species at each experimental temperature, species exhibited different thermal performance breadth patterns. For instance, an apparent trade-off is shown between *B.
*thamnobates* and *B.* sp. “emerald”, where the maintenance of mid temperature performance is sacrificed for improved maintenance of performance at lower temperatures in *B.* sp. “emerald”, which live in a colder environment. By maintaining a higher degree of performance at 25ºC and a performance decline in the 15-25ºC range, *B. thamnobates* broadened its thermal performance curve, possibly in response to a greater range of temperatures they are likely to remain active over than species from colder environments, such as *B.* sp. “emerald”. Alternatively, *B.* sp. “emerald” experienced a stronger decline in performance at 25ºC and then invariant performance in the 15-25ºC range. While this variation could not generally be tied to altitudinal gradation or differences in mean ambient temperature, it illustrates that both elastic-recoil-powered movements and muscle-powered movements experience changes in their thermal performance curves between populations, despite differences in how temperature affects the performance of these different movement types.

While variation in their performance curves are observed, the tongue projection mechanism of all chameleon species examined to date are still affected by temperature to a lesser degree than lizards that lack a prey capture mechanism that incorporates the recoil of elastic elements (Huey & Bennett, 1987; Herrel et al., 2007). As a result, chameleons are able to feed at very low body temperatures (Burrage, 1973; Reilly, 1982; Hebrard et al., 1982; Bennett, 2004; Andrews, 2008; Anderson & Deban, 2010), where other sympatric lizard species remain inactive (Hebrard et al., 1982). This ability enables chameleons to take advantage of feeding opportunities early in the morning when effective thermoregulation is not possible (Reilly, 1982), and may thus reduce thermoregulatory behavior and its ecological costs (Huey, 1974). Similarly, other
ectotherms that incorporate an elastic recoil mechanism into ecologically important movements may similarly benefit from the thermal robustness of these movements, although variation in their specific thermal performance curves is likely to exist despite the thermal robustness of the movements.

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References Cited


CHAPTER FIVE: CONCLUSIONS

The studies reported in this dissertation examined thermal effects on ballistic tongue projection and tongue retraction in chameleons at difference mechanistic levels and within the framework of how these thermal relationships may be affected by their local environment. As a result, considerable insight into the biology and performance of chameleons was gained, however many of the results apply more broadly to similar systems in other ectotherms. In that way, this dissertation serves to expand our understanding of how temperature effects highly dynamic movements powered by elastic-recoil in general and provide methodological approaches to studying similar systems in the future.

At the level of whole organism performance, I found that the elastic-recoil mechanism of tongue projection in chameleons circumvents the constraints that low temperature imposes on muscle rate properties, thereby reducing the thermal dependence of tongue projection. In all species examined, tongue projection was relatively thermally robust, maintaining a high degree of maximal performance at temperatures as low as 15°C. In fact, at 15°C tongue projection maintained at least 77% of its maximal peak velocity, at least 76% of its maximal peak acceleration, and at least 59% of its maximal peak power output. The maintenance of tongue projection performance resulted in temperature coefficient ($Q_{10}$) values as low as 1.00 in the 15-25°C temperature range, indicating in some cases complete temperature insensitivity. In contrast, the associated
muscle-powered tongue retraction was strongly effected by temperature and experienced substantial performance declines over the same temperature range. During tongue retraction at 15°C, for instance, peak velocity was at most maintained at 56% of its maximum, peak acceleration was at most maintained at 67% of its maximum, and peak power was at most maintained at 37% of its maximum. These results suggest that by incorporating an elastic-recoil mechanism, chameleons are able to project their tongue at high performance and effectively capture prey at low body temperature when sympatric species may remain inactive.

