The biomechanics of tongue projection in the frog _Rana pipiens_: dynamics and temperature effects

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The Biomechanics of Tongue Projection in the Frog *Rana pipiens*:

Dynamics and Temperature Effects

by

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A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science
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Abstract

Ectotherms such as frogs must either function within environments with temperatures amenable to their physiological needs, or find means to reduce the impact of temperature on their activities. Recent studies on reptile and amphibian feeding have shown convergent use of elastic recoil to drive feeding movements, thereby decoupling temperature’s effects on muscle from movement and allowing the animals to feed over broader temperature ranges. *Rana pipiens* specimens (n=5) were exposed to three ambient temperatures (10º, 15º, and 25º C) at which feeding behavior was imaged at 6000 Hz. The image sequences yielded detailed kinematic and dynamic information for jaw, tongue, and body movements, including velocity, acceleration, power, duration, and excursion. Previously published studies have examined feeding in ranid frogs; however, those studies employed slower frame rates that did not permit analysis of instantaneous accelerations and velocities, and depressor and jaw-tongue complex mass specific power outputs in *Rana* feeding have not yet been established. Specimens were dissected for morphological measurement and calculation of mass-specific dynamics relative to the *m. depressor mandibulae* and the center of mass of the jaw-tongue complex. Previous studies on tongue projection in *Bufo terrestris* have shown that the rapid jaw depression that inertially elongates the tongue relies on elasticity in the depressor muscles. Further, because this movement is elastically driven, it is less sensitive to temperature than a
completely muscle-driven movement would be. Because *Rana* also feeds through inertial elongation of the tongue (as does *Bufo*, in which the mechanism is convergent), I hypothesized that *Rana* would demonstrate thermal insensitivity in its feeding kinematics and dynamics in a pattern similar to that documented in *Bufo*. Experimental results indicated that portions of the feeding cycle related to the initial, ballistic phase were at least moderately thermally insensitive. At all temperatures studied, *Rana* reached approximately half of the depressor mass-specific power of *Bufo*, demonstrating that *Bufo*’s depressor mass-specific power output is not the minimum value necessary for inertial elongation. I further hypothesize that thermal independence and power output in excess of that achievable by muscle alone during the initial, ballistic mouth opening phase of feeding suggests the involvement of an elastic mechanism convergent with that of *Bufo terrestris*. 
Introduction

Ectotherms’ physiologic function relies on a thermally variable environment. Adapting these functions to these environments requires either limiting activity periods and/or ranges to those times and places with amenable temperatures, or physiologically specializing to reduce any functional disruption temperature may cause. Temperature’s influence is seen in the changes in physiological rates as an organism experiences varying temperatures. The change in a physiological rate over ten degrees celsius, or $Q_{10}$, is a quantitative representation of how sensitive a particular process is to changes in temperature (Hill, 1951; Rao, 1954; Belehradek, 1957; Bennett, 1984; Bennett, 1985). Muscular responses to temperature changes have been well documented, particularly in ectotherms (Putnam and Bennett, 1982; Rome and Kushmerick, 1983; Bennett and John-Alder, 1984; Bennett, 1984; Hirano and Rome, 1984; Renaud and Stevens, 1984; Bennett, 1985; Else and Bennett, 1987; John-Alder et al., 1989; Rome and Sosnicki, 1990; Altringham and Block, 1997; Wilson et al., 2000). Within the 20° - 30°C range, for example, muscle is prone to dramatic changes in performance rates such as contraction velocity ($Q_{10} = 2.0 - 2.5$), rate of tension development ($Q_{10} = 2.36$), and maximal power output ($Q_{10} = 2.42$) (Bennett, 1985). Heart rate can also increase alongside temperature, as observed in yellowfin tuna ($Q_{10} = 2.37$ over 18° - 28°C range) (Korsmeyer et al., 1997). Depending upon the variable and the temperature, an increase in rate may provide an advantage, such as greater blood perfusion; however, without some compensatory
mechanism, the necessary decrease in rate at lower temperatures can be detrimental to organismal performance.

Many species thermoregulate behaviorally, seeking temperatures that will meet their needs by basking or traveling between thermally differing areas to warm or cool their bodies (Dreisig, 1984; Bennett, 2004). Other animals will avoid temperature issues almost entirely by using thermally insensitive means, such as elastic recoil-based movements, to reduce the effects of temperature on their physiology. Jumping insects use resilin pads and “snapping” portions of their exoskeletons, rather than direct muscle contraction, to propel themselves (Sutton and Burrows, 2008; Burrows, 2011). Chameleons exploit an elastic recoil mechanism in their tongues to allow them to launch (and therefore feed) at consistent velocities across wide temperature ranges (de Groot and van Leeuwen, 2004; Anderson and Deban, 2010), and plethodontid salamanders have convergently evolved thermally insensitive tongue projection that also makes use of elastic recoil (Deban and Richardson, 2011). Recent research in ectotherm biology has shown a prominent role for elastic recoil in powering feeding mechanisms, with an added benefit of broadened capacity for feeding in thermally variable environments or conditions (Huey and Hertz, 1984; de Groot and van Leeuwen, 2004; Anderson and Deban, 2010; Deban and Lappin, 2011; Deban and Richardson, 2011). These elastic mechanisms generally provide amplification of mechanical power, which enhances performance (Roberts and Azizi, 2011).

Bufonid anurans use elastic recoil as a mechanism to depress their jaws rapidly, which drives their inertial tongue projection. Elastic recoil in the *m. depressor*
mandibulae, which wraps from the posterior portion of the mandible to the top of the head, lifts the retroarticular process of the mandible onto which the depressor inserts. This elevation applies a torque to the jaw joint and drops the front of the mandible, and the flap-like tongue, which is attached only at its rostral end, extends inertially (Nishikawa, 2000; Lappin et al., 2006). Portions of this feeding behavior, such as the velocity of the jaw during the initial mouth opening that propels the tongue, have been found not to be significantly affected by temperature in *Bufo terrestris* (Deban and Lappin, 2011). The elastic elements contributing to depressor recoil in *Bufo terrestris* are believed to provide thermal insensitivity because elastic rate properties are significantly less temperature sensitive than muscular ones (Alexander, 1966; Bennett, 1984; Bennett, 1985; Barnes and Ingalls, 1991; Denny and Miller, 2006).

