

RESEARCH ARTICLE

Dynamics and thermal sensitivity of ballistic and non-ballistic feeding in salamanders

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ABSTRACT

Low temperature reduces the performance of muscle-powered movements, but in movements powered by elastic recoil mechanisms, this effect can be mitigated and performance can be increased. To better understand the morphological basis of high performance and thermal robustness of elastically powered movements, we compared feeding dynamics at a range of temperatures (5–25°C) in two species of terrestrial plethodontid salamanders, *Plethodon metcalfi* and *Ensatina eschscholtzii*, which differ in tongue muscle architecture and the mechanism of tongue projection. We found that *Ensatina* is capable of ballistic projection with a mean muscle mass-specific power of 2100 W kg⁻¹, revealing an elastic mechanism. *Plethodon*, in contrast, projected its tongue non-ballistically with a mean power of only 18 W kg⁻¹, indicating it is muscle powered. *Ensatina* projected its tongue significantly farther than *Plethodon* and with dynamics that had significantly lower thermal sensitivity at temperatures below 15°C. These performance differences were correlated with morphological differences, namely elongated collagenous aponeuroses in the projector muscle of *Ensatina* as compared with *Plethodon*, which are likely the site of energy storage, and the absence in *Ensatina* of projector muscle fibers attaching to the tongue skeleton that allows projection to be truly ballistic. These findings demonstrate that, in these otherwise similar species, the presence in one species of elaborated connective tissue in series with myofibers confers not only 10-fold greater absolute performance but also greater thermal robustness of performance. We conclude that changes in muscle and connective tissue architecture are sufficient to alter significantly the mechanics, performance and thermal robustness of musculoskeletal systems.

KEY WORDS: Amphibian, Biomechanics, Temperature

INTRODUCTION

Temperature changes have a profound influence on physiological rate processes and are therefore among the most significant environmental challenges facing ectothermic animals (Cossins and Bowler, 1987; Hochachka and Somero, 2002). Studies of a diversity of ectothermic vertebrates from all major groups have shown that temperature has a strong effect on organismal performance, primarily via its effects on muscle contractile rates, which decline by at least half over a 10°C drop (i.e. $Q_{10} > 2$) (Bennett, 1984; Hirano and Rome, 1984; Marsh and Bennett, 1985; van Berkum, 1986; Else and Bennett, 1987; Huey and Bennett, 1987; John-Alder et al., 1988, 1989; Rome et al., 1990; Bauwens et al., 1995; Lutz and Rome, 1996; Altringham and Block, 1997;

Peplowski and Marsh, 1997; Navas et al., 1999; McLister, 2001; Marvin, 2003a,b; Donley et al., 2007; Herrel et al., 2007). Elastic recoil mechanisms can circumvent the effect of muscle thermal sensitivity on performance by temporally decoupling muscle contraction from movement. Elastically powered ballistic tongue projection movements in salamanders, chameleons, toads and frogs exhibit lower thermal dependence than movements powered directly by muscle contraction such as tongue retraction (Anderson and Deban, 2010, 2012; Deban and Lappin, 2011; Deban and Richardson, 2011; Sandusky and Deban, 2012; Anderson et al., 2014). These independently evolved ballistic systems demonstrate the thermal benefits of elastic recoil in diverse ectotherms, but because they are all elastically powered and divergent in their anatomy, comparisons among them fail to identify which specific morphological features confer thermal robustness. To better understand which anatomical features influence the feeding performance of salamanders and the thermal robustness of musculoskeletal systems more generally, we compared tongue projection at a range of temperatures in two closely related species of lungless salamander, ballistic-tongued *Ensatina eschscholtzii* and non-ballistic-tongued *Plethodon metcalfi*, which have the same basic tongue apparatus morphology and mechanism but with different tongue projector muscle architecture and different degrees of elaboration of the collagenous aponeuroses that lie in series with myofibers and thus can act as a spring in an elastic recoil mechanism.

