

# Extreme Performance and Functional Robustness of Movement are Linked to Muscle Architecture: Comparing Elastic and Nonelastic Feeding Movements in Salamanders

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## ABSTRACT

Muscle-powered movements are limited by the contractile properties of muscles and are sensitive to temperature changes. Elastic-recoil mechanisms can both increase performance and mitigate the effects of temperature on performance. Here, we compare feeding movements in two species of plethodontid salamanders, *Bolitoglossa franklini* and *Desmognathus quadramaculatus*, across a range of body temperatures (5–25°C) to better understand the mechanism of elastically powered, thermally robust movements. *Bolitoglossa* exhibited ballistic, elastically powered tongue projection with a maximum muscle mass specific power of 4,642 W kg<sup>-1</sup> while *Desmognathus* demonstrated nonballistic, muscle-powered tongue projection with a maximum power of 359 W kg<sup>-1</sup>. Tongue-projection performance in *Bolitoglossa* was more thermally robust than that of *Desmognathus*, especially below 15°C. The improved performance and thermal robustness of *Bolitoglossa* was associated with morphological changes in the projector muscle, including elaborated collagen aponeuroses and the absence of myofibers attaching directly to the tongue skeleton. The elongated aponeuroses likely increase the capacity for elastic energy storage, and the lack of myofibers inserting on the tongue skeleton permits ballistic projection. These results suggest that relatively simple changes in myofiber architecture and the amount of connective tissue can improve the performance and functional robustness of movements in the face of environmental challenges such as variable temperature. *J. Exp. Zool.* 0:1–17, 2016. © 2016 Wiley Periodicals, Inc.

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## INTRODUCTION

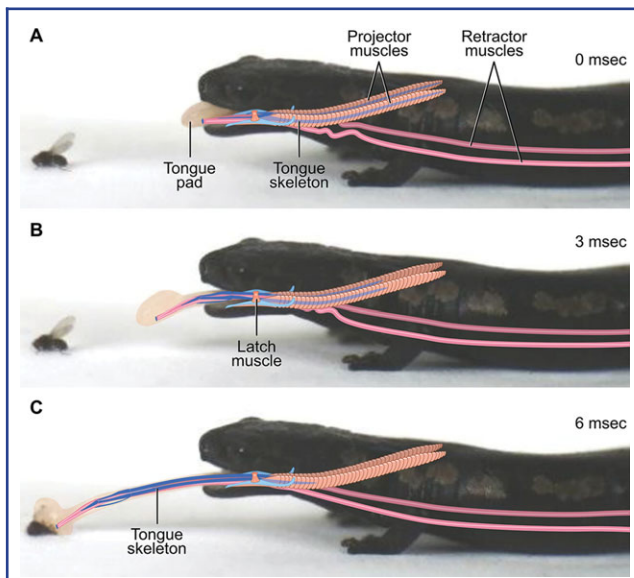
The speed and power of movement in animals can be limited by the contractile properties of skeletal muscle, such as the peak velocity and power of shortening (Josephson, '93; James et al., 2007). However, many organisms rely on rapid movements for survival (e.g., jumping, prey capture) that attain velocities and accelerations that require power well in excess of what muscle alone can produce (Bennett-Clark, '76; Aerts, '98; Askew and Marsh, 2001; de Groot and van Leeuwen, 2004; Deban et al., 2007; Zack et al., 2009). These high-powered movements are often accomplished via elastic mechanisms in which muscle performs the work required for movement on elastic tissue. The elastic tissue stores this energy and subsequently releases it at a much higher rate during recoil, thus amplifying muscle power (Alexander and Bennet-Clark, '77; Alexander, 2002; Deban et al., 2007). Elastic-recoil mechanisms can thereby overcome the limitations of muscle contractile rate properties and significantly improve performance.

Elastic systems clearly augment organismal performance, but they can also circumvent thermal constraints on muscle contractile performance. Decreases in temperature reduce muscle contractile rates, which can decline by half over a 10°C drop in temperature (Bennett, '85). Reduced temperature and the consequently lower muscle contractile rates can in turn cause substantial decreases in whole-organism performance (e.g., running, swimming) (Huey and Bennett, '87; Rome et al., '90). Elastic-recoil mechanisms mitigate these temperature effects by decoupling the rate of muscle contraction from the rate of movement, thereby reducing thermal sensitivity (Alexander and Bennet-Clark '77; Deban et al., '97, de Groot and van Leeuwen, 2004; Deban and Richardson, 2011). A striking example of this is elastically powered tongue projection in the salamander *Hydromantes platycephalus*, which exhibits thermal independence of dynamic performance across a roughly 20°C change in body temperature ( $Q_{10} \sim 1$ ) (Deban and Richardson, 2011). This thermal robustness of performance is a significant improvement compared to the high temperature sensitivity of muscle contraction ( $Q_{10} > 2$ ) (Bennett '85) and muscle-powered activities such as running ( $Q_{10} > 1.6$ ) (Huey and Stevenson, '79; Bennett, '84, '90; Hirano and Rome, '84; Herrel et al., 2007).

Elastically powered, ballistic tongue projection has evolved multiple times independently in chameleons, frogs, and salamanders, and in each of these cases, tongue-projection performance shows reduced thermal dependence compared to muscle-powered movements (Anderson and Deban, 2010, 2012; Deban and Lappin, 2011; Deban and Richardson, 2011; Sandusky and Deban, 2012; Anderson et al., 2014). Because these in-

dependently evolved systems are divergent in anatomy, it has been difficult to determine which morphological features confer enhanced performance and thermal robustness. To better understand the morphology underlying the improved thermal robustness and performance of elastic systems, we compare tongue-projection performance in two species of plethodontid salamanders across a range of body temperatures: ballistic-tongued *Bolitoglossa franklini* and nonballistic-tongued *Desmognathus quadramaculatus*. These species have similar gross morphology of the tongue projection apparatus but differ in performance and tongue-projector-muscle architecture.

Many species of salamanders in the family Plethodontidae exhibit high tongue-projection performance relative to other salamanders in both distance and velocity (Wake and Deban, 2000; Deban, 2002; Deban and Scales, 2016). Tongue projection often takes less than 10 msec and can reach a distance of up to 80% of body length (Deban et al., '97). The morphology and mechanism of tongue projection are thoroughly explained elsewhere (Lombard and Wake, '77; Deban et al., '97) and accordingly are only briefly described here. The general mechanism of tongue projection in plethodontid salamanders involves the articulated tongue skeleton and tongue pad being launched out of the mouth by cylindrical projector muscles (paired subarcualis rectus, SAR, Fig. 1). Each tongue projector muscle encircles one of the paired, elongated epibranchial cartilages that extend caudally from the buccal region. Each epibranchial attaches rostrally to paired ceratobranchials, which then connect rostrally to the unpaired basibranchial that sits in the floor of the mouth and supports the tongue pad (Fig. 1, Lombard and Wake, '77; Wake and Deban, 2000; Deban 2002). During nonelastic, nonballistic projection, activation of the SAR is coupled with tongue movement and the epibranchial does not leave the SAR (Deban et al. 2007; Deban and Scales, 2016). Conversely, in elastically powered projection, activation of the SAR muscle occurs 80–200 msec prior to tongue launch. This early activation of the SAR is thought to stretch collagenous aponeuroses within the muscles, which exert force on the epibranchials (Deban et al., 2007). The subsequent elastic recoil of these aponeuroses then accelerates the tongue out of the mouth with power of up to 18,000 W kg<sup>-1</sup> muscle mass (Deban et al., 2007). The epibranchials completely separate from the SAR and full tongue projection relies on momentum to carry the tongue skeleton and pad to the prey (Fig. 1, Deban et al., '97). Tongue retraction in all plethodontid salamanders is performed by retraction of the paired, elongated rectus cervicis profundus (RCP) muscles that originate on the pelvis (Fig. 1, Lombard and Wake, '77).



**Figure 1.** Tongue projection morphology of *Bolitoglossa franklini* showing the tongue skeleton (basibranchial, ceratobranchials and epibranchials, all dark blue) and the paired projector (SAR, peach) and retractor muscles (RCP, pink) in their approximate configurations at three stages of tongue projection: (A) Start of ballistic projection at 0 msec, in which the epibranchials are almost fully encompassed by the cylindrical SAR muscles, which originate on the paired ceratohyals (light blue) in the floor of the mouth; the RCP originates on the pelvis (not shown), is slack in the region of the heart, and inserts into the tongue pad (highlighted in beige). (B) At 3 msec into projection, the tongue skeleton has been pushed rostrally and the tongue pad is accelerating out of the mouth. (C) At 6 msec, the epibranchials have been projected almost completely out of the SAR muscle and the tongue pad has reached the prey. Note that the ceratohyals and SAR muscles move little during projection. The RCP is now taut and prepared to pull the tongue back into the mouth and to return the epibranchials to the SAR muscles. Image sequence is the same as in Fig. 3.

Elastically powered, ballistic tongue projection has evolved independently at least twice within the family Plethodontidae and has been documented in *Hydromantes*, *Eurycea*, *Ensatina*, and *Bolitoglossa* (Deban et al., 2007; Vieites et al., 2011; Anderson et al., 2014; Deban and Scales, 2016). Plethodontidae is divided basally into two subfamilies, Plethodontinae, which includes the genera *Ensatina*, *Hydromantes*, and *Desmognathus*, among others, and Hemidactyliinae, which includes *Eurycea* and *Bolitoglossa*, among several others. *Desmognathus* has epibranchials that extend only to the back of the head and a large tongue pad that is attached to the lower jaw by the genioglossus muscle, demonstrating a tongue morphology similar to the ancestral condition (Lombard and Wake, '77). Conversely,

*Bolitoglossa* has a relatively small tongue pad that is free from the lower jaw, coupled with long epibranchials that extend to the shoulder (Fig. 1) (Lombard and Wake, '77), and has demonstrated high-performance tongue projection (Deban et al., 2007). Therefore, a comparison between *Desmognathus* and *Bolitoglossa* affords an opportunity to evaluate which morphological traits are associated with high performance, thermally robust tongue projection as well as provide insights into how elastic-recoil mechanisms evolve in musculoskeletal systems.