Inertial elongation is not isolated to the Bufonidae. The Ranidae and Dendrobatidae, among others, employ this mechanism convergently (Nishikawa, 2000; Hoegg et al., 2004; van der Meijden, 2006). While performance and morphological observations have established the convergence, whether or to what degree ranids and bufonids use the same elastic recoil system to drive their tongue projection has yet to be established. Outwardly the feeding behavior in *Rana* and *Bufo* appears different, even before kinematics and dynamics are compared. Whereas *Bufo* will closely approach prey and whip its tongue in a tight, low-profile trajectory, *Rana* throws its body forward and gapes as its tongue lifts out of the mouth and flips. Both approaches, however, result in the tongue inertially elongating to catch, and then pull back, a prey item (Nishikawa, 2000).
While *Rana pipiens* is convergent with *Bufo* for inertial elongation, convergence in the elastic recoil mechanism that drives *Bufo*’s jaw depression has not been established. The thermal sensitivity of the steps of the feeding gape cycle in *Rana*, as determined by the Q₁₀ values for rate properties relevant to inertial elongation, would be a useful tool for uncovering such a convergence. For instance, *Bufo*’s initial mouth opening, termed “ballistic” for its rapid achievement of peak velocity after the start of movement (Lappin et al., 2006; Deban and Lappin, 2011), has been shown to be more thermally neutral than the primarily muscle-driven final closing of the mouth after the prey item has been pulled back into the buccal cavity (Deban and Lappin, 2011). Q₁₀ trends can be used to compare other phases in the gape cycle for convergence in performance and physiology. Movements that rely more on muscle will have higher Q₁₀ values for their excursions, velocities, and accelerations and higher inverse Q₁₀ (1/Q₁₀) values for their durations, because durations can be expressed as rates through their reciprocals. Conversely, movements that rely primarily on elastic recoil will have lower Q₁₀ values for their rate properties, reflecting less of a performance difference across the range of experimental temperatures (Hill, 1951; Bennett, 1984; Burrows and Sutton, 2008; Anderson and Deban, 2010; Burrows, 2011; Deban and Lappin, 2011; Deban and Richardson, 2011). In those cases in which there is no difference between the measured rates at the compared temperatures, the Q₁₀ would be expected to be 1.

I hypothesize that the thermal sensitivity of tongue projection in *Rana* will show that an elastic recoil mechanism is involved, and that elastically amplified power in *Rana* will be similar to that of *Bufo*. 
Methods and Materials

Five *Rana pipiens* Schreber 1782 were purchased commercially and housed as a group in a plastic bin with *ad libitum* access to water deep enough for swimming. The animals were maintained at approximately 22°C and were fed a diet of crickets, beetles (*Dermestes maculatus*), and beetle larvae in random combination. All procedures in this study were approved by the University of South Florida Institutional Animal Care and Use Committee (protocol W3620).

Feeding Experiments

Frogs’ feedings were imaged at 6000 Hz (1/12,000 shutter speed) with a Fastcam SA4 or Fastcam 1024 PCI camera (Photron USA, San Diego, California, USA). The image frame was calibrated to a centimeter scale before each imaging session.

The animals were imaged individually, with the right side of the frog always facing the camera on a plastic foam stage covered with wet paper towels and set against a dark background. Crickets were presented at haphazard distances in front of the frog (2.345 - 10.026 cm (5.305 ± 0.287 cm)). Frogs were presented with one cricket for each feeding event, and the animals were permitted to continue feeding until they no longer demonstrated an interest in the prey. Feedings continued with five to ten minute intervals between a feeding and the next presentation of prey.
A total of 103 feeding events were recorded, and these were evaluated for visibility of digitizing landmarks (see below) and correct orientation of the animal within the frame (± 15° relative to the imaging plane) to form a subset of 46 feeding events comprised of three or four for each animal that would be analyzed to provide data for every individual at each of the three experimental temperatures. Events were included in the analysis if valid kinematic data could be extracted from their image sequences.

Frogs fed at three different ambient temperatures (10, 15, and 25°C) to form the basis of the comparisons in the study. While attempts were made to include a broader biologically relevant temperature range by including 5 and 30°C in the study, eliciting feedings via inertial elongation proved difficult at those temperatures; thus, the 10-25°C range was employed instead.

Imaging took place in a programmable environmental chamber (Environmental Growth Chambers, Chagrin Falls, Ohio, USA) with additional halogen lighting (Source Four PAR MCM, Electronic Theatre Controls, Middleton, Wisconsin, USA) to improve image quality. This lighting was turned off after each successfully imaged feeding to prevent elevating the animal’s body temperature artificially. The chamber’s set temperature and the animal’s body temperature were documented for every feeding recorded. Frog body temperatures were measured with a handheld infrared thermometer (Sixth Sense LT300, Williston, Vermont, USA; ±1°C accuracy) held directly either above the head or facing the tympanum to ensure measurement of temperature in the animal’s body rather than from an environmental surface. Body and chamber temperature were

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1 At 5°C, the frogs fed by jaw prehension, rather than tongue prehension. At 30°C, the frogs were extremely active and did not reliably stay in the analysis frame.
within 0.38±0.18°C. Individuals experienced haphazardly determined temperature sequences over the duration of the experiment. Animals were acclimated to chamber temperatures for one to two hours prior to each imaging session, until body temperature reached chamber temperature.

**Anatomy**

Following completion of imaging, animals were euthanized *via* overdoses of MS-222. Specimens were stored in ice prior to dissection. Snout-vent lengths and body masses were recorded for each individual upon thawing. The muscle fibers of the *m. depressor mandibulae* were removed as completely as possible from both sides and the top of each frog’s head. The left and right depressor muscles were soaked in amphibian Ringer’s solution for 15-30 minutes, lightly toweled to reduce extraneous fluid mass, and weighed on a balance (Virtual Measurements and Control model VB-302A, Santa Rosa, California, USA, ±0.001g accuracy).