Many species of lungless salamanders of the Plethodontidae have enhanced tongue projection performance relative to other salamanders, in terms of distance and velocity (Wake and Deban, 2000; Deban, 2002), with tongue projection often taking less than 10 ms and reaching a distance of up to 80% of body length (Deban et al., 1997). The morphology and mechanism of tongue projection are described and illustrated in detail elsewhere (Lombard and Wake, 1977; Deban et al., 1997), so we describe it only briefly here. The basic mechanism involves the articulated tongue skeleton and attached tongue pad being accelerated out of the mouth by the cylindrical projector muscles (the paired subarcualis rectus, SAR). Each tongue projector muscle surrounds one of the paired, elongated, tapered epibranchial cartilages that extend caudally from the buccal region. Each epibranchial connects rostrally to paired ceratobranchials, which in turn articulate rostrally with the medial, unpaired basibranchial that lies in the floor of the mouth and supports the sticky tongue pad (Lombard and Wake, 1977; Wake and Deban, 2000; Deban, 2002; Deban and Dicke, 2004). In elastically powered, ballistic tongue projection, activation of the SAR muscles 80–200 ms prior to tongue launch stretches collagenous tissues within the muscles. Subsequent recoil of these elastic structures launches the tongue with accelerations of up to 460 g and power of up to 18,000 W kg⁻¹ muscle mass (Deban et al., 2007). The tongue projector muscle exerts force on the epibranchial, which separates from it entirely during ballistic tongue projection;

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thus, full tongue projection relies on momentum to carry the tongue skeleton and pad to the target (Deban et al., 1997). In non-elastic systems, tongue projection velocity and power are much lower, the (SAR) projector muscle and epibranchial do not separate because a direct myofiber connection exists, and projection is not ballistic. Tongue retraction in ballistic and non-ballistic species is typically slower than projection and is accomplished by contraction of the lengthy retractor muscles (the rectus cervicis profundus, RCP) which originate on the pelvis.

Ballistic tongue projection via elastic recoil has evolved repeatedly within the Plethodontidae, being found in the taxa *Hydromantes*, *Bolitoglossa* and *Eurycea* (Deban et al., 2007). The family Plethodontidae is divided basally into two subfamilies: the Hemidactyliinae, which includes the genera *Eurycea* and *Bolitoglossa* among others, and the Plethodontinae, which includes *Hydromantes*, *Ensatina* and *Plethodon*, among others. *Plethodon* is basal within Plethodontinae whereas *Ensatina* is deeply nested (Vieites et al., 2011). Based on this phylogenetic arrangement and the results of this study in which we document that *Plethodon* has a non-elastic mechanism whilst that of *Ensatina* is both ballistic and elastic, we can be reasonably certain that *Ensatina* has evolved elastic, ballistic projection independently from *Eurycea* and *Bolitoglossa*. We chose *Plethodon* and *Ensatina* for comparison of performance and thermal robustness because they are closely related, ecologically similar woodland salamanders of similar body size, and are fully terrestrial and direct developing (Stebbins, 1985; Conant and Collins, 1991). *Ensatina* and *Plethodon* both retain a muscular attachment of the large tongue pad to the lower jaw (i.e. the genioglossus muscle) and have epibranchials that extend only to the back of the head, revealing that the tongue morphology of these two taxa is not as divergent from the ancestral condition as that of the ballistic taxa mentioned above, which have complete freedom of the relatively small tongue pad and epibranchials that extend to the shoulder or beyond (Lombard and Wake, 1977). *Ensatina*, however, has an elongated genioglossus muscle relative to that of *Plethodon* and is known to have fast tongue projection (Lombard and Wake, 1977; Deban, 1997; Wake and Deban, 2000), indicating that it has intermediate performance between that of *Plethodon* and ballistic taxa such as *Eurycea* and *Bolitoglossa*. Comparison of *Ensatina* and *Plethodon* can therefore improve our understanding of how ballistic tongues evolved within salamanders and thus how high performance and thermal robustness may evolve in musculoskeletal systems.