## MATERIALS AND METHODS

### Specimens

*Bolitoglossa franklini* and *D. quadramaculatus* were collected from natural populations in Chiapas, Mexico and North Carolina, USA, respectively, and housed individually in plastic containers with a substrate of moist paper towels at 14–17°C. *Bolitoglossa* was maintained on a diet of fruit flies, while *Desmognathus* was fed gut-loaded crickets. Six individuals of *Bolitoglossa* (Snout-vent length [SVL] 54.3–64.6 mm) and six individuals of *Desmognathus* (SVL 78.5–99.2 mm) that fed readily under observation were used for feeding experiments. All procedures were approved by the Institutional Animal Care and Use Committee of the University of South Florida.

### Morphology

Three *Bolitoglossa* and nine *Desmognathus* were euthanized by immersion in a 2 g/L aqueous solution of MS-222 (tricaine methanesulfonate; Sigma, St Louis, MO, USA) buffered with sodium bicarbonate. For each individual, body mass was measured using a balance (Virtual Measurements and Control model VB-302A, Santa Rosa, CA, USA;  $\pm 0.001$  g accuracy), and SVL (the distance from the rostrum tip to the posterior extent of the vent) was measured using digital calipers (Mitutoyo 700-126, Kawasaki-shi, Kanagawa, Japan;  $\pm 0.1$  mm accuracy). The salamander's tongue skeleton was then extended out of the mouth to maximum projection distance; maximum tongue extension was achieved by suspending the body weight of the specimen from the tongue, and was a repeatable measure of the maximum possible extent of tongue projection. Three lengths were measured: (1) the length of the extended tongue from basibranchial tip to the rostral tip of the mandible, (2) the length of the extended tongue skeleton from basibranchial tip to epibranchial tip, and (3) the length of the longer epibranchial. The tongue apparatus was removed by cutting the retractor (RCP) muscles at the level of the epibranchial tips, severing the genioglossus muscle, if present, at the origin on the lower jaw, and peeling the tongue projector (SAR) muscles from their origins on the ceratohyals. The paired SAR muscles were removed from the excised tongue apparatus and their mass was measured. The mass of the excised tongue apparatus, including tongue skeleton plus tongue pad and a portion of the RCP but without the SAR muscles, was mea-

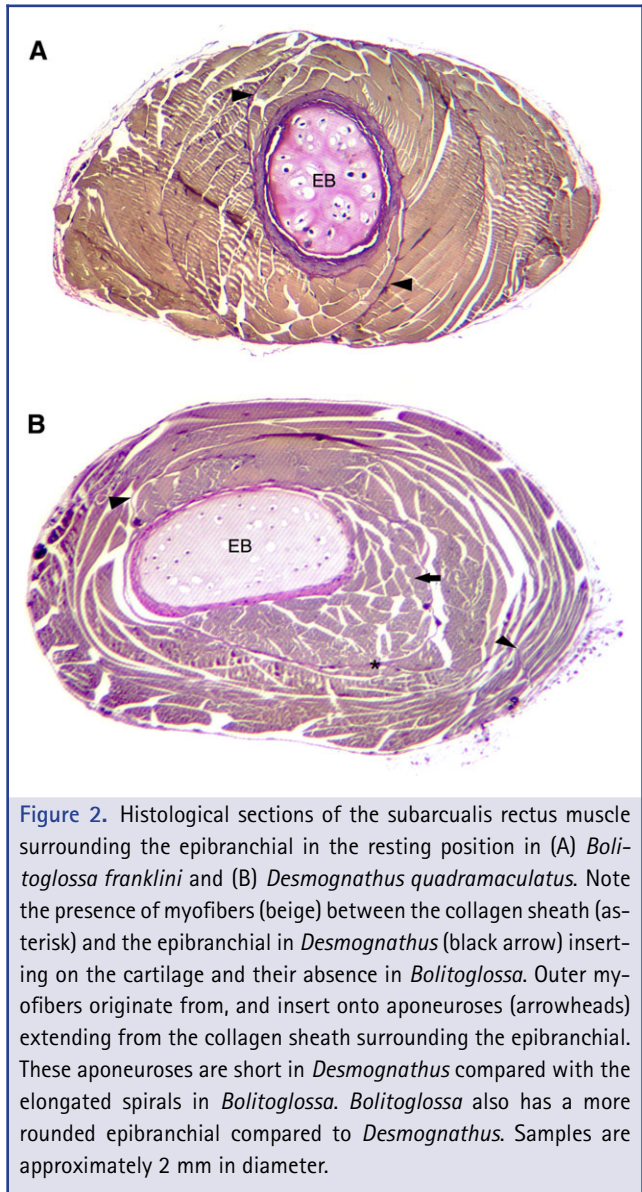
sured. The remaining lengths of RCP were severed at their origin on the pelvis, freed from surrounding tissue, and their mass was measured. The ratios of tongue apparatus to muscle mass were used in the calculations of muscle mass specific power and work.

The SAR muscles from two *Desmognathus* and one *Bolitoglossa* were fixed in neutral-buffered formalin and embedded in paraffin for histological staining. Samples were sectioned at 5–10  $\mu\text{m}$  thickness and stained with Verhoeff and Van Gieson stains for elastin and collagen, with a Weigert's iodine counterstain to allow visualization of myofibers and their relationships to the epibranchial cartilage and connective tissue. Sections were examined for the presence of SAR myofibers inserting directly onto the epibranchial cartilage (i.e., inner myofibers) in addition to the myofibers surrounding the epibranchial, but not attaching directly to it (i.e., outer myofibers). The outer myofibers insert on a pair of aponeuroses that form arches or spirals that extend from the collagen sheath surrounding the epibranchial to the periphery of the SAR muscle. The spirality of each aponeuroses was measured as the angle subtended by the beginning and end of the spiral with the center of the epibranchial at the vertex (Fig. 2), and the average spirality angle was taken for each species.

#### Feeding Experiments

Salamanders were imaged individually in dorsal view at 6 kHz frame rate and 1/12,000 sec shutter speed with a Fastcam 1024 PCI camera (Photron USA Inc., San Diego, CA, USA) as they captured individual prey, which included fruit flies for *Bolitoglossa* and crickets (0.015–0.030 g and 5.8–8.5 mm long) for *Desmognathus*. Salamanders were placed on moistened paper printed with a 5 mm grid for distance calibration, set on the surface of a temperature-controlled platform (ThermoElectric Cooling America Corporation #AHP-1200CPV, Chicago, IL, USA). Prey items were placed at varying distances in front of the salamanders and salamanders were permitted to approach the prey prior to the start of prey capture. Salamanders, prey, and substrate were illuminated by two infrared LED lights (LED infrared illuminator IR-200; Speco Technologies, Amityville, NY, USA) that provided cool light to avoid warming the salamanders.

Feeding trials were conducted across a range of experimental temperatures (5, 10, 15, 20, and 25°C) by varying the surface temperature of the feeding platform. Each salamander was allowed to acclimate at the experimental temperature for a period of at least 20 min prior to feeding trials. The salamander rested with its ventral surface against a moistened paper on the temperature platform, so its body temperature closely matched the temperature of the platform ( $\pm 1^\circ\text{C}$ ). Body temperature was measured by directing a calibrated infrared thermometer (Sixth Sense LT300, Williston, VT, USA;  $\pm 1^\circ\text{C}$  accuracy) at the dorsal surface of the head following each feeding event. The temperature sequence of feeding trials for each individual was randomized with one to three feedings recorded per experimental tempera-



**Figure 2.** Histological sections of the subarcualis rectus muscle surrounding the epibranchial in the resting position in (A) *Bolitoglossa franklini* and (B) *Desmognathus quadramaculatus*. Note the presence of myofibers (beige) between the collagen sheath (asterisk) and the epibranchial in *Desmognathus* (black arrow) inserting on the cartilage and their absence in *Bolitoglossa*. Outer myofibers originate from, and insert onto aponeuroses (arrowheads) extending from the collagen sheath surrounding the epibranchial. These aponeuroses are short in *Desmognathus* compared with the elongated spirals in *Bolitoglossa*. *Bolitoglossa* also has a more rounded epibranchial compared to *Desmognathus*. Samples are approximately 2 mm in diameter.

ture before attempting a different, randomly selected temperature. Salamander body temperatures ranged from 4.4 to 25.6°C. Digital image sequences were obtained from 151 feedings of *Bolitoglossa* and 141 feedings of *Desmognathus*.

#### Kinematic and Dynamic Analysis

The digital image sequences were used to quantify the projection and retraction movements of the tongue during prey capture. The  $x$  and  $y$  coordinates of the tongue tip and the tip of the upper jaw were recorded from the image sequences using ImageJ software (National Institute of Health, Bethesda, MD, USA) running on an Apple iMac computer. Tongue-projection

distance was computed as the straight-line distance between the upper jaw tip and tongue tip in each image of the feeding sequence. Coordinates were recorded beginning with the first appearance of the tongue beyond the upper jaw during tongue projection and ending with the withdrawal of the tongue pad into the mouth at the end of tongue retraction. A 5-mm grid printed on the substrate was used to calibrate distances for each feeding. The times of two events in the image sequences were measured relative to the start of tongue projection at time zero: (1) maximum tongue projection, the time at which the leading edge of the tongue pad was the greatest distance from the tip of the upper jaw, and (2) the end of tongue retraction, the time at which the tongue pad was withdrawn past the jaw tips following tongue projection. Duration of tongue projection is equivalent to time of event 1 and the duration of tongue retraction was calculated as time of event 2 minus time of event 1. Average velocity of tongue projection and tongue retraction was calculated as the tongue-projection distance divided by these durations, respectively.