The jaw and tongue complex (including skin, buccal musculature, the majority of the hyoid plate, and the retroarticular processes) was removed from each specimen and soaked in amphibian Ringer’s solution, then the mass measured as above. The distance along a rostrocaudal axis of the jaw-tongue complex from the center of the jaw joint to the mandible tips and the distance from the joint center to the tip of the retroarticular process were measured with calipers (Mitutoyo Corporation, Kanagawa, Japan; ±0.1 mm accuracy). These measurements represented in-lever and out-lever portions of the mandible. The location of the center of mass relative to the point of jaw articulation was
determined through the use of a balance and a ruler using published methods (Deban and Lappin, 2011). Mean values were calculated for combined depressor mass, jaw-tongue complex mass, and jaw length for use in the dynamic analyses.

**Kinematic and Dynamic Analyses**

Image sequences were imported into NIH ImageJ (Abramoff et al., 2004) on a MacBook Pro computer (Apple, Inc., Cupertino, California, USA) for digitization of dynamically relevant points and for measurement of durations and excursions. The x,y positions of four anatomical landmarks were recorded in each frame of the feeding sequences. The landmarks were (1) the tip of the upper jaw, (2) the tip of the mandible, (3) the jaw joint, visible as a bulge just ventral to the caudal edge of the tympanum, and (4) the anatomic tongue tip. Digitizing for each sequence began 100-200 frames before the frog began to lunge and ended once the mouth closed.

To determine excursions, durations, average and instantaneous velocities, accelerations, and power, image sequences were analyzed in phases with reference to the inertial elongation gape cycle (Nishikawa, 2000; Deban and Lappin, 2011). First, the animal starts a full-body lunge toward its prey. Before finishing the lunge, the animal opens its mouth in an initially rapid, ballistic opening phase, and the tongue moves forward to begin exiting the mouth. The gape stabilizes briefly while the tongue continues moving and makes contact with its target, and then the gape widens further for prey transport as the tongue retracts to bring the prey into the buccal cavity. Once the
prey has passed through the gape, the mouth closes. The animal then returns to its crouched resting posture.

To standardize measurement of durations, the gape cycle was treated as a series of timestamped events. The first frame in which the mouth was seen to open (as in ballistic mouth opening) was event 1. The body of the tongue first crossing the gape was event 2. The earliest observed gape stabilization at the end of ballistic opening was event 3. The tongue tip reaching the substrate was event 4. The first observed widening of the gape was event 5. The onset of tongue shortening was event 6. Achievement of maximum gape was event 7. The return of the tongue tip to the buccal cavity and disappearing into the pharynx was event 8. Final mouth closing was event 9. Durations were thus calculated through subtraction of timestamp values. Ballistic mouth opening was event 3 minus event 1. Transport mouth opening was event 7 minus event 5. Mouth closing was event 9 minus event 7. Tongue projection was event 4 minus event 2. Tongue retraction was event 8 minus event 6.

Excursions were measured with reference to the scale frame (see above) in ImageJ as linear distances between landmarks at given frames of interest, and these frames were the same as the start/end frames for the durations measured. Initial prey distance was the distance between the frog’s upper jaw tip and the nearest edge of the prey item, measured in the first digitized frame of the feeding sequence (and therefore before the animal began its lunge). The gape at the end of ballistic mouth opening was the distance between the jaw tips in the final frame of ballistic mouth opening. Maximum gape, similarly, was the distance between the jaw tips at the conclusion of transport.
mouth opening. Maximum tongue reach was the distance between the tip of the lower jaw and the anatomical tongue tip, measured at the end of tongue projection (i.e., once the tongue tip had made contact with the substrate).

Average velocities of ballistic opening, tongue projection, transport opening, tongue retraction, and mouth closing were calculated by dividing the applicable gape or tongue excursions by the durations over which they were achieved.

Peak velocity, acceleration, and power values for ballistic mouth opening were calculated for all feeding sequences relative to the mean placement of the center of mass of the jaw-tongue complex. These dynamics variables were calculated with a custom script in R statistical software (R Development Core Team, 2009) designed to determine the gape distance based upon the length of the arc traveled through mouth opening and centered at the jaw joint. Arc radii extended to the tips of the upper jaw and mandible for accurate gape arc measurement. A quintic spline (using the PSpline package, available through CRAN) was fit to this instantaneous gape distance relative to time, which was calculated from the frame rate of the image sequence. The script evaluated the location of the center of mass of the jaw-tongue complex as a percentage of the total jaw length from the jaw joint, which it then “followed” through its arc trajectory to prepare a displacement function to be rendered along with its first and second derivatives. The first and second derivatives of the displacement function represented the instantaneous velocity and acceleration, respectively, of the jaw-tongue center of mass. Mass specific power was calculated as the product of the instantaneous velocity and acceleration values. Peak values for all three dynamic variables were reported.
Absolute power was calculated by multiplying the peak power by the mean mass of the jaw-tongue complex. Depressor muscle mass-specific power was calculated by dividing the absolute power by the mean mass of the paired *mm. depressores mandibulae* muscles.

Lunge distance, duration, and mean velocity were calculated in a similar fashion, based upon a quintic spline applied to the location of the jaw joint within the image frame over the course of the sequence.

**Statistical Analysis**

All kinematic and dynamic variables (Table 1) were log$_{10}$ transformed in Excel® prior to statistical analysis. Statistical analyses were performed in JMP 5.1 software (SAS Institute, Cary, North Carolina, USA) on Apple MacBook Pro and iMac computers. Analyses of covariance (ANCOVAs) were performed to examine the effects of individual animal, measured body temperature, and prey distance on the log$_{10}$-transformed kinematic and dynamic values. The interaction of individual and temperature was also tested for all variables, but when the results of these interaction analyses proved not statistically significant for all but one variable, the remaining variables were tested without the interaction terms to conserve statistical power. A total of 17 variables were tested and assessed for significance using a Bonferroni corrected $\alpha$ of 0.0029 (0.05 / 17).