We imaged tongue projection and retraction during feeding in *Ensatina* and *Plethodon* at a range of body temperatures (5–25°C) and calculated the temperature coefficients (Q_{10}) of a number of kinematic and dynamic performance parameters. Additionally, we examined the morphology of the tongue apparatus and projector muscles using dissection and histological sectioning and staining to correlate morphological features with performance differences.

MATERIALS AND METHODS

Specimens

Ensatina eschscholtzii Gray 1850 and *Plethodon metcalfi* Brimley 1912 were collected from natural populations in California and North Carolina, respectively, housed individually in plastic containers with a substrate of moist paper towels at 14–17°C and maintained on a diet of termites and gut-loaded crickets. Seven individuals of *Ensatina* (snout–vent length, SVL 54–66 mm) and

six individuals of *Plethodon* (SVL 45–61 mm) that fed readily under observation were selected for feeding experiments. All procedures were approved by the Institutional Animal Care and Use Committee of the University of South Florida.

Morphology

Six specimens of *Ensatina* and 15 specimens of *Plethodon* were killed by immersion in a 2 g l⁻¹ aqueous solution of MS-222 (tricaine methanesulfonate; Sigma, St Louis, MO, USA) buffered with sodium bicarbonate and specimens were massed. The salamander's tongue skeleton was extended out of the mouth to maximum projection distance. Four lengths were measured with digital calipers (Mitutoyo 700-126, Kawasaki-shi, Kanagawa, Japan; ±0.1 mm accuracy): SVL, the distance from the rostral tip of the extended tongue skeleton (i.e. the basibranchial's rostral tip) to the rostral tip of the lower jaw, the length of the extended tongue skeleton from the basibranchial tip to the epibranchial tip, and the length of the epibranchials. The tongue apparatus was removed by severing the retractor (RCP) muscles at the level of the epibranchial tips, severing the submentalis muscle at the origin on the lower jaw, and by peeling the tongue projector (SAR) muscles from their origins on the ceratohyals. The paired SAR muscles were removed from the excised tongue apparatus and massed (Virtual Measurements and Control model VB-302A, Santa Rosa, CA, USA; ±0.001 g accuracy). The excised tongue apparatus including the tongue skeleton plus tongue pad and a portion of the RCP were massed. The remaining lengths of RCP were severed at their origin on the pelvis, freed from surrounding tissue and massed. 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 specimens of each species were fixed in neutral-buffered formalin and embedded in paraffin for histological staining. Samples were sectioned midway along their length at 5–10 µm 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 on 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 which form arches or spirals that extend from the collagen sheath surrounding the epibranchial to the periphery of the SAR muscle. The spirality of each aponeurosis was measured as the angle subtended by the beginning and end of the spiral with the center of the epibranchial at the vertex (Fig. 1), and the average spirality angle was taken.

Feeding experiments

Salamanders were imaged individually in dorsal view at 6 kHz frame rate and 1/12,000 s shutter speed with a Fastcam 1024 PCI camera (Photron USA Inc., San Diego, CA, USA) as they captured termites. Salamanders were placed on moistened paper printed with a 5 mm grid for distance calibration, set on the surface of a temperature-controlled platform (AHP-1200CPV; ThermoElectric Cooling America Corporation, Chicago, IL, USA) and termites were placed at varying distances in front of them. Salamanders were permitted to approach the prey and choose their distance to the prey at the start of prey capture. Salamanders, prey and substrate were illuminated by two infrared LED lights (LED infrared illuminator IR-200, Speco Technologies,