The dynamics of tongue movements were calculated using published methods (Deban and Lappin, 2011; Anderson et al., 2014) by fitting a quintic spline to the distance–time data using the Pspline package in R statistical software (R Development Core Team, 2013) ([www.r-project.org](http://www.r-project.org)). First and second derivatives of the spline function were computed to yield instantaneous velocity and acceleration, respectively, at an interpolated rate of 10 kHz. The smoothing parameter of the spline was adjusted separately for tongue projection and tongue retraction of each feeding event to remove secondary oscillation artifacts from the velocity and acceleration traces. Instantaneous mass-specific power was calculated as the product of the velocity at each point in time and its corresponding acceleration at the same point in time.

Total tongue mass specific kinetic energy during tongue projection was then calculated as half the squared maximum projection velocity (kinetic energy calculated as the time integral of the power curve yielded virtually identical values). Total muscle mass specific kinetic energy and maximum muscle mass specific power during tongue projection were calculated by multiplying these tongue mass specific values by the average ratio of the mass of the tongue projectile to the mass of the SAR muscles ( $3.7 \pm 0.2$  [mean  $\pm$  SEM] for *Desmognathus* and  $0.7 \pm 0.2$  for *Bolitoglossa*). Muscle mass specific power achieved during tongue retraction was calculated by multiplying the power by the average ratio of the mass of the tongue projectile to the mass of the RCP muscles ( $0.8 \pm 0.1$  for *Desmognathus* and  $0.6 \pm 0.3$  for *Bolitoglossa*). Mean and maximum values of velocity and acceleration, and total kinetic energy and maximum power were used to examine the effects of temperature and projection distance in each species.

### Statistical Analyses

Kinematic and dynamic data examined for temperature effects were  $\log_{10}$  transformed prior to statistical analysis because biological rates are expected to have an exponential relationship with temperature. Data were divided into four overlapping intervals (5–15, 10–20, 15–25, and 5–25°C, each  $\pm 1^\circ\text{C}$ ) based on the body temperature at which the data were gathered to examine whether the effects of temperature varied across the full temperature range. An analysis of covariance (ANCOVA) was conducted separately on each subset of the data.

Performance data were tested for three effects: (1) temperature, (2) individual, and (3) projection distance. Individual was included in the model as a random effect to account for body size and other random individual differences. Measured body temperature as a continuous variable was included as a fixed effect to examine how kinematic and dynamic variables responded to changes in body temperature. Projection distance was included as a covariate because it has been found to correlate with performance measures in salamander feeding (Deban and Richardson, 2011; Deban and Scales, 2016), but was dropped from the model when not significant for a given variable to increase statistical power.

Temperature coefficients ( $Q_{10}$ ) were computed for each variable across each temperature interval as the base 10 antilogarithm of the partial regression coefficients of the temperature effect in the ANCOVAs multiplied by 10 (Deban and Lappin, 2011; Deban and Richardson, 2011; Anderson and Deban, 2012; Sandusky and Deban, 2012; Anderson et al., 2014). The ANCOVA models include effects of individual (and projection distance for relevant performance data) that influence the estimate of the relationship between the variable and temperature, so calculation of  $Q_{10}$  values from the partial regression coefficients accounts for these effects as well. The temperature coefficients for durations were reported as inverse  $Q_{10}$  values (i.e.,  $1/Q_{10}$ ) to express them as responses of rates.

Differences in the thermal robustness of feeding movements between *Desmognathus* and *Bolitoglossa* were examined using ANCOVA for each variable to test for effects of species  $\times$  temperature interactions in each of the four temperature intervals, including individual as a random effect and projection distance as a covariate in the model. Species differences in feeding movements at each nominal experimental temperature were examined using a separate analysis of variance (ANOVA) for each temperature that included effects of species with individual nested within species.

Differences in morphological measurements of the tongue apparatus between *Desmognathus* and *Bolitoglossa* were examined using ANOVA as well as ANCOVA with body length (SVL), body mass, or muscle masses as covariates to compare relative dimensions (Packard and Boardman, '99). Measurements compared between species included tongue mass, SAR mass, SAR

**Table 1.** Morphological measurements of *Desmognathus quadramaculatus* and *Bolitoglossa franklini* and tests of species differences

	<i>Desmognathus</i>		<i>Bolitoglossa</i>		Species		Species with covariate		
	Mean $\pm$ SEM	N	Mean $\pm$ SEM	N	F-ratio	P-value	F-ratio	P-value	Covariate
Body mass (g)	14.16 $\pm$ 1.94	6	4.44 $\pm$ 0.4	6	28.82	<0.0001*	-	-	-
Snout-vent length (mm)	89.1 $\pm$ 3.5	6	60.8 $\pm$ 1.5	6	63.15	<0.0001*	-	-	-
Extended tongue length (mm)	7.8 $\pm$ 0.9	9	19.0 $\pm$ 1.7	3	37.86	0.0002*	34.66	0.0004*	SVL
Tongue skeleton length (mm)	17.9 $\pm$ 0.5	9	17.1 $\pm$ 1.2	3	0.46	0.5120	0.81	0.3919	SVL
Epibranchial length (mm)	6.2 $\pm$ 0.5	9	11.0 $\pm$ 0.7	3	26.07	0.0005*	91.35	<0.0001*	SVL
Tongue mass (mg)	125.3 $\pm$ 18.3	9	26.0 $\pm$ 3.1	3	9.20	0.0126*	15.34	0.0035*	Body mass
SAR mass (mg)	34.2 $\pm$ 4.8	9	40.0 $\pm$ 10.0	3	0.34	0.5760	0.63	0.4467	Body mass
RCP mass (mg)	169.1 $\pm$ 18.4	9	65.0 $\pm$ 21.1	3	8.98	0.0134*	21.44	0.0012	Body mass
Tongue mass vs. SAR mass	-	9	-	3	-	-	18.17	0.0021*	SAR mass
Tongue mass vs. RCP mass	-	9	-	3	-	-	10.13	0.0111*	RCP mass
Tongue mass/SAR mass	3.7 $\pm$ 0.2	9	0.7 $\pm$ 0.2	3	-	-	-	-	-
Tongue mass/RCP mass	0.8 $\pm$ 0.1	9	0.6 $\pm$ 0.3	3	-	-	-	-	-
SAR aponeurosis spirality ( $^{\circ}$ )	17.4 $\pm$ 8.8	2	85.2	1	-	-	-	-	-

\*Significant species difference after adjusting for false discovery rate. ANCOVAs comparing species include indicated covariates.

aponeurosis spirality angle, RCP mass, tongue skeleton length, epibranchial length, and extended tongue length.

All statistical analyses were performed using R statistical software version 3.1.1 (R Development Core Team, 2013) (www.r-project.org) on an Apple iMac computer. Significance levels were adjusted to control for false discovery rate (Benjamini and Hochberg, '95) when multiple comparisons were made within each temperature or temperature interval.

## RESULTS

### Morphology of *Desmognathus* and *Bolitoglossa*

Tongue morphology differs between *Desmognathus*, which exhibits nonballistic tongue projection, and *Bolitoglossa*, which has ballistic tongue projection. The *Desmognathus* used in this study was significantly larger than the *Bolitoglossa* in both body mass and SVL (Table 1). *Bolitoglossa*, however, had significantly longer relative and absolute extended tongue lengths than *Desmognathus* (Table 1). The greater extended tongue lengths of *Bolitoglossa* are not a result of a longer tongue skeleton, because the two species did not differ in this trait. However, *Bolitoglossa* did have significantly longer epibranchials that make up a greater portion of their tongue skeleton length (Table 1).

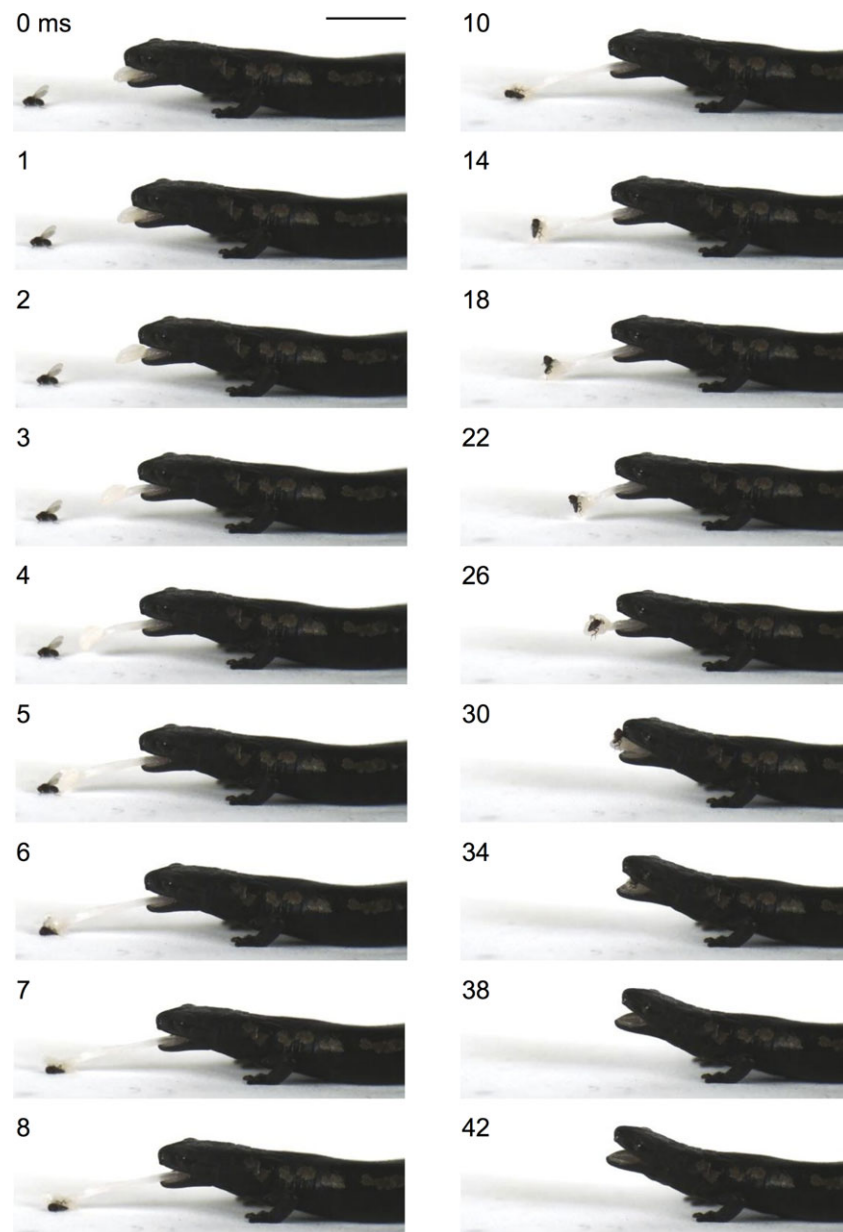
*Bolitoglossa* has more muscle mass for both tongue projection and retraction relative to tongue size compared to *Desmognathus*. The mass of the SAR muscles did not differ significantly between *Bolitoglossa* and *Desmognathus* (Table 1), but the tongue mass of *Desmognathus* was significantly greater than that of *Bolitoglossa* when accounting for SAR mass as a covariate. In addition, the ratio of tongue mass to SAR mass was more than five times smaller in *Bolitoglossa* (0.7) than in *Desmog-*