While tongue projection performance was itself thermally robust, muscle contractile dynamics of the tongue projector muscle, which preloads the elastic elements responsible for powering projection, was strongly affected by temperature. In fact, all dynamic contractile properties of both the tongue projector muscle and the tongue retractor muscle exhibited significant effects of temperature in the 15-25°C range. These muscles exhibited $Q_{10}$ values of 1.48 or higher on their dynamic contractile properties in the 15-25°C range, while peak force was less thermally dependent, with $Q_{10}$ values of 1.34 or less over the same temperature range. These results suggest that at low temperature, it would take longer for these muscles to exert close to the same amount of force as at higher temperatures. Supporting this expectation, I found that at cooler temperatures the tongue projector muscle became active earlier relative to the onset of tongue projection, due to the reduced rate of tension buildup and the resulting increase in time required to load the elastic elements of the tongue with the sufficient force to subsequently power tongue projection. At the same time, there was no increase in muscle activation intensity at 15°C in the tongue projector muscle. These 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 temperature to overcome these thermal constraints on their muscle physiology. Further, the tongue projector muscle also shows no evidence of physiological specializations that would explain the reduced thermal dependence of tongue projection. These results ultimately suggest that evolutionary modifications of gross morphology (i.e., the incorporation of an elastic recoil mechanism) and motor control, in the absence of changes in muscle contractile physiology, are sufficient to produce high-performance and thermal robustness.

Finally, while behaviors and physiological processes that are less affected by environmental conditions may similarly experience selective pressures of lower intensity acting on them, I found that both tongue projection and tongue retraction performance varied between species living in different thermal environments. While some species appeared to broaden the thermal performance breadth of both their tongue projection and retraction performance, others appeared to substitute increased tongue projection performance at mid temperatures for complete thermal insensitivity across lower temperature ranges. These results suggest that despite differences in how temperature affects the performance of these different movement types, both elastic-recoil-powered movements and muscle-powered movements may experience selective pressure to optimize their performance to their environments.

Based on these studies, I suggest that the relative thermal independence of tongue projection in chameleons is a more general characteristic of elastic-recoil-powered mechanisms and organisms that use elastic recoil mechanisms for ecologically important
movements such as feeding and locomotion may benefit from an expanded thermal niche. Further, given the prevalence of elastic power-amplification mechanisms in ectotherms, the benefit of reduced thermal sensitivity may promote the evolution of these mechanisms in other ectothermic animals. Finally, I propose that temperature manipulations may be a useful methodological approach to testing for the presence or prevalence of elastic recoil in powering other biomechanical systems.

Future studies could continue to develop our understanding of this system, both at the level of chameleon biology and more broadly at the mechanistic level. Examination of thermal effects of tongue projection and tongue retraction in a wider variety of species, for instance, may reveal a variety of interesting trends, including patterns of adaptation to local environments in feeding performance, specific environmental drivers of species or population level differences and phylogenetic variation in thermal robustness. Further, examination of acclimation effects on thermal dependence in this system may provide insight into additional means that thermal constraints are mitigated in these types of movement. Finally, comparison of these findings to similar elastically powered systems may help solidify the generality of these finding among other taxa.
Table A1. Results of ANCOVA examining effects on projection performance variables in *Bradypodion melanoccephalum* over three temperature ranges.