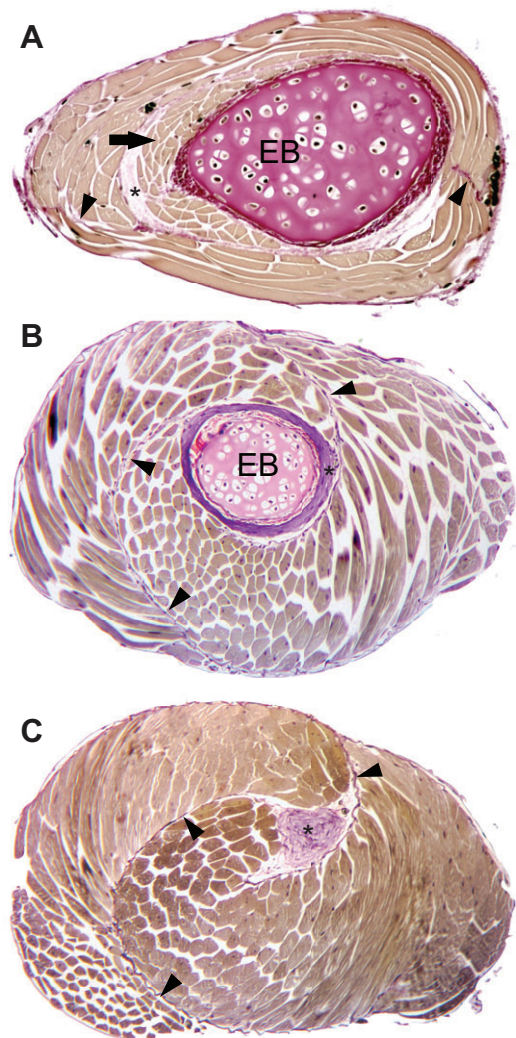


Fig. 1. Histological sections of the epibranchial, surrounded by the subarcualis rectus muscle of salamanders in the resting and projected condition. Sections are from *Plethodon metcalfi* (A) and *Ensatina eschscholtzii* in the resting condition (B) and the projected condition (C) in which the epibranchial (EB) is absent, having been forced out of the SAR during projection. Note the presence of myofibers (beige) between the collagen sheath (asterisk) and the epibranchial in *Plethodon* (black arrow) inserting on the cartilage, and their absence in *Ensatina*, as well as the round epibranchial in *Ensatina*. Aponeuroses (arrowheads) extending from the collagen sheath surrounding the epibranchial are short in *Plethodon* compared with the elongated spirals in *Ensatina*. Samples are approximately 2 mm in diameter.

Amityville, NY, USA) that provided cool light to avoid warming the salamanders.

Feeding trials were conducted across a range of nominal 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's ventral surface was pressed against a moistened surface on top of 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 every feeding event. The temperature

sequence of feeding trials for each individual was in random order with one to three feedings per experimental temperature, depending on the willingness of the salamander to feed, before attempting a different randomly selected temperature. Salamander body temperatures ranged from 4.3 to 25.1°C. Digital image sequences were obtained from 178 feedings of *Ensatina* and 138 feedings of *Plethodon*.

Kinematic and dynamic analysis

The digital image sequences were used to quantify movements of the tongue during prey capture, with respect to the upper jaw tip as a fixed reference point. The x , y coordinates of the tongue tip and the tip of the upper jaw were recorded from the image sequences using ImageJ software (National Institutes of Health, Bethesda, MD, USA) running on an Apple iMac computer. Tongue projection distance was computed as the geometric resultant 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 fully withdrawn into the mouth following tongue projection. The duration of tongue projection is equivalent to time 1 and the duration of tongue retraction was calculated as time 2 minus time 1. The average velocity of tongue projection and that of tongue retraction were calculated as these durations, respectively, divided by the tongue projection distance.