*nathus* (3.7). *Desmognathus* also had significantly more massive RCP muscles and tongue than *Bolitoglossa* both relative to body mass and absolutely (Table 1). Tongue mass remained significantly greater in *Desmognathus* even when accounting for RCP mass as a covariate (Table 1). Thus, *Desmognathus* also has lower relative muscle mass for tongue retraction compared to *Bolitoglossa*.

The morphology of the SAR muscles also differed between *Desmognathus* and *Bolitoglossa*. While both species have myofibers surrounding the epibranchial that are separated from it by a collagenous sheath, the SAR of *Desmognathus* has additional myofibers within this sheath that insert directly onto the epibranchial (Fig. 2). These inner fibers are absent in *Bolitoglossa*. Two collagenous aponeuroses extend from the collagenous sheath to the periphery of the muscle in both species (Fig. 2). The aponeuroses of *Bolitoglossa* subtended an angle of 85.2 $^{\circ}$ , higher than the mean angle of 17.4 $^{\circ}$  in *Desmognathus* (Table 1) indicating that *Bolitoglossa* has more elastic tissue in series with myofibers in the SAR than does *Desmognathus*.

### Feeding in *Desmognathus* and *Bolitoglossa*

*Desmognathus* and *Bolitoglossa* successfully fed at all experimental temperatures (5, 10, 15, 20, and 25 $^{\circ}$ C) using tongue projection. Feedings generally consisted of salamanders orienting toward the prey and rapidly projecting the tongue from the mouth toward the prey (Fig. 3). Upon contact, the tongue and prey were retracted into the mouth. The two species differed during feedings in that a whole-body lunge toward the prey typically accompanied tongue projection and retraction in *Desmognathus* while this behavior was absent in *Bolitoglossa*.



**Figure 3.** Image sequence of *Bolitoglossa* demonstrating ballistic tongue projection to capture a fruit fly at 19°C. This projection of 14 mm or 24% of SVL was completed in 8 msec, while retraction required approximately 32 msec. Note the small tongue pad and its lack of muscular connection to lower jaw. The time step is 1 msec for projection (left column) and 4 msec for retraction (right). Scale bar is 1 cm.

#### Prey Capture in *Desmognathus*

A total of 141 feedings of *Desmognathus* were recorded from six individuals across the entire experimental range of temperatures (5–25°C). Tongue-projection distance ranged from 4.0 to 11.9 mm over durations of 20.2–154.7 msec (Table 2). Average projection velocity reached  $0.53 \text{ msec}^{-1}$  (25°C) and maximum velocity peaked at  $0.94 \text{ msec}^{-1}$  (20°C). Maximum projection acceleration

reached  $153 \text{ msec}^{-2}$  (20°C) with maximum instantaneous muscle mass specific power peaking at  $358.71 \text{ W kg}^{-1}$  (20°C) and maximum tongue mass specific projection kinetic energy reaching  $0.438 \text{ J kg}^{-1}$  (20°C, Table 2).

The velocity and acceleration of tongue retraction tended to be greater than those of projection in *Desmognathus*; however, there was considerable overlap in performance

Table 2. Summary statistics of performance variables at each experimental temperature in *Desmognathus quadramaculatus* and *Bolitoglossa franklini*

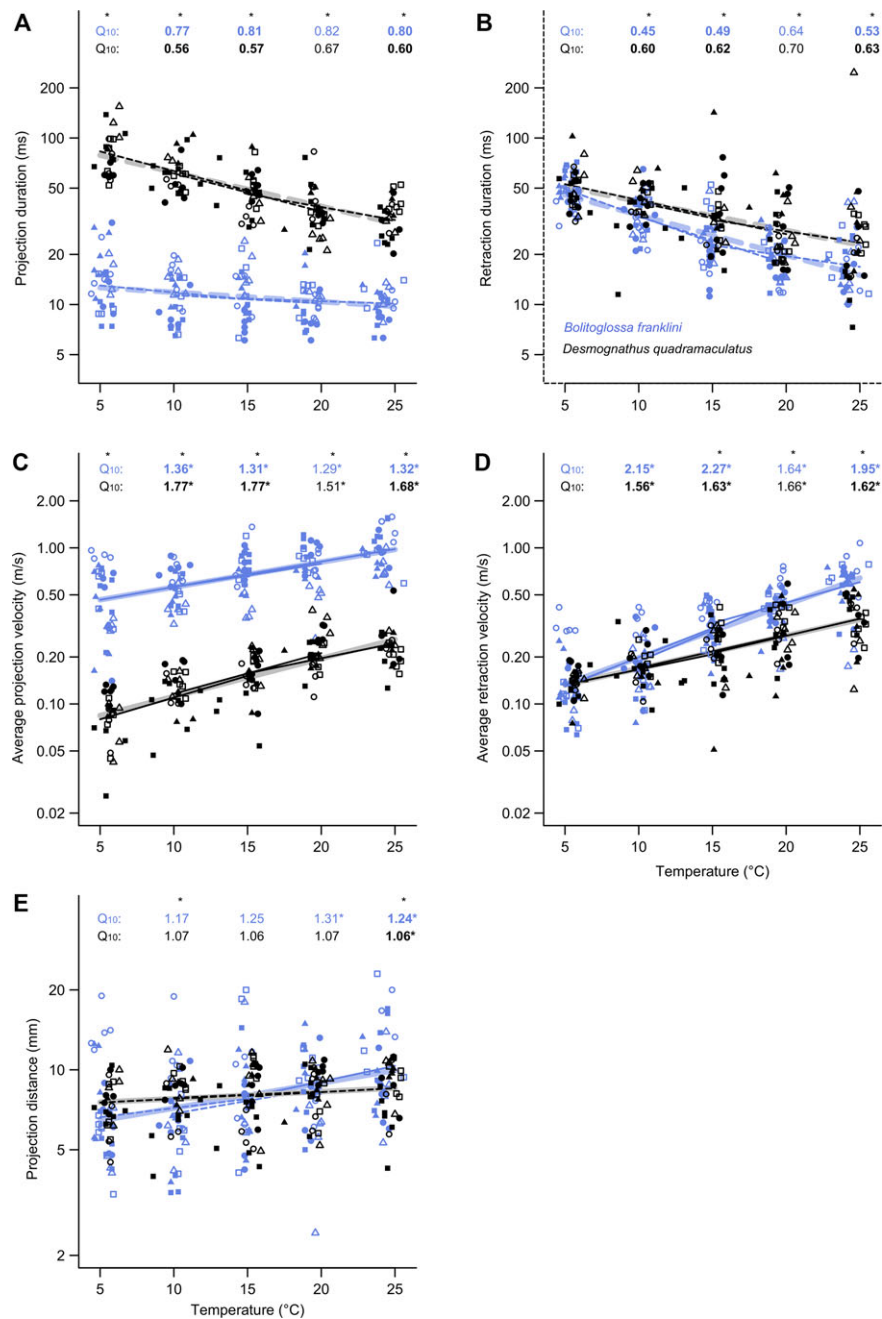
	5°C					10°C					15°C					20°C					25°C					
	Mean	SEM	N	Min	Max	Mean	SEM	N	Min	Max	Mean	SEM	N	Min	Max	Mean	SEM	N	Min	Max	Mean	SEM	N	Min	Max	
<i>Desmognathus</i>																										
Projection distance (mm)	7.6	0.3	25	4.5	10.4	8.1	0.3	28	4.0	11.9	8.2	0.4	31	4.3	11.6	8.5	0.3	31	5.2	10.9	8.6	0.4	26	4.3	11.2	
Projection duration (msec)	84.2	5.3	25	52.0	154.7	62.3	3.1	28	41.0	104.5	48.9	2.7	31	29.2	88.4	38.4	2.5	31	21.1	82.9	35.0	1.7	26	20.2	52.4	
Average projection velocity (msec <sup>-2</sup> )	0.09	0.01	25	0.03	0.13	0.13	0.01	28	0.05	0.19	0.16	0.01	31	0.05	0.23	0.22	0.01	31	0.11	0.40	0.23	0.01	26	0.13	0.53	
Maximum projection velocity (msec <sup>-2</sup> )	0.18	0.01	25	0.10	0.38	0.24	0.01	28	0.10	0.37	0.33	0.01	31	0.21	0.47	0.44	0.03	31	0.19	0.94	0.47	0.03	26	0.21	0.71	
Maximum projection acceleration (msec <sup>-2</sup> )	24.0	5.3	25	3.5	105.0	33.6	4.4	28	3.6	95.7	45.2	5.0	31	9.7	118.0	65.5	6.9	31	6.8	153.0	66.3	6.2	26	8.8	137.0	
Maximum tongue mass specific projection power (W/kg)	3.64	1.43	25	0.32	34.50	5.79	1.02	28	0.17	20.90	10.28	1.32	31	1.61	32.30	22.21	3.98	31	1.54	97.30	20.72	2.82	26	2.35	65.30	
Maximum muscle mass specific projection power (W/kg)	13.43	5.28	25	1.19	127.19	21.34	3.76	28	0.62	77.05	37.91	4.85	31	5.94	119.08	81.90	14.67	31	5.68	358.71	76.39	10.41	26	8.66	240.74	
Projection energy (mJ)	2E-06	4E-07	25	6E-07	9E-06	4E-06	4E-07	28	7E-07	8E-06	7E-06	5E-07	31	3E-06	1E-05	1E-05	2E-06	31	2E-06	5E-05	1E-05	2E-06	26	3E-06	3E-05	
Tongue mass specific projection energy (J/kg)	0.019	0.003	25	0.005	0.073	0.030	0.003	28	0.005	0.067	0.058	0.004	31	0.021	0.111	0.107	0.015	31	0.019	0.438	0.116	0.012	26	0.022	0.251	
Muscle mass specific projection energy (J/kg)	0.068	0.011	25	0.018	0.269	0.111	0.011	28	0.019	0.246	0.214	0.015	31	0.078	0.411	0.393	0.055	31	0.069	1.615	0.429	0.045	26	0.081	0.924	
Retraction duration (ms)	51.2	3.2	25	31.5	102.3	42.4	2.2	28	11.5	65.5	36.1	4.2	31	16.0	142.1	28.8	2.1	31	16.1	61.3	32.7	8.8	26	7.3	247.1	
Average retraction velocity (m/s)	0.14	0.01	25	0.07	0.19	0.18	0.01	28	0.09	0.34	0.23	0.02	31	0.05	0.42	0.30	0.02	31	0.11	0.59	0.36	0.02	26	0.12	0.54	
Maximum retraction velocity (m/s)	0.30	0.02	25	0.17	0.59	0.38	0.02	28	0.22	0.76	0.49	0.03	31	0.16	1.01	0.68	0.04	31	0.23	1.10	0.74	0.04	26	0.20	1.18	
Maximum retraction acceleration (msec <sup>-2</sup> )	43.3	8.9	25	7.3	171.0	47.60	8.3	28	15.6	250.0	65.0	6.9	31	4.2	182.0	112.3	11.9	31	13.0	302.0	144.5	17.8	26	11.6	343.0	
Maximum tongue mass specific retraction power (W/kg)	0.14	3.61	25	0.94	66.30	15.85	4.15	28	2.17	119.00	24.94	3.86	31	0.46	102.00	64.93	9.23	31	3.03	229.00	77.68	12.03	26	2.31	259.00	
Maximum muscle mass specific retraction power (W/kg)	9.06	2.78	25	0.73	51.18	12.24	3.21	28	1.68	91.87	19.25	2.98	31	0.36	78.75	50.13	7.13	31	2.34	176.79	59.97	9.29	26	1.78	199.95	