$Q_{10}$ values were calculated based upon the partial regression coefficient estimating the slope of the linear relationship between log$_{10}$ transformed response variables versus the frogs’ measured body temperatures, including the influence of initial
prey distance and individual animal. The partial regression coefficient was then multiplied by 10 to form the exponent of a base-10 antilogarithm as follows:

\[ Q_{10} = 10^{(PRC \times 10)} \]  \hspace{1cm} (1)

Excursions and dynamic values were evaluated for thermal sensitivity by their \( Q_{10} \) values; in particular, the statistical tests evaluated the variation from an expected \( Q_{10} \) of 1 (Table 2). Temporal properties, such as durations, were evaluated as rates, and inverse \( Q_{10} \) values (\( 1/Q_{10} \)) reflected those temperature effects.
Results

Prey Capture Kinematics

Over all 46 feedings, spanning frog body temperatures of 10.1 - 26.7°C, frogs spent 9 - 24 msec for ballistic mouth opening, ending with a maximum gape of 10 - 16 mm. The tongue took 17 - 92 msec after first passing through the gape to reach its full extent, which was 9 - 38 mm. Transport opening of the mouth required 55.3 - 180.5 msec, and the maximum gape was 14 - 31 mm. Tongue retraction ended (and the tongue returned to the buccal cavity) within 58 - 172 msec, and the mouth closed within 48 - 258 msec after the animal reached its maximum gape (Table 1).

Temperature variation did not produce significantly different results for the duration of ballistic mouth opening, gape width at the end of ballistic mouth opening, or the width of the gape at the end of mouth opening for prey transport. Temperature did produce significantly different results for the maximum tongue extension after projection, the durations of tongue projection and transport mouth opening, and the durations of tongue retraction and final mouth closing (Table 2).

The duration of ballistic mouth opening was not statistically significant for any of the covariates ($1/Q_{10} = 1.16$). The gape at the end of ballistic mouth opening was significant for individual ($p < 0.0001$). Maximum tongue projection length was significant for individual ($p < 0.0001$) and increased with temperature ($p < 0.0001$, $Q_{10} = 1.24$). Duration of tongue projection was significant for individual ($p < 0.0001$) and
interaction of individual and temperature (p = 0.0006) and decreased with temperature (p < 0.0001, \(1/Q_{10} = 1.43\)). Duration of transport mouth opening decreased with temperature (p < 0.0001, \(1/Q_{10} = 1.35\)). Final gape at the end of transport mouth opening was significant for individual (p < 0.0001). Duration of tongue retraction decreased with temperature (p < 0.0001, \(1/Q_{10} = 1.33\)). Duration of mouth closing was significant for temperature (p < 0.0001, \(1/Q_{10} = 2.08\)) and initial prey distance (p = 0.0020) (Table 2).

**Prey Capture Dynamics**

Across all feedings, the mean velocity of ballistic mouth opening (as measured at the jaw tips) was 0.37 - 1.58 m sec\(^{-1}\), while the maximum instantaneous velocity of the mandibular center of mass was 0.25 - 0.80 m sec\(^{-1}\) with a peak acceleration of 31.3 - 99.3 m sec\(^{-1}\) sec\(^{-1}\). Maximum mass-specific power of the *m. depressor mandibulae* was 115.10 - 1783.23 W kg\(^{-1}\) of depressor muscle mass. The mean velocity of tongue projection was 0.14 - 2.10 m sec\(^{-1}\), and the mean velocity of tongue retraction was 0.06 - 0.54 m sec\(^{-1}\). The mean velocity of transport mouth opening was 0.10 - 0.54 m sec\(^{-1}\). The mean velocity of mouth closing was 0.06 - 0.46 m sec\(^{-1}\) (Table 1).

Temperature variation produced statistically significant results for all prey capture dynamic variables. The mean velocities of tongue projection, tongue retraction, transport mouth opening, and mouth closing showed strongly significant p-values (p = < 0.0001) (Table 2).

All dynamic variables showed significance for effects of temperature. Mean velocity of ballistic opening increased at warmer temperatures (\(Q_{10} = 1.20, p = 0.0011\)).
Maximum instantaneous velocity of ballistic opening increased at cooler temperatures \( (Q_{10} = 1.19, p = 0.0016) \), as did peak acceleration \( (Q_{10} = 1.33, p = 0.0007) \). Maximum mass-specific power of ballistic opening increased considerably at warmer temperatures \( (Q_{10} = 1.57, p = 0.0007) \) (Figure 2). The mean velocities of tongue projection and retraction had \( Q_{10} \) values of 1.66 and 1.65, respectively \( (p < 0.0001 \text{ for both}) \), and the mean velocity of transport opening had a \( Q_{10} \) value of 1.23 \( (p < 0.0001) \) (Figures 2 and 3). The mean velocity of mouth closing had a \( Q_{10} \) value of 2.17 \( (p < 0.0001) \) (Figure 3). All three increased significantly with warmer temperatures.

**Lunge Kinematics and Dynamics**

The average feeding lunge was 2.2 - 8.7 cm in length and lasted 77 - 351 msec, as measured over 41 feedings. The average lunge velocity was 0.01 - 0.36 m sec\(^{-1}\), as measured over 40 feedings (Table 1).

Only lunge duration had any statistical significance for temperature \( (p = 0.0021, 1/Q_{10} = 1.31) \), and it decreased with increasing temperature. Lunge distance \( (p < 0.0001) \), lunge duration \( (p < 0.0001) \), and average lunge velocity \( (p = 0.0021) \) were significant for initial prey distance. Average lunge velocity increased with increasing temperature \( (Q_{10} = 1.64, p = 0.0118) \) (Figure 5).