<table>
<thead>
<tr>
<th>Temperature Range</th>
<th>Individual P-value</th>
<th>Temperature P-value</th>
<th>Projection Distance P-value</th>
<th>Temperature Slope</th>
<th>Temperature Q&lt;sub&gt;10&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>14-26°C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Velocity</td>
<td>0.0399</td>
<td>0.2480</td>
<td>0.9367</td>
<td>0.0047713</td>
<td>1.12</td>
</tr>
<tr>
<td>Acceleration</td>
<td>0.0689</td>
<td>0.5327</td>
<td>0.7458</td>
<td>0.0047591</td>
<td>1.12</td>
</tr>
<tr>
<td>Power</td>
<td>0.0495</td>
<td>0.3760</td>
<td>0.8237</td>
<td>0.0102207</td>
<td>1.27</td>
</tr>
<tr>
<td>24-31°C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Velocity</td>
<td><strong>0.0014</strong></td>
<td>0.2608</td>
<td>0.4347</td>
<td>0.0061468</td>
<td>1.15</td>
</tr>
<tr>
<td>Acceleration</td>
<td><strong>0.0004</strong></td>
<td>0.1518</td>
<td>0.1031</td>
<td>0.013844</td>
<td>1.38</td>
</tr>
<tr>
<td>Power</td>
<td><strong>0.0005</strong></td>
<td>0.2097</td>
<td>0.1788</td>
<td>0.0186924</td>
<td>1.54</td>
</tr>
<tr>
<td>29-36°C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Velocity</td>
<td>0.0328</td>
<td>0.3305</td>
<td>0.8462</td>
<td>-0.006388</td>
<td>0.86</td>
</tr>
<tr>
<td>Acceleration</td>
<td><strong>0.0058</strong></td>
<td>0.0697</td>
<td>0.3665</td>
<td>-0.023302</td>
<td>0.58</td>
</tr>
<tr>
<td>Power</td>
<td><strong>0.0072</strong></td>
<td>0.1070</td>
<td>0.5060</td>
<td>-0.030616</td>
<td>0.49</td>
</tr>
</tbody>
</table>

*P*-values are shown for individual and temperature, as in the partial regression coefficient for the temperature effect (i.e., slope) from the model from which *Q*<sub>10</sub> 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 Hochberg, 1995). Bold *Q*<sub>10</sub> values indicate significant temperature effects.
Table A2. Results of ANCOVA examining effects on projection performance variables in *Bradypodion thamnobates* over three temperature ranges.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Individual P-value</th>
<th>Temperature P-value</th>
<th>Projection Distance P-value</th>
<th>Temperature Slope</th>
<th>$Q_{10}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>14-26ºC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Velocity</td>
<td>0.0001</td>
<td>&lt;.0001</td>
<td>0.1799</td>
<td>0.0086148</td>
<td>1.22</td>
</tr>
<tr>
<td>Acceleration</td>
<td>&lt;.0001</td>
<td>0.0037</td>
<td>0.9492</td>
<td>0.0117204</td>
<td>1.31</td>
</tr>
<tr>
<td>Power</td>
<td>&lt;.0001</td>
<td>0.0002</td>
<td>0.7883</td>
<td>0.0207694</td>
<td>1.61</td>
</tr>
<tr>
<td>24-31ºC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Velocity</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>0.0039112</td>
<td>1.09</td>
</tr>
<tr>
<td>Acceleration</td>
<td>&lt;.0001</td>
<td>0.9752</td>
<td>0.2377</td>
<td>0.0002038</td>
<td>1.00</td>
</tr>
<tr>
<td>Power</td>
<td>&lt;.0001</td>
<td>0.4254</td>
<td>0.5254</td>
<td>0.0045493</td>
<td>1.11</td>
</tr>
<tr>
<td>29-36ºC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Velocity</td>
<td>&lt;.0001</td>
<td>0.0245</td>
<td><strong>0.0022</strong></td>
<td>-0.006659</td>
<td>0.86</td>
</tr>
<tr>
<td>Acceleration</td>
<td>&lt;.0001</td>
<td>0.0865</td>
<td><strong>0.0058</strong></td>
<td>-0.011531</td>
<td>0.77</td>
</tr>
<tr>
<td>Power</td>
<td>&lt;.0001</td>
<td>0.0411</td>
<td><strong>0.0013</strong></td>
<td>-0.018117</td>
<td>0.66</td>
</tr>
</tbody>
</table>

*P*-values are shown for individual and temperature, as in 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 A3. Results of ANCOVA examining effects on projection performance variables in *Bradypodion* sp. “emerald” over three temperature ranges.