(Continued)



Table 2. Continued

	5°C					10°C					15°C					20°C					25°C					
	Mean	SEM	N	Min	Max	Mean	SEM	N	Min	Max	Mean	SEM	N	Min	Max	Mean	SEM	N	Min	Max	Mean	SEM	N	Min	Max	
	<i>Bolitoglossa</i>																									
Projection distance (mm)	7.6	0.7	32	3.4	19.0	7.2	0.6	30	3.5	18.9	8.9	0.8	29	4.1	20.0	8.6	0.5	30	2.4	14.9	11.2	0.8	30	5.3	23.0	
Projection duration (msec)	14.4	1.1	32	7.4	31.0	11.8	0.7	30	6.5	19.7	11.8	0.9	29	6.1	24.0	10.5	0.6	30	6.1	20.3	10.7	0.6	30	6.3	23.4	
Average projection velocity (m/sec)	0.53	0.04	32	0.14	0.96	0.57	0.03	30	0.32	0.89	0.72	0.04	29	0.36	1.36	0.79	0.04	30	0.26	1.21	1.00	0.06	30	0.58	1.58	
Maximum projection velocity (m/sec)	1.61	0.10	32	0.71	3.26	1.34	0.08	30	0.73	2.61	1.68	0.13	29	0.97	3.87	1.81	0.08	30	1.12	3.05	2.34	0.13	30	1.26	3.97	
Maximum tongue projection acceleration (msec <sup>-2</sup> )	916	108	32	184	2,660	621	55	30	224	1,560	779	62	29	252	1,600	921	94	30	302	2,470	1,100	93	30	368	3,140	
Maximum tongue mass specific projection power (W/kg)	1,128	171	32	111	3,780	589	94	30	143	2,610	947	168	29	190	4,310	1,184	161	30	216	3,650	1,800	243	30	317	6,510	
Maximum muscle mass specific projection power (W/kg)	805	122	32	79	2,695	420	67	30	102	1,861	675	119	29	135	3,073	844	115	30	154	2,602	1,284	173	30	226	4,642	
Projection energy (mJ)	4E-05	4E-06	32	6E-06	1E-04	2E-05	3E-06	30	6E-06	8E-05	4E-05	7E-06	29	1E-05	2E-04	4E-05	4E-06	30	2E-05	1E-04	7E-05	8E-06	30	2E-05	2E-04	
Tongue mass specific projection energy (J/kg)	1.46	0.18	32	0.25	5.31	0.99	0.13	30	0.26	3.41	1.65	0.29	29	0.47	7.49	1.73	0.15	30	0.63	4.65	2.98	0.33	30	0.79	7.88	
Muscle mass specific projection energy (J/kg)	1.04	0.13	32	0.18	3.79	0.71	0.09	30	0.19	2.43	1.17	0.21	29	0.34	5.34	1.23	0.11	30	0.45	3.32	2.12	0.24	30	0.57	5.62	
Retraction duration (msec)	49.9	1.9	32	29.6	71.7	35.3	1.8	30	21.0	65.0	25.1	1.8	29	11.2	52.4	19.6	1.1	30	11.7	32.1	19.1	1.5	30	10.0	41.4	
Average retraction velocity (m/sec)	0.16	0.02	32	0.06	0.42	0.20	0.02	30	0.08	0.42	0.34	0.02	29	0.16	0.50	0.44	0.02	30	0.17	0.76	0.61	0.04	30	0.17	1.07	
Maximum retraction velocity (m/sec)	0.36	0.05	32	0.11	1.34	0.42	0.03	30	0.20	0.90	0.69	0.05	29	0.26	1.37	0.73	0.04	30	0.33	1.42	0.99	0.05	30	0.64	1.73	
Maximum retraction acceleration (msec <sup>-2</sup> )	31.5	4.9	32	4.4	103.0	50.2	7.7	30	10.0	162.0	90.8	9.0	29	19.9	199.0	145.2	33.7	30	22.9	986.0	190.5	23.0	30	12.5	632.0	
Maximum tongue mass specific retraction power (W/kg)	7.6	1.7	32	39.4	32.0	16.7	3.8	30	2.20	90.2	38.2	5.0	29	4.2	98.0	103.8	34.6	30	5.6	996.0	130.1	16.0	30	20.4	286.0	
Maximum muscle mass specific retraction power (W/kg)	2.2	0.5	32	0.2	11.4	4.8	1.1	30	0.64	26.2	11.1	1.5	29	1.2	28.4	30.1	10.0	30	1.6	289.0	37.8	4.6	30	5.9	83.0	



**Figure 4.** Scatterplots of kinematic parameters versus body temperature including all feedings of *Desmognathus* (black) and *Bolitoglossa* (blue). Y-axes have log<sub>10</sub> scales and are the same for a given parameter to facilitate comparison between projection and retraction. Symbols represent individual salamanders. *Bolitoglossa* exhibit significantly higher tongue projection performance compared to *Desmognathus*. Asterisks across the top of each graph indicate significant differences in performance between species at each nominal experimental temperature. Regression lines from ANOVA including temperature and individual effects are shown for each temperature interval as solid lines when significant and as dashed lines when not significant; thick lines are for the full 5–25°C range.  $Q_{10}$  values are shown for each temperature interval (5–15, 10–20, 15–25, and 5–25°C from left to right), with asterisks on  $Q_{10}$  values indicating a significant temperature effect. Bold  $Q_{10}$  values indicate significantly different thermal sensitivity between species across each temperature interval.