**Anatomy**

The mean snout-vent length of the frogs was 8.42 cm \( (± 0.15 \text{ cm}) \). The mean body mass was 35.51 gm, although body mass was not recorded for one gravid female (SVL
8.81 cm). The mean jaw-tongue complex mass was 0.90 gm (± 0.049 gm). The mean location of the center of mass of the jaw-tongue complex relative to the articulation point of the jaw, expressed as a percentage of jaw length, was 37.86% (± 4.12%). The mean mass of the left *m. depressor mandibulae* was 0.02 gms (± 0.0041 gms), and the mean mass of the right *m. depressor mandibulae* was 0.02 gms (± 0.0033 gms). The combined mean depressor mass used for calculation of mass specific power was 0.05 gms (± 0.0073 gms). The mean jaw length was 2.29 cm (± 0.072 cm), and the mean retroarticular process length was 1.6 mm (± 0.045 mm). Anatomical values are reported with their standard errors.
Discussion

Prey Capture Kinematics

All feedings recorded took place with the frogs elongating their tongues inertially, in which the dropping of the jaw propels the flap-like tongue in a targeted direction, generally that in which the animal is facing (Nishikawa, 2000; Mallett et al., 2001; Lappin et al., 2006; Deban and Lappin, 2011). When attempts were made to elicit feedings at 5°C, the frogs captured the crickets through jaw prehension, which is otherwise associated with the acquisition of larger, more unwieldy prey such as earthworms (Anderson, 1993). In this case, tongue use was not observed. The thermal sensitivity of the duration of tongue projection suggests that the tongue’s projection is driven by muscle contraction as well as elasticity.

The effect of prey distance upon duration of mouth closing (p = 0.0020, Table 2) is curious, and it suggests a behavioral coupling between the animal’s closing its mouth and “resetting” itself at rest after withdrawing from its feeding lunge. Because final gape is not significantly affected by prey distance, this effect would not be driven by a greater gape at a longer lunge distance. Maximum tongue reach was not significantly affected by prey distance, suggesting that it also does not necessarily influence the duration of mouth closing.

The overall thermal sensitivity of mouth closing (duration $1/Q_{10} = 2.08$,
p = < 0.0001; mean velocity $Q_{10} = 2.17$, $p = < 0.0001$) relative to ballistic mouth opening (duration $1/Q_{10} = 1.16$, $p = 0.0082$; mean velocity $Q_{10} = 1.20$, $p = 0.0011$) is interesting for its comparative value (Figures 2, 4, and 6; Table 2). Inertial elongation is generally associated with elastic recoil in the *m. depressor mandibulae*, which applies a torque about the jaw joint to drop the jaw and inertially elongate the tongue (Lappin et al., 2006; Deban and Lappin, 2011). Elastic recoil-based ballistic opening is believed to be associated with a reduced sensitivity to temperature based upon the properties of the components necessary for elastic energy storage, such as connective biomaterials like collagen that accompany muscle tissue (Alexander 1966; Denny and Miller 2006; Rigby et al., 1959; Deban and Lappin, 2011). The expectation is that greater reliance on elastic mechanisms reduces thermal sensitivity; conversely, the more the movement relies on muscular drive, the greater its thermal sensitivity will be (Bennett, 1985; Barnes and Ingalls, 1991).

Whereas depressor activity is associated with elastic recoil, levator activity, which closes the mouth, is not. Based upon the inverse $Q_{10}$ value (1.16) found, the duration of ballistic mouth opening appears to have more of a thermally insensitive (and ostensibly elastic) drive than does the duration of mouth closing ($1/Q_{10} = 2.08$) (Figure 6). Since the gape width at the end of ballistic opening showed low thermal response ($Q_{10} = 1.04$), the thermally insensitive duration of ballistic opening represents the animal widening its gape at nearly the same rate and degree, regardless of temperature. Were the movement more muscle-dependent, temperature effects on shortening velocity would have changed the
duration significantly (Bennett 1984, 1985). The $Q_{10}$ of mean ballistic opening velocity (1.20) supports this relationship.

**Prey Capture Dynamics**

The relatively low thermal sensitivity for both mean ($Q_{10} = 1.20$, $p = 0.0011$) and instantaneous velocity ($Q_{10} = 1.19$, $p = 0.0016$) of ballistic mouth opening suggests elastic mechanisms driving the rapid, initial jaw depression in *Rana pipiens*, in a manner similar to that seen in *Bufo terrestris* (Lappin et al., 2006; Deban and Lappin, 2011). Given the dramatic, thermally-induced changes in velocity observed in purely muscular movement (Bennett, 1984; Barnes and Ingalls, 1991), as well as the more modest (or absent) changes seen with elastic recoil systems (Anderson and Deban 2010, Deban and Lappin, 2011, Deban and Richardson 2011), we can compare performance in two gape movements and assess which is more elastically or muscularly powered. Based on this pattern, the mean velocities of ballistic and transport mouth opening ($Q_{10} = 1.20$ and 1.23, respectively) show greater elastic involvement than does the mean velocity of mouth closing ($Q_{10} = 2.17$), which is more muscularly driven.

The mean velocities of tongue projection ($Q_{10} = 1.66$) and tongue retraction ($Q_{10} = 1.65$) show intermediate thermal sensitivity. The results suggest a muscular involvement in projection and an elastic involvement in retraction, resulting in movements that are neither completely elastic nor completely muscular. Electromyographic data from the retraction musculature would be useful in determining the motor control of the tongue during the feeding cycle, and particularly during
projection, when no muscular input from the tongue was expected, according to the inertial elongation model of tongue projection (Nishikawa 2000). These findings suggest a possible difference in feeding motor control between *Rana pipiens* and *Bufo terrestris* (Deban and Lappin, 2011).