The dynamics of tongue movements were calculated using published methods (Deban and Richardson, 2011; Anderson et al., 2014) by fitting a quintic spline to the distance–time data using the Pspline package in R statistical software (R Core Team, 2013; www.r-project.org). First and second derivatives of the spline function were computed to yield instantaneous velocity and acceleration 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 product of 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 (3.0 ± 0.2 for *Plethodon* and 2.2 ± 0.2 for *Ensatina*, means \pm s.e.m.). 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 (1.1 ± 0.2 for *Plethodon* and 1.3 ± 0.2 for *Ensatina*). 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

Plethodon and *Ensatina* data were analyzed separately prior to comparing species. 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), but it 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 (PRCs) of the temperature effect in the ANCOVA multiplied by 10 (Deban and Lappin, 2011; Deban and Richardson, 2011; Sandusky and Deban, 2012; Anderson 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 PRC 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 *Plethodon* and *Ensatina* were examined using ANCOVA for each variable to test for effects of species \times temperature interactions in each of the four temperature intervals, including in the model individual as a random effect and projection distance as a covariate. Species differences in feeding movements at each nominal experimental temperature were examined using a separate ANOVA for each temperature that included the effects of species with individual nested within species.

Differences in morphological measurements of the tongue apparatus between *Plethodon* and *Ensatina* were examined using ANOVA as well as ANCOVA with body length, body mass or muscle mass as covariates to compare relative dimensions (Packard and Boardman, 1999). Measurements compared between species included tongue mass, SAR mass, SAR aponeurosis spirality angle, RCP mass, tongue skeleton length, epibranchial length and extended tongue length.

All statistical analyses were performed using R statistical software version 1.65 (R 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, 1995) when multiple comparisons were made within each temperature or temperature interval.

RESULTS

Morphology of *Plethodon* and *Ensatina*

The *Ensatina* used in this study were of significantly greater mass than the *Plethodon* (Table 1); however, the species were similar in SVL. Mean lengths of the tongue skeleton and epibranchial of *Plethodon* were 11.5 and 3.5 mm, respectively. These lengths contributed to a short extended tongue length of only 7.0 mm beyond the jaws (with the remainder staying in the mouth). The tongue skeleton and epibranchial were significantly longer in *Ensatina*, both absolutely and relative to SVL, with mean values of 18.8 and 7.4 mm, respectively. *Ensatina* also had a significantly greater extended tongue length, absolutely and relatively, with a mean value of 14.8 mm, roughly twice that of *Plethodon*.

The tongue mass of *Ensatina* was also significantly greater than that of *Plethodon*, both absolutely and relative to body mass. *Ensatina* had a mean tongue mass of 78.5 mg and SAR and RCP muscle masses averaged 37.3 and 66.7 mg, respectively (Table 1). *Plethodon*, in contrast, had a mean tongue mass of 25.9 mg while the SAR and RCP muscles weighed an average of 9.2 and 27.5 mg. These tongue and muscle masses reveal that *Ensatina* had higher relative muscle mass for tongue projection and tongue retraction when compared with *Plethodon*. The RCP mass of *Ensatina* was greater in absolute terms than that of *Plethodon*, but not relative to body mass. The tongue mass of *Ensatina* was also significantly greater than that of *Plethodon* when accounting for SAR and RCP mass as a covariate, even though the ratio of tongue mass to SAR mass was higher in *Plethodon* (3.0 versus 2.2) and the ratio of tongue mass to RCP mass was higher in *Ensatina* (1.3 versus 1.1).

Table 1. Morphological measurements of *Plethodon metcalfei* and *Ensatina eschscholtzii* and tests of species differences