<table>
<thead>
<tr>
<th>Temperature Range</th>
<th>Individual</th>
<th>Temperature</th>
<th>Projection Distance</th>
<th>Temperature Slope</th>
<th>Q_{10}</th>
</tr>
</thead>
<tbody>
<tr>
<td>14-26ºC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Velocity</td>
<td><strong>0.0440</strong></td>
<td>0.6968</td>
<td>0.8178</td>
<td>0.0008478</td>
<td>1.02</td>
</tr>
<tr>
<td>Acceleration</td>
<td><strong>0.0069</strong></td>
<td>0.9827</td>
<td>0.3073</td>
<td>-0.000096</td>
<td>1.00</td>
</tr>
<tr>
<td>Power</td>
<td><strong>0.0096</strong></td>
<td>0.9289</td>
<td>0.3510</td>
<td>0.0005882</td>
<td>1.01</td>
</tr>
<tr>
<td>24-31ºC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Velocity</td>
<td>0.0513</td>
<td><strong>0.0032</strong></td>
<td>0.7607</td>
<td>0.0132078</td>
<td><strong>1.36</strong></td>
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<tr>
<td>Acceleration</td>
<td>0.0603</td>
<td><strong>0.0044</strong></td>
<td>0.2330</td>
<td>0.0254094</td>
<td><strong>1.80</strong></td>
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<tr>
<td>Power</td>
<td>0.0692</td>
<td><strong>0.0039</strong></td>
<td>0.2758</td>
<td>0.0387738</td>
<td><strong>2.44</strong></td>
</tr>
<tr>
<td>29-36ºC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Velocity</td>
<td><strong>0.0167</strong></td>
<td>0.0791</td>
<td>0.8265</td>
<td>-0.008703</td>
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<tr>
<td>Acceleration</td>
<td><strong>0.0144</strong></td>
<td>0.1298</td>
<td>0.2214</td>
<td>-0.014188</td>
<td>0.72</td>
</tr>
<tr>
<td>Power</td>
<td><strong>0.0192</strong></td>
<td>0.1018</td>
<td>0.4577</td>
<td>-0.022957</td>
<td>0.59</td>
</tr>
</tbody>
</table>

*P*-values are shown for individual and temperature, as in 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 A4. *P*-values of species x temperature interaction effect in ANCOVAs for species pairs over three temperature ranges.

<table>
<thead>
<tr>
<th></th>
<th>Bmelan &amp; Btham</th>
<th>Bmelan &amp; Bsp</th>
<th>Btham &amp; Bsp</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>P</em>-value</td>
<td><em>P</em>-value</td>
<td><em>P</em>-value</td>
</tr>
<tr>
<td>14-26°C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Projection Velocity</td>
<td>0.3220</td>
<td>0.3624</td>
<td><strong>0.0079</strong></td>
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<tr>
<td>Projection Acceleration</td>
<td>0.3681</td>
<td>0.5682</td>
<td>0.0485</td>
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<tr>
<td>Projection Power</td>
<td>0.3385</td>
<td>0.4489</td>
<td><strong>0.0197</strong></td>
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<tr>
<td>Retraction Velocity</td>
<td>0.4770</td>
<td>0.3238</td>
<td><strong>0.0070</strong></td>
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<tr>
<td>Retraction Acceleration</td>
<td>0.1084</td>
<td>0.4138</td>
<td>0.2414</td>
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<tr>
<td>Retraction Power</td>
<td>0.0338</td>
<td>0.9678</td>
<td><strong>0.0041</strong></td>
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<tr>
<td>24-31°C</td>
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<td></td>
<td></td>
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<tr>
<td>Projection Velocity</td>
<td>0.5209</td>
<td>0.3095</td>
<td><strong>0.0226</strong></td>
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<tr>
<td>Projection Acceleration</td>
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<td>0.3867</td>
<td><strong>0.0194</strong></td>
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<td>Projection Power</td>
<td>0.2754</td>
<td>0.3197</td>
<td><strong>0.0126</strong></td>
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<tr>
<td>Retraction Velocity</td>
<td>0.4137</td>
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<td>0.0748</td>
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<tr>
<td>Retraction Acceleration</td>
<td>0.2221</td>
<td>0.7936</td>
<td><strong>0.0283</strong></td>
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<tr>
<td>Retraction Power</td>
<td>0.0183</td>
<td>0.8680</td>
<td><strong>0.0079</strong></td>
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<tr>
<td>29-36°C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Projection Velocity</td>
<td>0.8165</td>
<td>0.7791</td>
<td>0.6672</td>
</tr>
<tr>
<td>Projection Acceleration</td>
<td>0.4712</td>
<td>0.5614</td>
<td>0.9759</td>
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<tr>
<td>Projection Power</td>
<td>0.6228</td>
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<td>0.9489</td>
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<tr>
<td>Retraction Velocity</td>
<td>0.0393</td>
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<td>0.6542</td>
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<tr>
<td>Retraction Acceleration</td>
<td>0.8110</td>
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<td>0.9651</td>
</tr>
<tr>
<td>Retraction Power</td>
<td>0.3250</td>
<td>0.3577</td>
<td>0.8916</td>
</tr>
</tbody>
</table>