**Lunge Kinematics and Dynamics**

The dynamics and kinematics of the lunge are statistically significant when considered relative to initial prey distance, which is to be expected. Thermal sensitivity (mean velocity $Q_{10} = 1.64$, duration $1/Q_{10} = 1.31$) (Table 2), however, suggests that the lunge itself is not entirely muscually driven (Bennett, 1984). Jumping, a related explosive behavior, has been shown to rely strongly on an elastic element arranged in series with the musculature of the lower leg (Roberts and Marsh, 2003). In turn, jumping is temperature-sensitive over a subset of the overall range of biologically relevant temperatures (Hirano and Rome, 1984). In this case, however, the animal is not actually producing a jump so much as it is extending its body quickly toward its prey. The need for jumping power would then likely be damped by decreased muscular loading of the elastic element (i.e., the Achilles’ tendon). Were the lunge completely reliant on muscle and without a contributing elastic element, the $Q_{10}$ and inverse $Q_{10}$ values corresponding to the kinematics and dynamics of the behavior (0.85 for distance, 1.31 for duration, and 1.64 for mean velocity) would probably be considerably higher (Bennett, 1984). These results suggest modulated recruitment of elastic recoil based upon the movement required, although electromyographic data is necessary for confirmation.
Anatomy and convergence with Bufo

Rana is considered an inertial elongator convergent with Bufo for its tongue protraction (Gans, 1961; Gans and Gorniak, 1982; Nishikawa, 2000; Lappin et al., 2006), with numerous non-inertial elongators more closely related to each genus than Rana and Bufo are to each other, a conclusion borne out by continually improving phylogenies (Nishikawa, 2000; Hoegg et al., 2004; Van der Meijden, 2006). Both Rana pipiens and Bufo terrestris manage similar performance through the same behavior, but with considerably different dynamic properties (Lappin et al., 2006; Deban and Lappin, 2011). The peak depressor mass-specific power output found in Bufo when tested across the temperature range of 10° to 38°C was determined to span 449.6 to 4,348.5 W kg⁻¹ of depressor muscle mass (Deban and Lappin, 2011). Over all the feedings conducted for this study with Rana, the peak depressor mass-specific power output was appreciably lower; in fact, Rana’s values (115.1 - 1,783.2 W kg⁻¹) were less than half that produced by Bufo. The quality of the m. depressor mandibulae may itself provide the answer that explains the difference in dynamics. First, Bufo appears to have more muscle to use than does Rana. The mean mass of the paired mm. depressores mandibulae in sampled Bufo was found to be 0.13 g (Deban and Lappin, 2011), while Rana’s mean paired depressor mass (0.045 g) was less than half of Bufo’s, with animals of comparable size (mean Rana SVL = 8.42 cm (± 0.15 cm), Bufo SVL range = 3.9 - 8.2 cm (Deban and Lappin, 2011)). Additionally, the ratio of depressor mass to jaw/tongue mass was considerably greater for Bufo (17 - 22% (19±1%)) (Deban and Lappin, 2011) than for Rana (2.85 - 6.0% (4.77±0.68%)).
Both *Rana* and *Bufo* demonstrate some power amplification from elastic elements, as their mass-specific power output surpasses 373 W kg\(^{-1}\), or that of muscle alone (Lutz and Rome, 1984; Altringham and Block, 1997; Aerts, 1998; Roberts and Marsh, 2003). *Rana*, however, achieves successful performance with appreciably less muscle and power. The difference in the depressors may also explain the kinematic differences between the protrusion of the tongue in specimens from these genera. Although this study does not include quantitative, comparative results relating both *Rana* and *Bufo*, based upon appearance in the feeding films, *Rana* projects its tongue in a higher arc than that seen in *Bufo*.

The higher performance values observed in *Bufo* do not necessarily impart an additional advantage in the pursuit of the same prey *Rana* would consume. Higher performance values do not appear to limit feeding efficacy, although more powerful feeding might not become a constraint if typical prey items tend to move quickly. The result may be a neuromechanical “arms race” of sorts in which the capacity to feed more powerfully could broaden the range of possible prey that are themselves more powerful in their capacities to escape, although answering that question would involve more research into the effects of temperature on the prey species on which the inertial elongators feed.

Feeding ecology is informative in this regard. *Rana* is more aquatic, with an occasional need to lunge over water to capture aerial prey (Gans, 1961), while *Bufo* is more terrestrial and feeds more cryptically on prey that may not jump or fly. In any case, *Rana* appears to feed with a convergent, yet less powerful system than *Bufo* employs,
with apparently little functional constraint imposed by the difference, except for a higher
temperature sensitivity ($Q_{10}$ of 1.06 and 1.20 for the mean velocity of ballistic mouth
opening in *Bufo terrestris* and *Rana piniens*, respectively (Deban and Lappin, 2011)).
Conclusion

*Rana pipiens* possesses an inertial elongation system for tongue prehension of prey. The mechanism’s performance rates demonstrate graded thermal sensitivity depending upon whether a given movement is more elastically or muscually. More muscually dependent movements, such as mouth closing (duration $1/Q_{10} = 2.08$, mean velocity $Q_{10} = 2.17$) have a greater thermal sensitivity than do movements thought to be more reliant on elastic recoil, such as ballistic mouth opening (duration $1/Q_{10} = 1.16$, mean velocity $Q_{10} = 1.20$), suggesting that *Rana* relies upon some elastic recoil to power its jaw depression (and therefore feeding) mechanism. Elastic mechanisms in feeding are an emerging pattern in ectotherms, and the mechanism in *Rana* is convergent with that found in toads, chameleons, and salamanders (Lappin et al., 2006; Anderson and Deban, 2010; Deban and Lappin, 2011; Deban and Richardson, 2011). Insensitivity to temperature in feeding mechanisms allows ectotherms to live in thermally variable environments without consequences for prey capture (Anderson and Deban, 2010).

*Rana pipiens* may use the elastic elements in its legs for some resilience against thermal effects when it lunges at its prey. $Q_{10}$ (for mean velocity, 1.64) and $1/Q_{10}$ (for duration, 1.31) values for lunge-related kinematic and dynamic values are not commensurate with an entirely muscually driven lunge. A partially elastic lunge may offer an advantage over the thermal dependencies of the prey species *Rana* lunges to
capture. The intermediate thermal sensitivity may be a side effect of the engagement of the legs to propel the lunge in a manner similar to jumping (Roberts and Marsh, 2003).