	Species		Species with covariate				
	<i>Plethodon</i>	<i>Ensatina</i>	F-ratio	P-value	F-ratio	P-value	Covariate
Body mass (g)	2.42 \pm 0.18 (15)	3.61 \pm 0.41 (6)	9.41	0.0063*	–	–	–
SVL (mm)	55.7 \pm 1.6 (15)	59.4 \pm 1.4 (6)	1.86	0.1887	–	–	–
Extended tongue length (mm)	7.0 \pm 0.6 (12)	14.8 \pm 1.3 (6)	41.34	<0.0001*	56.84	<0.0001*	SVL
Tongue skeleton length (mm)	11.5 \pm 0.4 (13)	18.8 \pm 0.5 (6)	135.77	<0.0001*	197.94	<0.0001*	SVL
Epibranchial length (mm)	3.5 \pm 0.2 (13)	7.4 \pm 0.4 (6)	131.63	<0.0001*	146.11	<0.0001*	SVL
Tongue mass (mg)	25.9 \pm 2.1 (13)	78.5 \pm 7.5 (6)	81.11	<0.0001*	130.44	<0.0001*	Body mass
SAR mass (mg)	9.2 \pm 0.8 (13)	37.3 \pm 5.7 (6)	51.08	<0.0001*	40.44	<0.0001*	Body mass
RCP mass (mg)	27.5 \pm 4.8 (15)	66.7 \pm 12.5 (6)	10.97	0.0041*	2.45	0.1368	Body mass
Tongue mass vs SAR mass	– (13)	– (6)	–	–	196.15	0.0136*	SAR mass
Tongue mass vs RCP mass	– (13)	– (6)	–	–	214.17	<0.0001*	RCP mass
Tongue mass/SAR mass	3.0 \pm 0.2 (13)	2.2 \pm 0.2 (6)	–	–	–	–	–
Tongue mass/RCP mass	1.1 \pm 0.2 (13)	1.3 \pm 0.2 (6)	–	–	–	–	–
SAR aponeurosis spirality (deg)	17.4 \pm 0.7 (2)	95.1 \pm 4.3 (2)	322.98	0.0031*	–	–	–

Data are means \pm s.e.m. (N is given in parentheses).

SAR, subarcualis rectus; RCP, rectus cervicis profundus; SVL, snout–vent length.