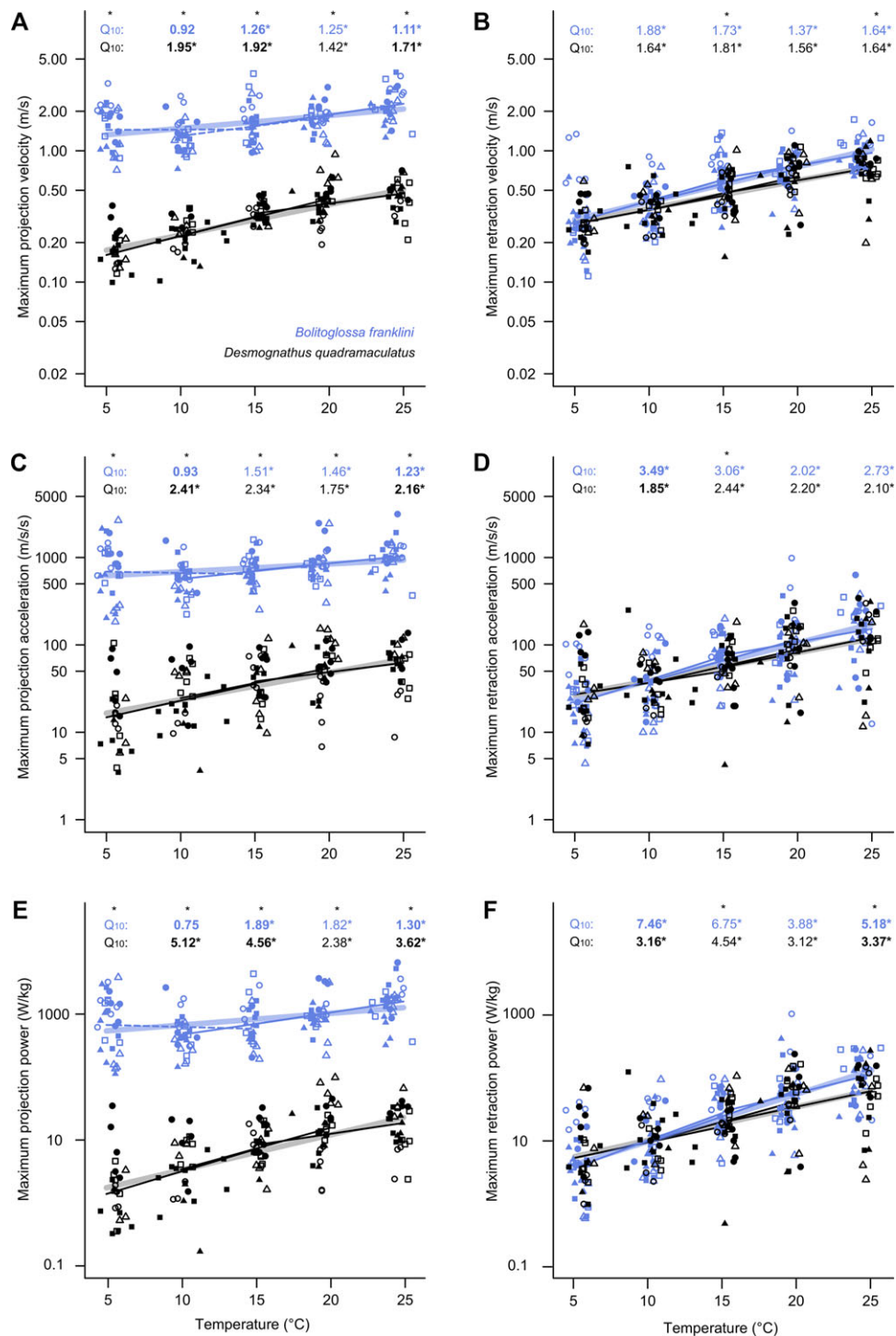


Figure 5. Scatterplots of dynamic parameters versus body temperature including all feedings of *Desmognathus* (black) and *Bolitoglossa* (blue). See Fig. 4 for full description.

between these two movements at all temperatures. Over the entire temperature range, retraction duration reached 247.1 msec, but was as fast as 7.3 msec (Table 2, Fig. 4). Higher velocities were attained during retraction than projection with average and maximum velocities reaching 0.59 (20°C) and 1.18 msec<sup>-1</sup> (25°C), respectively (Table 2, Figs. 4 and 5). Maximum retraction accelerations were as high as 343 msec<sup>-2</sup> and maximum muscle mass specific power peaked at 200 W kg<sup>-1</sup> (25°C, Table 2, Fig. 5).

Tongue projection and retraction performance generally declined with decreasing temperature in *Desmognathus*. Tongue-projection distance was significantly reduced at lower temperature over the entire temperature range (5–25°C), but this effect was not observed at smaller temperature intervals (Table S1 in the Supporting Information, Fig. 4). All other projection and retraction performance measures showed significantly reduced performance at lower temperature across all temperature intervals, with  $Q_{10}$  values typically over 1.5, and frequently above 2.0 (Table S1 in the Supporting Information, Figs. 4 and 5). Overall, tongue projection and retraction showed similar thermal dependence.

#### Prey Capture in *Bolitoglossa*

A total of 151 feedings of *Bolitoglossa* were recorded from six individuals over all temperatures. *Bolitoglossa* achieved high-performance tongue projection, extending the tongue up to 23 mm with durations that ranged from 6.1 to 31 msec (Table 2, Fig. 4). During projection, average and maximum velocities reached 1.58 and 3.97 msec<sup>-1</sup> (25°C), respectively, and maximum projection acceleration peaked at 3,140 msec<sup>-2</sup> (25°C; Table 2, Figs. 4 and 5). Maximum instantaneous tongue mass specific power was also high, reaching 6,510 W kg<sup>-1</sup>, which resulted in muscle mass specific power of up to 4,642 W kg<sup>-1</sup> (25°C, Table 2, Fig. 5). Finally, tongue mass specific projection kinetic energy reached a maximum of 7.88 J kg<sup>-1</sup> (25°C) but was as low as 0.25 J kg<sup>-1</sup> (5°C).

Tongue retraction kinematic and dynamic values revealed much lower performance compared to projection in *Bolitoglossa*. Tongue retraction could be quick, taking only 10 msec, but could require as long as 71.7 msec (Table 2, Fig. 4). Average and maximum retraction velocities reached 1.07 and 1.73 msec<sup>-1</sup>, respectively (25°C), with maximum retraction accelerations reaching 986 msec<sup>-2</sup> (Table 2, Figs. 4 and 5). Maximum tongue mass specific retraction power was also low compared to projection, reaching only 996 W kg<sup>-1</sup> (20°C; Table 2, Fig. 5) and a muscle mass specific power of 289 W kg<sup>-1</sup> (20°C).

Tongue projection was less temperature sensitive than tongue retraction in *Bolitoglossa*. Projection kinematic and dynamic variables were largely unaffected by temperature across the 5–15°C interval, with only projection duration and average projection velocity showing a significant effect of temperature (Figs. 4 and 5, Table S2 in the Supporting Information). Temper-

ature significantly affected all projection kinematics at other temperature ranges (Table S2 in the Supporting Information). However,  $Q_{10}$  values were typically low, never reaching 2.0 across any 10°C temperature interval and remaining under 1.5 over the entire temperature range (Figs. 4 and 5, Table S2 in the Supporting Information). Conversely, all tongue retraction kinematic and dynamic variables were significantly affected by temperature across all temperature intervals (Figs. 4 and 5, Table S2 in the Supporting Information). Furthermore,  $Q_{10}$  values were generally above 1.5 and frequently higher than 2.0 (Figs. 4 and 5, Table S2 in the Supporting Information).

#### Comparison of *Desmognathus* and *Bolitoglossa*

*Bolitoglossa* achieved higher tongue-projection performance than *Desmognathus*, attaining significantly higher projection velocity, acceleration, power, and work (Table S3 in the Supporting Information, Figs. 4 and 5). Only absolute tongue-projection distance did not consistently differ between the two species (Table S3 in the Supporting Information). Tongue retraction performance was more similar in the two species, but the relationship varied with temperature. Retraction kinematics and dynamics were especially similar at lower temperatures (5 and 10°C); only retraction duration differed at 10°C (Table S3 in the Supporting Information, Figs. 4 and 5). Conversely, *Bolitoglossa* achieved faster, more powerful tongue retraction performance compared to *Desmognathus* at both 15 and 25°C (Table S3 in the Supporting Information, Figs. 4 and 5). At these temperatures, only maximum retraction acceleration did not differ between the two species. At 20°C, *Bolitoglossa* tongue retraction was shorter in duration with higher average velocity than *Desmognathus*, but the two species did not differ in maximum velocity, acceleration or power (Table S3 in the Supporting Information, Figs. 4 and 5). Overall, *Bolitoglossa* exhibited higher projection and retraction performance than *Desmognathus*, but differences in retraction performance were fewer and less pronounced.

The thermal dependence of feeding performance also differed between *Bolitoglossa* and *Desmognathus*. Over the entire 5–25°C temperature range, the  $Q_{10}$  values of all projection variables were significantly lower for *Bolitoglossa* compared to *Desmognathus*, but this was not the case across 10°C temperature intervals (Table S4 in the Supporting Information, Figs. 4 and 5). *Bolitoglossa* had lower  $Q_{10}$  values than *Desmognathus* for all projection variables except projection distance and maximum projection acceleration at the 5–15°C and 10–20°C intervals (Table S4 in the Supporting Information, Figs. 4 and 5). However, the  $Q_{10}$  values of projection variables did not differ between species at 15–25°C (Table S4 in the Supporting Information, Figs. 4 and 5). Thus, tongue projection of *Bolitoglossa* was generally more thermally robust than that of *Desmognathus*, especially at lower temperatures.

The relative thermal sensitivity for *Desmognathus* and *Bolitoglossa* was reversed for tongue retraction performance; over the entire temperature range, tongue retraction in *Desmognathus*

was less thermally dependent.  $Q_{10}$  values for retraction duration, average velocity, and power were all significantly lower in *Desmognathus* compared to *Bolitoglossa* (Table S4 in the Supporting Information, Figs. 4 and 5). At the lowest temperature range, *Desmognathus* had lower  $Q_{10}$  values compared to *Bolitoglossa* for all retraction variables except maximum retraction velocity. *Desmognathus* showed significantly lower  $Q_{10}$  values for retraction duration and average velocity at 10–20°C (Table S4 in the Supporting Information, Figs. 4 and 5). *Bolitoglossa* tongue retraction was more temperature sensitive compared to *Desmognathus*, with the largest differences between species occurring at lower temperatures.