*Bmelan*, *Bradypodion melanocephalum*; *Btham*, *Bradypodion thamnobates*; *Bsp*, *Bradypodion* sp. “emerald”.

*P*-values are shown for species x temperature interaction effect from ANCOVA models run for species pairs with individual (nested within species), species, temperature and projection distance included in the models as effects. 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).
Table A5. Results of ANCOVA examining effects on retraction performance variables in *Bradypodion melanocephalum* over three temperature ranges.

<table>
<thead>
<tr>
<th></th>
<th>Individual P-value</th>
<th>Temperature P-value</th>
<th>Projection Distance P-value</th>
<th>Temperature Slope</th>
<th>Q_{10}</th>
</tr>
</thead>
<tbody>
<tr>
<td>14-26°C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Velocity</td>
<td>0.1626</td>
<td>0.0059</td>
<td>0.4997</td>
<td>0.017376</td>
<td>1.49</td>
</tr>
<tr>
<td>Acceleration</td>
<td>0.4708</td>
<td>0.1858</td>
<td>0.9489</td>
<td>0.0101533</td>
<td>1.26</td>
</tr>
<tr>
<td>Power</td>
<td>0.1755</td>
<td>0.0236</td>
<td>0.6250</td>
<td>0.0262583</td>
<td>1.83</td>
</tr>
<tr>
<td>24-31°C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Velocity</td>
<td>0.3588</td>
<td>0.1521</td>
<td>0.7113</td>
<td>0.0165227</td>
<td>1.46</td>
</tr>
<tr>
<td>Acceleration</td>
<td>0.4343</td>
<td>0.3553</td>
<td>0.5634</td>
<td>0.0164096</td>
<td>1.46</td>
</tr>
<tr>
<td>Power</td>
<td>0.2329</td>
<td>0.0175</td>
<td>0.4057</td>
<td>0.0444359</td>
<td>2.78</td>
</tr>
<tr>
<td>29-36°C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Velocity</td>
<td>0.3452</td>
<td>0.0275</td>
<td>0.9815</td>
<td>0.024121</td>
<td>1.74</td>
</tr>
<tr>
<td>Acceleration</td>
<td>0.1841</td>
<td>0.2693</td>
<td>0.8904</td>
<td>-0.017513</td>
<td>0.67</td>
</tr>
<tr>
<td>Power</td>
<td>0.4380</td>
<td>0.8109</td>
<td>0.8504</td>
<td>0.0026341</td>
<td>1.06</td>
</tr>
</tbody>
</table>

*P*-values are shown for individual and temperature, as in 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 A6. Results of ANCOVA examining effects on retraction performance variables in *Bradypodion thamnobates* over three temperature ranges.