*Rana* and *Bufo* have converged on the inertial elongation mechanism for tongue projection, and measurements of mass-specific power output of the *m. depressor mandibulae* suggest that the differences that distinguish inertial elongation between the taxa accompany differences in the relative mass of the depressor muscle. *Bufo* demonstrates more than twice the mass-specific power output *Rana* does, and *Bufo* appears to have a greater depressor muscle mass-specific mass overall (Deban and Lappin, 2011). The difference in mass-specific power output suggests that the outstanding power values *Bufo* produces are not mandatory for inertial elongation performance, but may explain differences in overall kinematics between the tongue movement in *Rana* and *Bufo*. Why these convergent feeding mechanisms are so divergent in dynamics is still open to question, but *Rana pipiens* appears to have less reliance on elastic recoil than does *Bufo terrestris*, as evidenced by its lower peak mass-specific depressor power output and its higher thermal dependence throughout the gape cycle.
References


Figure 1. Anatomical features and digitized points. The *m. depressor mandibulae* is drawn in approximate anatomical position. Digitizing points are drawn where they were found for analysis. The tips of the upper and lower jaws, the jaw joint, the anatomical tongue tip, and the nearest edge of the cricket were digitized. This figure represents an advanced stage in the feeding cycle, and the nearest edge of the cricket was used for measurement of initial prey distance on only the first frame of the image sequence. The distance between the nearest edge of the cricket and the tip of the upper jaw is the initial prey distance.
Figure 2. Scatterplots of ballistic mouth opening kinematic and dynamic results at experimental temperatures. Q10 trends are represented by red logarithmic fit lines applied to each response variable. Scatterplot columns are aligned along common temperature gridlines. Different markers represent different individuals, and all analyzed feeding events are represented by single points in each plot.
Figure 3. Scatterplots of tongue projection kinematic and dynamic results at experimental temperatures. The plots are formatted as described in the legend of figure 2.
Figure 4. Scatterplots of prey transport and tongue retraction kinematic and dynamic results at experimental temperatures. All plots are formatted as described in the legend of figure 2.
Figure 5. Scatterplots of lunge kinematic and dynamic results at experimental temperatures. All plots are formatted as described in the legend of figure 2.
Figure 6. Montage comparing feedings at 10°C and 25°C. Both image sequences feature the same animal. Projection is shown in 10 msec timesteps, and retraction is shown in 30 msec timesteps. The line drawn after the frames at 30 msec distinguishes the ballistic initial feeding stages from the remainder of the gape cycle. For brevity, the sequences shown begin with the frame immediately before the start of ballistic mouth opening. The timesteps shown may have missed peak or landmark events (e.g., end of tongue projection or ballistic opening). For the sequences shown, the animal finished ballistic mouth opening after approximately 14 msec at 10°C and 10 msec at 25°C. The frog reached peak tongue projection after approximately 27 msec at 10°C and 26 msec at 25°C. The animal completed the full feeding sequence in approximately 282 msec at 10°C and 149 msec at 25°C.
Table 1. Minimum and maximum values for variables measured in the *Rana pipiens* feeding experiments.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Feedings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body Temperature (°C)</td>
<td>10.10</td>
<td>26.70</td>
<td>46 (9, 10, 9, 9, 9)</td>
</tr>
<tr>
<td>Duration of ballistic mouth opening (s)</td>
<td>0.01</td>
<td>0.02</td>
<td>46 (9, 10, 9, 9, 9)</td>
</tr>
<tr>
<td>Gape at end of ballistic mouth opening (m)</td>
<td>0.01</td>
<td>0.02</td>
<td>46 (9, 10, 9, 9, 9)</td>
</tr>
<tr>
<td>Duration of tongue projection (s)</td>
<td>0.02</td>
<td>0.09</td>
<td>46 (9, 10, 9, 9, 9)</td>
</tr>
<tr>
<td>Maximum tongue reach (m)</td>
<td>0.01</td>
<td>0.04</td>
<td>46 (9, 10, 9, 9, 9)</td>
</tr>
<tr>
<td>Duration of transport mouth opening (s)</td>
<td>0.06</td>
<td>0.19</td>
<td>46 (9, 10, 9, 9, 9)</td>
</tr>
<tr>
<td>Final gape at end of transport mouth opening (m)</td>
<td>0.01</td>
<td>0.03</td>
<td>46 (9, 10, 9, 9, 9)</td>
</tr>
<tr>
<td>Duration of tongue retraction (s)</td>
<td>0.06</td>
<td>0.17</td>
<td>46 (9, 10, 9, 9, 9)</td>
</tr>
<tr>
<td>Duration of mouth closing (s)</td>
<td>0.05</td>
<td>0.26</td>
<td>46 (9, 10, 9, 9, 9)</td>
</tr>
<tr>
<td>Mean velocity of ballistic opening (m/s)</td>
<td>0.37</td>
<td>1.58</td>
<td>46 (9, 10, 9, 9, 9)</td>
</tr>
<tr>
<td>Maximum instantaneous velocity of ballistic opening (m/s)</td>
<td>0.25</td>
<td>0.80</td>
<td>46 (9, 10, 9, 9, 9)</td>
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<tr>
<td>Maximum instantaneous acceleration of ballistic opening (m/s/s)</td>
<td>31.30</td>
<td>99.30</td>
<td>46 (9, 10, 9, 9, 9)</td>
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<tr>
<td>Maximum depressor mass-specific power of ballistic opening (W/kg)</td>
<td>115.09</td>
<td>1783.23</td>
<td>46 (9, 10, 9, 9, 9)</td>
</tr>
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<td>Mean velocity of tongue projection (m/s)</td>
<td>0.14</td>
<td>2.10</td>
<td>46 (9, 10, 9, 9, 9)</td>
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<tr>
<td>Mean velocity of tongue retraction (m/s)</td>
<td>0.06</td>
<td>0.54</td>
<td>46 (9, 10, 9, 9, 9)</td>
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<td>Mean velocity of transport opening (m/s)</td>
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<td>0.54</td>
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<tr>
<td>Mean velocity of mouth closing (m/s)</td>
<td>0.06</td>
<td>0.46</td>
<td>46 (9, 10, 9, 9, 9)</td>
</tr>
<tr>
<td>Lunge distance (m)</td>
<td>0.02</td>
<td>0.09</td>
<td>41 (8, 10, 8, 8, 7)</td>
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<tr>
<td>Lunge duration (s)</td>
<td>0.08</td>
<td>0.35</td>
<td>41 (8, 10, 8, 8, 7)</td>
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<tr>
<td>Average lunge velocity (m/s)</td>
<td>0.01</td>
<td>0.36</td>
<td>40 (8, 10, 8, 7, 7)</td>
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</table>