## DISCUSSION

### Morphology

Although *Desmognathus* and *Bolitoglossa* share most anatomical components of the feeding apparatus with other plethodontids (Lombard and Wake, '77), they demonstrate distinct projector-muscle architecture indicative of different projection mechanisms. *Desmognathus* exhibits several morphological traits that reveal tongue projection is nonballistic and directly powered by muscle: short epibranchials, a relatively short extended tongue length, the presence of myofibers that insert directly on the epibranchials (Fig. 2, Table 1), and a genioglossus muscle attaching the tongue pad to the lower jaw, all of which restrict projection distance. *Desmognathus* also exhibits a ratio of tongue mass to SAR mass of 3.7, indicating a relatively small amount of muscle available to accelerate a relatively massive tongue, which limits performance (i.e., lower projection velocity, acceleration, and power). Finally, *Desmognathus* has short collagen aponeuroses within the SAR muscle (Table 1, Fig. 2) suggesting that the muscle is capable of little if any elastic energy storage and must rely on myofiber shortening to extend the tongue with relatively low performance. *Desmognathus* tongue and muscle morphology is similar in these features to *Plethodon*, in which tongue projection is both muscle powered and nonballistic (Deban and Scales, 2016). This suite of traits is likely ancestral for plethodontids and is retained in the nonballistic, muscle-powered tongues of other plethodontids and other families of terrestrial salamanders (Wake and Deban, 2000).

In contrast, *Bolitoglossa* exhibits long epibranchials and relatively greater extended and projected tongue length (Table 1). These greater lengths coupled with a relatively small tongue mass and larger projector muscle (Table 1) likely improve dynamic performance (e.g., higher projection velocity, acceleration, and power) regardless of whether projection is powered by muscle contraction or elastic recoil. The SAR muscles of *Bolitoglossa* also lack inner myofibers that insert on the epibranchials and have augmented collagen aponeuroses compared to *Desmognathus* (Fig. 2). The freedom of the epibranchials to leave the SAR lumen improves projection distance. Longer collagen aponeu-

ros encircling the epibranchials likely improve the capacity for elastic energy storage, although the details of how the aponeuroses are strained by myofiber contraction remain unknown.

The present findings, together with previous findings (Deban et al., 2007; Anderson et al., 2014; Deban and Scales, 2016), suggest the mechanism of tongue projection involves the activation of myofibers in series with collagen aponeuroses. The shortening and other shape changes in the myofibers deforms or stretches the collagen aponeuroses, storing the work performed by the myofibers as elastic strain energy. The stretched aponeuroses transmit force radially to the tapered epibranchial. When the tongue skeleton is allowed to move rostrally by release of a catch mechanism, the elastic tissues recoil and force the tongue skeleton from the SAR and from the mouth. In such a system, elongated collagen aponeuroses provide increased potential for elastic storage, much as elongated tendons increase energy storage in locomotor systems (Alexander, 2002).

The morphological traits seen in *Bolitoglossa* that differentiate it from *Desmognathus* are repeatedly found in plethodontids with elastically powered, ballistic tongue projection. For example, ballistic-tongued species all have relatively low tongue mass to SAR mass ratios, ranging from 0.7 to 2.2, compared to ratios of 3.0 and 3.7 in muscle-powered, nonballistic plethodontids (Deban et al., 2007; Deban and Scales, 2016). Additionally, all plethodontids with ballistic tongues have SAR muscles that lack inner myofibers inserting on the tongue skeleton and possess elongated collagen aponeuroses (Deban et al., 2007; Deban and Scales, 2016). The presence of elaborated collagen aponeuroses in distantly related species with elastic tongue projection provides evidence that these collagen spirals within the SAR are the site of energy storage and elastic recoil during tongue projection.

Epibranchial cross-sectional shape also differs between *Bolitoglossa* and *Desmognathus*. The epibranchials of *Desmognathus* are an irregular shape that may provide a greater area of attachment for inner myofibers (Fig. 2). Conversely, the epibranchials of *Bolitoglossa* are round in cross-section (Fig. 2), which may enhance projection because muscle forces are likely applied radially to the epibranchial via the collagen sheath (in the absence of myofibers inserting on the epibranchial). This shape is also observed in *Hydromantes* and *Ensatina*, species that also exhibit elastic and ballistic tongue projection that has probably evolved independently of *Bolitoglossa* (Deban et al., 2007; Deban and Scales, 2016).

### Prey Capture in *Desmognathus*

Our analyses of feeding dynamics of *Desmognathus* provide several lines of evidence that tongue protraction is nonballistic and powered directly by muscle contraction. Maximum muscle mass specific power peaked at 359 W kg<sup>-1</sup>, a value below the maximum instantaneous power that amphibian muscle has been found to generate (373 W kg<sup>-1</sup> at 25°C) (Lutz and Rome, '96).

Maximum projection velocity and acceleration reached only 0.71 msec<sup>-1</sup> and 153 msec<sup>-2</sup>, respectively (Table 2). These values are low compared to species with elastically powered tongue projection (Deban et al., '97; Wake and Deban, 2000; Deban et al., 2007; Deban and Richardson, 2011; Anderson et al., 2014; Deban and Scales, 2016), but similar to the projection velocity and acceleration observed in the muscle-powered tongue projection of *Plethodon* (0.4 msec<sup>-1</sup>, and 74 msec<sup>-2</sup>, respectively, Deban and Scales, 2016). Lastly, the maximum tongue reach observed in *Desmognathus* was 13% of SVL. This distance is comparable to values from other species with nonballistic tongues (7–15.6%, Wake and Deban, 2000; Deban and Scales, 2016), but well short of distances observed in ballistic tongued species (see below). Additionally, tongue projection and retraction performance was similar, with comparable values of velocity, acceleration, and power output (Figs. 4 and 5).

Our calculated kinetic energy of projection shows that tongue projection in *Desmognathus* was accomplished with low mechanical work. Maximum muscle mass specific energy of 1.62 J kg<sup>-1</sup> is higher than that observed in *Plethodon* (0.24 J kg<sup>-1</sup>) (Deban and Scales, 2016), but these values fall short of those of the SAR during ballistic projection in *Eurycea*, *Ensatina*, *Hydromantes*, and *Bolitoglossa* (3.9, 8.2, 12.3, and 19.5 J kg<sup>-1</sup>, respectively) (Deban et al., 2007; Anderson et al., 2014), as well as those of the depressor mandibulae during ballistic mouth opening in *Rana* and *Bufo* (6.3 and 3.6 J kg<sup>-1</sup>, respectively), which also incorporate elastic recoil (Deban and Lappin, 2011; Sandusky and Deban, 2012). The low mechanical work estimated for *Desmognathus* reinforces previous findings that muscle-powered tongue projection is achieved with lower muscle energy than in elastic systems (Deban and Scales, 2016).

Tongue projection and retraction in *Desmognathus* are significantly and similarly influenced by changes in temperature (Table S1 in the Supporting Information), indicating that both result from direct muscle power. *Desmognathus* has a high thermal sensitivity at lower temperatures with  $Q_{10}$  values above 1.75 and commonly reaching 3.0, but these values decrease at higher temperatures. This pattern of high thermal sensitivity at low temperatures combined with a plateau of optimal performance is common across muscle-powered movements, including feeding in other amphibians (Bennett, '84, '85; Deban and Lappin, 2011; Anderson et al., 2014). The thermal dependence of projection in *Desmognathus* is similar to that of *Plethodon*, which also has muscle-powered tongue projection (Deban and Scales, 2016).

Retraction kinematics and dynamics suggest a greater emphasis on tongue retraction in *Desmognathus* compared to several other plethodontids. *Desmognathus* achieved higher retraction velocity, acceleration, and muscle mass specific power (1.18 msec<sup>-1</sup>, 343 msec<sup>-2</sup>, and 200 W kg<sup>-1</sup>, respectively) compared to *Plethodon*, *Eurycea*, and *Hydromantes*, but similar to that of *Ensatina* and *Bolitoglossa* (Deban et al., 2007; Deban and Scales, 2016). This high performance may in part be attributed

to a large RCP and a relatively low ratio of tongue mass to RCP mass (0.8) in *Desmognathus*. This ratio is smaller than those found in *Ensatina* (1.3) and *Plethodon* (1.1), but still higher than that of *Bolitoglossa* (0.6) (Deban and Scales, 2016). Higher retraction performance coupled with a large tongue pad (also seen in *Ensatina*) might be related to feeding on large prey, which may place greater importance on tongue retraction. In support of this idea, *Desmognathus* has been known to eat prey such as crayfish and juvenile conspecifics in addition to the arthropods that make up the diet of most salamanders (Davic '91; Camp, '97; Wake and Deban, 2000).

#### Prey Capture in *Bolitoglossa*

Our analyses of feeding kinematics and dynamics indicate that *Bolitoglossa* achieves high performance tongue projection via an elastic-recoil mechanism. Muscle mass specific projection power reached 4,642 W kg<sup>-1</sup> (at 25°C), a value much higher than the maximum instantaneous power measured in amphibian striated muscle (373 W kg<sup>-1</sup> at 25°C) (Lutz and Rome, '96), but falls within the range observed in other plethodontids that utilize elastic recoil. Conversely, tongue retraction in *Bolitoglossa* is achieved with a maximum muscle mass specific power of 289 W kg<sup>-1</sup>, which falls within the limits of muscle power (Lutz and Rome, '96). The large difference in power output between projection and retraction indicate different mechanisms underlying projection and retraction. In addition, *Bolitoglossa* tongue projection achieved high performance, reaching a maximum velocity of 3.97 msec<sup>-1</sup> and acceleration of 3,140 msec<sup>-2</sup> (Table 2, Fig. 5). These values are similar to or higher than other plethodontids that possess elastic and ballistic tongue projection including *Eurycea*, *Ensatina*, *Bolitoglossa dofleini*, and *Hydromantes*, and greatly surpass those of nonballistic species such as *Desmognathus* and *Plethodon* (Deban et al., 2007; Anderson et al., 2014; Deban and Scales, 2016). Finally, the observed maximum tongue reach of *Bolitoglossa* (43% SVL) is higher than that of *Ensatina* (29% SVL), *Eurycea* (33% SVL), and *Bolitoglossa doefleni* (31% SVL) (Deban et al., 2007), all of which have elastically powered, ballistic tongues.