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

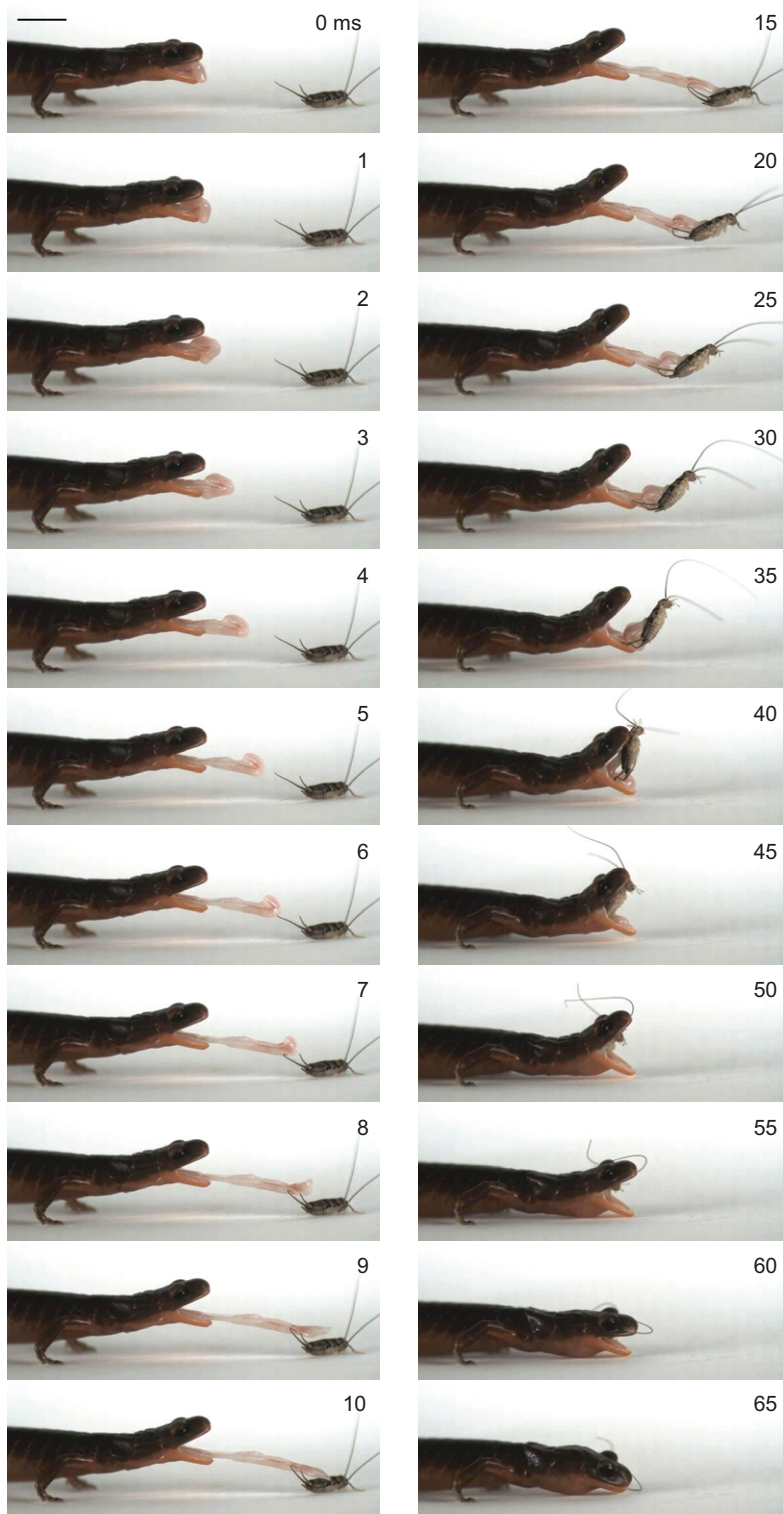


Fig. 2. Image sequence of *Ensatina* demonstrating ballistic tongue projection to capture a cricket at 20°C. This exceptionally long projection of 22 mm or 40% of SVL was completed in 10 ms, while retraction required approximately 40 ms. Note the forward lunge of the salamander and its large tongue pad. The time step is 1 ms for projection (left column) and 5 ms for retraction (right). Scale bar (first frame), 1 cm.

The morphology of the SAR also differed markedly between the two species. Both species possess myofibers that encircle the epibranchial yet are separated from it by a collagenous sheath (Fig. 1). The SAR of *Plethodon* additionally has myofibers within this sheath that insert on the epibranchial; these inner myofibers are absent in *Ensatina*. Extending from this sheath to the periphery of the muscle are two aponeuroses, which in *Plethodon* form a mean spirality angle of only 17.4 deg (Table 1). The SAR

of *Ensatina* had a significantly higher mean aponeurosis spirality angle of 95.1 deg.

Feeding in *Plethodon* and *Ensatina*

Plethodon and *Ensatina* fed successfully at all experimental temperatures (5, 10, 15, 20 and 25°C) using tongue projection to capture prey. During a typical feeding session, the salamander oriented towards the prey and rapidly projected its tongue from its

Table 2. Summary statistics of performance variables at each experimental temperature in *Plethodon metcalfi* and *Ensatina eschscholtzii*