The number of involved feedings is shown for each variable, in total and relative to each animal.
<table>
<thead>
<tr>
<th></th>
<th>Individual Effects</th>
<th>Temperature Effects</th>
<th>Initial Prey Distance</th>
<th>Temperature</th>
<th>Q₁₀</th>
<th>1/Q₁₀</th>
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<tr>
<td>Duration of ballistic mouth opening (s)</td>
<td>4.28 0.0057</td>
<td>7.76 0.0082</td>
<td>0.43 0.5138</td>
<td>6, 45</td>
<td>-0.006</td>
<td>0.86 1.16</td>
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<tr>
<td>Gape at end of ballistic mouth opening (m)</td>
<td>35.47 &lt;0.0001</td>
<td>2.90 0.0963</td>
<td>1.62 0.2102</td>
<td>6, 45</td>
<td>0.002</td>
<td>1.04 0.96</td>
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<tr>
<td>Maximum tongue reach (m)</td>
<td>25.76 &lt;0.0001</td>
<td>22.05 &lt;0.0001</td>
<td>4.16 0.0482</td>
<td>6, 45</td>
<td>0.009</td>
<td>1.24 0.81</td>
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<tr>
<td>Duration of tongue projection (s)</td>
<td>13.15 &lt;0.0001</td>
<td>36.53 &lt;0.0001</td>
<td>0.03 0.8708</td>
<td>10, 45</td>
<td>-0.015</td>
<td>0.70 1.43</td>
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<td>Duration of transport mouth opening (s)</td>
<td>1.90 0.1301</td>
<td>21.24 &lt;0.0001</td>
<td>3.02 0.9010</td>
<td>6, 45</td>
<td>-0.013</td>
<td>0.74 1.35</td>
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<tr>
<td>Final gape at end of transport mouth opening (m)</td>
<td>9.60 &lt;0.0001</td>
<td>1.20 0.2794</td>
<td>1.58 0.2160</td>
<td>6, 45</td>
<td>0.002</td>
<td>1.04 0.96</td>
</tr>
<tr>
<td>Duration of tongue retraction (s)</td>
<td>2.08 0.1022</td>
<td>34.83 &lt;0.0001</td>
<td>2.58 0.1165</td>
<td>6, 45</td>
<td>-0.012</td>
<td>0.75 1.33</td>
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<tr>
<td>Duration of mouth closing (s)</td>
<td>0.72 0.5867</td>
<td>104.63 &lt;0.0001</td>
<td>10.96 0.0020</td>
<td>6, 45</td>
<td>-0.032</td>
<td>0.48 2.08</td>
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<td>Mean velocity of ballistic opening (m/s)</td>
<td>18.03 &lt;0.0001</td>
<td>12.32 0.0011</td>
<td>0.01 0.9058</td>
<td>6, 45</td>
<td>0.008</td>
<td>1.20 0.83</td>
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<tr>
<td>Maximum instantaneous velocity of ballistic opening (m/s)</td>
<td>16.29 &lt;0.0001</td>
<td>11.50 0.0016</td>
<td>4.01 0.0522</td>
<td>6, 45</td>
<td>0.007</td>
<td>1.19 0.84</td>
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<tr>
<td>Maximum instantaneous acceleration of ballistic opening (m/s²)</td>
<td>7.29 0.0002</td>
<td>13.42 0.0007</td>
<td>5.31 0.0266</td>
<td>6, 45</td>
<td>0.012</td>
<td>1.33 0.75</td>
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<tr>
<td>Maximum depressor mass-specific power of ballistic opening (W/kg)</td>
<td>12.17 &lt;0.0001</td>
<td>13.68 0.0007</td>
<td>5.62 0.0228</td>
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<td>0.020</td>
<td>1.57 0.64</td>
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<tr>
<td>Mean velocity of tongue projection (m/s)</td>
<td>27.91 &lt;0.0001</td>
<td>40.99 &lt;0.0001</td>
<td>1.42 0.2403</td>
<td>6, 45</td>
<td>0.022</td>
<td>1.66 0.60</td>
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<td>Mean velocity of tongue retraction (m/s)</td>
<td>16.51 &lt;0.0001</td>
<td>56.86 &lt;0.0001</td>
<td>6.62 0.0140</td>
<td>6, 45</td>
<td>0.022</td>
<td>1.65 0.61</td>
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<td>Mean velocity of transport opening (m/s)</td>
<td>14.89 &lt;0.0001</td>
<td>42.50 &lt;0.0001</td>
<td>0.89 0.3522</td>
<td>6, 45</td>
<td>0.009</td>
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<tr>
<td>Mean velocity of mouth closing (m/s)</td>
<td>2.90 0.0341</td>
<td>72.98 &lt;0.0001</td>
<td>9.94 0.0031</td>
<td>6, 45</td>
<td>0.034</td>
<td>2.17 0.46</td>
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<td>Lunge distance (m)</td>
<td>2.81 0.0409</td>
<td>8.11 0.0074</td>
<td>98.50 &lt;0.0001</td>
<td>6, 40</td>
<td>-0.007</td>
<td>0.85 1.18</td>
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<td>Lunge duration (s)</td>
<td>1.25 0.3099</td>
<td>11.09 0.0021</td>
<td>25.23 &lt;0.0001</td>
<td>6, 40</td>
<td>-0.012</td>
<td>0.76 1.31</td>
</tr>
<tr>
<td>Average lunge velocity (m/s)</td>
<td>3.88 0.0109</td>
<td>7.11 0.0118</td>
<td>11.09 0.0021</td>
<td>6, 39</td>
<td>0.021</td>
<td>1.64 0.61</td>
</tr>
</tbody>
</table>

P-values are presented in bold type for those values that were statistically significant against a Bonferroni-corrected alpha of 0.0029 (0.05 / 17). Inverse Q₁₀ values are highlighted for significant non-rate values, while Q₁₀ values are highlighted for significant rate values. Q₁₀ and inverse Q₁₀ values associated with significance for effect of temperature are shown in bold type.