*Bolitoglossa* achieved moderate SAR muscle work during tongue projection peaking at 5.62 J kg<sup>-1</sup> at 25°C (Table 2). These values overlap with those from the elastic feeding systems in frogs and salamanders (3.6–19.5 J kg<sup>-1</sup>) (Deban et al., 2007; Deban and Lappin, 2011; Deban and Richardson, 2011; Sandusky and Deban, 2012; Anderson et al., 2014), and are significantly higher than in nonelastic projection by *Desmognathus* and *Plethodon* (1.62 and 0.24) (Deban and Scales, 2016) indicating that elastic systems such as tongue projection in *Bolitoglossa* can extract greater work from muscle than nonelastic systems. However, even the highest muscle work observed in these elastic feeding systems is much lower than the maximum theoretical muscle work of 67 J kg<sup>-1</sup> (for frog striated muscle; Peplowski and

Marsh, '97). Thus, muscles in a range of feeding systems may be constrained to operate at low strain, stress, or both.

Compared to tongue projection, tongue retraction maximum velocity, acceleration, and muscle mass specific power in *Bolitoglossa* were much lower, that is,  $1.07 \text{ msec}^{-1}$ ,  $632 \text{ msec}^{-2}$ , and  $286 \text{ W kg}^{-1}$ , respectively (Table 2). Retraction performance is higher than that of *Eurycea*, *Hydromantes*, and *Plethodon*, but comparable to that observed in *Desmognathus* and *Ensatina* (Anderson et al., 2014; Deban and Scales, 2016). The higher retraction performance in *Ensatina* and *Desmognathus* may indicate specialization for feeding on larger prey, consistent with these species possessing large tongue pads (see above; Deban and Scales, 2016). *Bolitoglossa*, however, has a relatively small tongue pad to enhance projection (Table 1) suggesting that the improved retraction performance is a consequence of a large RCP relative to tongue mass.

Tongue-projection kinematics and dynamics are relatively thermally robust in *Bolitoglossa*, especially at lower temperatures. Performance was thermally independent at the lowest temperatures and maintained high performance across the other temperature ranges with 24 of the 28  $Q_{10}$  values below 1.5 and little change in  $Q_{10}$  values between 10 and  $25^\circ\text{C}$  (Table S2 in the Supporting Information). Tongue retraction, on the other hand, showed higher thermal dependence with all but one  $Q_{10}$  value above 1.5 (Table S2 in the Supporting Information). The higher thermal sensitivity of retraction compared with projection indicates that elastic recoil mechanisms confer higher thermal stability of performance, which is consistent with previous studies of elastically powered tongue projection in the salamanders *Eurycea*, *Ensatina*, and *Hydromantes*, as well as in toads and true frogs, and chameleons (Anderson and Deban, 2010; Deban and Lappin, 2011; Deban and Richardson, 2011; Sandusky and Deban, 2012; Anderson et al., 2014, Deban and Scales, 2016). Temperature dependence, however, varies across these elastic systems. Projection performance declines significantly at lower temperatures in *Eurycea* and *Ensatina* (Anderson et al. 2014, Deban and Scales, 2016), less strongly in *Bolitoglossa*, and not at all in *Hydromantes* (Deban and Richardson, 2011). This difference in temperature sensitivity may be linked to differences in relative muscle work; *Hydromantes* and *Bolitoglossa* have higher mass-specific muscle work than *Eurycea* and *Ensatina*.

The high-performance projection observed in the *B. franklini* of this study is comparable to that found in the congener *Bolitoglossa doefleini* (Deban et al., 2007). These two species also exhibit relatively similar ratios of tongue mass to SAR mass, lack of inner myofibers in the SAR, and have similar elaborated collagen aponeuroses within the SAR. *Bolitoglossa franklini* and *B. doefleini* occur in different clades within the genus (Parra-Olea et al., 2004), indicating that feeding performance and its underlying morphology and mechanisms may be conserved within the genus.

### Comparison of Mechanisms

The elastically powered, ballistic tongue of *Bolitoglossa* achieves higher performance than the muscle-powered, nonballistic projection of *Desmognathus* across all temperatures. The longer relative tongue reach of *Bolitoglossa* can be explained by the lack of SAR myofibers inserting directly to the epibranchials, allowing the tongue skeleton to fully leave the SAR, and the absence of a genioglossus muscle attaching the tongue pad to the lower jaw. The increased dynamic performance of *Bolitoglossa* likely results from the rapid recoil of elaborated collagen aponeuroses within the SAR that return strain energy stored by the muscle prior to tongue projection. The elastic recoil mechanism of *Bolitoglossa* can clearly produce more total muscle work compared to the muscle-powered system of *Desmognathus* (e.g.,  $2.12$  vs.  $0.43 \text{ J kg}^{-1}$ , Table 2). The increase in work allows *Bolitoglossa* to achieve much higher peak tongue velocity compared to *Desmognathus* ( $2.34$  vs.  $0.47 \text{ msec}^{-1}$ , Table 2). Additionally, the elastic tissue can release energy at a much higher rate than muscle ( $1,284$  vs.  $81.9 \text{ W kg}^{-1}$ , Table 2), resulting in higher acceleration.

In addition to improving performance, the elastic-recoil mechanism of *Bolitoglossa* also reduces the temperature dependence of tongue projection. *Bolitoglossa* has significantly lower  $Q_{10}$  values than *Desmognathus* across the entire temperature range and generally lower  $Q_{10}$  values at all temperature intervals except for the  $15\text{--}25^\circ\text{C}$  range, where performance tends to plateau in muscle-powered and elastically powered movements alike. This trend of reduced thermal dependence of elastically powered tongue projection is common within plethodontids; *Hydromantes*, *Eurycea*, *Ensatina*, and *Bolitoglossa* all have relatively low  $Q_{10}$  values compared to *Plethodon* and *Desmognathus*, indicating that improved thermal robustness of muscle function and performance is a general benefit of elastic mechanisms (Deban and Richardson, 2011; Anderson et al., 2014; Deban and Scales, 2016).

Important morphological differences, especially with the SAR muscle, accompany the performance differences in *Bolitoglossa* and *Desmognathus*. Notably, *Bolitoglossa* lacks inner myofibers attaching to the epibranchials and has increased collagen aponeuroses in the SAR. In addition, *Bolitoglossa* has a much lower ratio of SAR mass to tongue mass and longer epibranchials. These traits serve to increase projection distance and enhance performance in *Bolitoglossa*.

The lack of myofiber attachment to the tongue skeleton and increased collagen is similar to the tongue-projection mechanism of chameleons. The accelerator muscle of chameleons is also free of a tight connection to the tongue skeleton, and muscle work is also stored in elastic tissues of the tongue that surrounds the tongue skeleton, likely the nested collagen sheaths that lie between the accelerator muscle and the entoglossal process of the hyoid (de Groot and van Leeuwen, 2004). The mechanism of tongue projection is reversed compared to salamanders; in

chameleons the tongue skeleton remains in the mouth during projection while the accelerator muscle and associated tongue pad are propelled outward, while in plethodontid salamanders the muscle remains behind while the tongue skeleton and tongue pad are launched from the mouth. In both systems, however, a tongue skeleton free of a close attachment with the projector muscle and increased amounts of elastic tissue in the form of collagen sheets are key traits associated with high-powered, long-distance tongue projection. Chameleon and salamander tongue projection thus provide a striking example of convergent evolution.

Morphology similar to *Bolitoglossa* occurs in *B. dofleini*, *Hydromantes*, and *Ensatina*, all of which have ballistic tongue projection, unlike *Desmognathus* and *Plethodon* (Deban et al., 2007; Deban and Scales 2016). *Hydromantes* and *Ensatina* are in the clade Plethodontinae while *Bolitoglossa* is within the Hemidactyliinae (Vieites et al., 2011). This phylogenetic pattern suggests that elastically powered, ballistic tongue projection has evolved at least twice in the family Plethodontidae, each time arriving at a similar underlying morphology and mechanism. This high-performance, elastically powered, and thermally robust feeding in lungless salamanders is thus an example of parallel evolution.

## CONCLUSIONS

Elastic-recoil mechanisms have been found to confer enhanced performance and increased thermal robustness in a number of feedings systems with diverse morphologies, including those of salamanders, toads and true frogs, and chameleons (Anderson and Deban, 2010; Deban and Lappin, 2011; Deban and Richardson, 2011; Sandusky and Deban, 2012; Anderson and Deban, 2012; Anderson et al., 2014). The diverse morphologies have made it challenging to identify the key morphological features responsible for improved performance. However, the comparison of *Desmognathus* and *Bolitoglossa* presented here reveals that freedom of the epibranchials from the myofibers of the SAR together with augmented collagen tissue in series with myofibers is central to the high performance of the elastically powered system. Comparison of *Ensatina* and *Plethodon* showed this same correspondence between these morphological features and tongue-projection performance and functional robustness (Deban and Scales, 2016). Elaboration of collagen tissue in series with muscle fibers allows the temporal decoupling of muscle shortening from the subsequent movement (Deban et al., 2007; Roberts and Azizi 2011) so that muscle can increase work output, while the rapid recoil of thermally insensitive elastic tissue maintains high performance across a range of temperatures. These findings indicate that relatively minor changes in the morphology of muscles and associated connective tissue can significantly enhance the performance of musculoskeletal systems as well as their functional robustness in the face of environmental challenges.

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