<table>
<thead>
<tr>
<th>Temperature Range</th>
<th>Individual P-value</th>
<th>Temperature P-value</th>
<th>Projection Distance P-value</th>
<th>Temperature Slope</th>
<th>Q_{10}</th>
</tr>
</thead>
<tbody>
<tr>
<td>14-26°C</td>
<td>0.2825</td>
<td>&lt;.0001</td>
<td>0.0424</td>
<td>0.0211006</td>
<td>1.63</td>
</tr>
<tr>
<td>Velocity</td>
<td>0.0208</td>
<td>&lt;.0001</td>
<td>0.4738</td>
<td>0.0235192</td>
<td>1.72</td>
</tr>
<tr>
<td>Acceleration</td>
<td>0.1550</td>
<td>&lt;.00001</td>
<td>0.6264</td>
<td>0.0490307</td>
<td>3.09</td>
</tr>
<tr>
<td>Power</td>
<td>0.5222</td>
<td>0.0273</td>
<td>0.0048</td>
<td>0.0091798</td>
<td>1.24</td>
</tr>
<tr>
<td>24-31°C</td>
<td>0.4121</td>
<td>0.7316</td>
<td>0.4882</td>
<td>-0.002257</td>
<td>0.95</td>
</tr>
<tr>
<td>Velocity</td>
<td>0.0617</td>
<td>0.0994</td>
<td>0.5516</td>
<td>0.0095627</td>
<td>1.25</td>
</tr>
<tr>
<td>Acceleration</td>
<td>0.0635</td>
<td>0.3988</td>
<td>0.0637</td>
<td>0.003967</td>
<td>1.10</td>
</tr>
<tr>
<td>Power</td>
<td>0.1912</td>
<td>0.0625</td>
<td>0.0369</td>
<td>-0.01448</td>
<td>0.72</td>
</tr>
<tr>
<td>29-36°C</td>
<td>0.0122</td>
<td>0.3283</td>
<td>0.0002</td>
<td>-0.007065</td>
<td>0.85</td>
</tr>
</tbody>
</table>

*P*-values are shown for individual and temperature, as in 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 A7. Results of ANCOVA examining effects on retraction performance variables in *Bradypodion* sp. “emerald” over three temperature ranges.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Individual P-value</th>
<th>Temperature P-value</th>
<th>Projection Distance P-value</th>
<th>Temperature Slope</th>
<th>Q&lt;sub&gt;10&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>14-26°C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Velocity</td>
<td>&lt;0.0310</td>
<td>&lt;0.0002</td>
<td>0.1851</td>
<td>0.0114889</td>
<td>1.30</td>
</tr>
<tr>
<td>Acceleration</td>
<td>&lt;0.0006</td>
<td>&lt;0.0001</td>
<td>0.5289</td>
<td>0.0166034</td>
<td>1.47</td>
</tr>
<tr>
<td>Power</td>
<td>&lt;0.0047</td>
<td>&lt;0.0001</td>
<td>0.7302</td>
<td>0.0267148</td>
<td>1.85</td>
</tr>
<tr>
<td>24-31°C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Velocity</td>
<td>&lt;0.0001</td>
<td>&lt;0.0011</td>
<td>0.2455</td>
<td>0.0189374</td>
<td>1.55</td>
</tr>
<tr>
<td>Acceleration</td>
<td>&lt;0.0021</td>
<td>0.0136</td>
<td>0.8324</td>
<td>0.0207811</td>
<td>1.61</td>
</tr>
<tr>
<td>Power</td>
<td>&lt;0.0005</td>
<td>&lt;0.0003</td>
<td>0.5161</td>
<td>0.0412557</td>
<td>2.59</td>
</tr>
<tr>
<td>29-36°C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Velocity</td>
<td>&lt;0.0043</td>
<td>0.2289</td>
<td>0.1104</td>
<td>0.0073602</td>
<td>1.18</td>
</tr>
<tr>
<td>Acceleration</td>
<td>&lt;0.0012</td>
<td>0.0459</td>
<td>&lt;0.0020</td>
<td>-0.013628</td>
<td>0.73</td>
</tr>
<tr>
<td>Power</td>
<td>&lt;0.0230</td>
<td>0.2247</td>
<td>0.1871</td>
<td>-0.012856</td>
<td>0.74</td>
</tr>
</tbody>
</table>

*P*-values are shown for individual and temperature, as in the partial regression coefficient for the temperature effect (i.e., slope) from the model from which *Q*<sub>10</sub> 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*<sub>10</sub> values indicate significant temperature effects.
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