	5°C			10°C			15°C			20°C			25°C		
	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.
<i>Plethodon</i>															
Projection distance (mm)	5.0±0.4	1.5	8.7	5.5±0.4	1.1	8.1	5.1±0.3	3.0	8.1	4.8±0.4	1.5	8.5	4.9±0.4	1.4	8.6
Projection duration (ms)	85.9±4.2	43.7	132.4	68.4±4.3	29.5	132.8	46.1±2.0	25.5	67.8	40.2±2.0	22.6	73.1	34.9±2.7	17.9	64.0
Average projection velocity (m s ⁻¹)	0.05±0.00	0.02	0.09	0.08±0.01	0.02	0.13	0.11±0.01	0.06	0.16	0.12±0.01	0.04	0.21	0.15±0.01	0.05	0.25
Max. projection velocity (m s ⁻¹)	0.10±0.01	0.04	0.19	0.14±0.01	0.04	0.23	0.20±0.01	0.12	0.30	0.21±0.01	0.09	0.34	0.26±0.02	0.07	0.40
Max. projection acceleration (m s ⁻²)	5.9±0.9	0.8	19.3	11.0±1.6	1.1	28.6	25.2±3.4	3.0	72.7	27.1±3.3	3.0	71.5	34.6±4.4	4.5	74.0
Max. tongue mass-specific projection power (W kg ⁻¹)	0.42±0.09	0.03	2.17	1.11±0.20	0.10	3.46	3.49±0.61	0.31	13.80	3.92±0.69	0.41	13.90	6.21±1.06	0.31	18.10
Max. muscle mass-specific projection power (W kg ⁻¹)	1.23±0.28	0.09	6.42	3.28±0.60	0.30	10.24	10.33±1.81	0.91	40.85	11.61±2.04	1.21	41.14	18.38±3.15	0.91	53.58
Projection energy (mJ)	2E-04±2E-05	2E-05	4E-04	3E-04±4E-05	2E-05	7E-04	6E-04±6E-05	2E-04	1E-03	6E-04±8E-05	1E-04	1E-03	1E-03±1E-04	7E-05	2E-03
Tongue mass-specific projection energy (J kg ⁻¹)	0.006±0.001	0.001	0.017	0.011±0.001	0.001	0.026	0.022±0.002	0.007	0.045	0.024±0.003	0.004	0.057	0.040±0.005	0.003	0.082
Muscle mass-specific projection energy (J kg ⁻¹)	0.018±0.003	0.003	0.051	0.033±0.004	0.003	0.077	0.064±0.007	0.022	0.133	0.072±0.009	0.011	0.169	0.117±0.016	0.008	0.242
Retraction duration (ms)	76.4±4.3	36.1	135.0	66.8±4.8	40.7	136.8	39.4±1.7	23.8	60.6	32.3±1.6	19.5	50.0	28.7±1.8	16.9	45.8
Average retraction velocity (m s ⁻¹)	0.07±0.01	0.02	0.12	0.09±0.01	0.02	0.14	0.13±0.01	0.08	0.24	0.15±0.01	0.05	0.30	0.17±0.02	0.06	0.31
Max. retraction velocity (m s ⁻¹)	0.11±0.01	0.03	0.23	0.15±0.01	0.04	0.23	0.23±0.01	0.14	0.34	0.26±0.02	0.10	0.43	0.31±0.03	0.11	0.53
Max. retraction acceleration (m s ⁻²)	4.8±0.7	1.2	16.9	8.4±0.9	2.2	18.5	17.2±1.7	3.1	42.8	28.9±2.4	8.4	57.5	34.7±4.2	10.4	75.0
Max. tongue mass-specific retraction power (W kg ⁻¹)	0.42±0.08	0.03	2.19	0.93±0.13	0.06	2.48	2.95±0.34	0.66	7.26	5.63±0.86	0.70	16.10	8.97±1.76	0.90	31.90
Max. muscle mass-specific retraction power (W kg ⁻¹)	0.44±0.09	0.03	2.31	0.98±0.14	0.06	2.61	3.11±0.36	0.69	7.65	5.93±0.91	0.73	16.97	9.45±1.86	0.94	33.62

Continued

Table 2. Continued

	5°C			10°C			15°C			20°C			25°C		
	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.
<i>Ensatina</i>															
Projection distance (mm)	7.0±0.3	5.2	11.6	7.4±0.4	2.7	13.1	7.1±0.3	3.8	11.7	8.3±0.5	3.0	17.1	8.7±0.5	4.1	15.5
Projection duration (ms)	22.6±3.7	7.1	62.3	12.6±1.9	4.8	67.2	13.3±1.4	5.7	42.0	10.9±0.9	5.5	29.8	9.6±0.5	6.2	20.8
Average projection velocity (m s ⁻¹)	0.45±0.05	0.07	0.84	0.68±0.05	0.11	1.41	0.63±0.05	0.12	1.50	0.84±0.07	0.10	1.68	0.93±0.07	0.24	1.94
Max. projection velocity (m s ⁻¹)	0.96±0.07	0.27	1.68	1.35±0.07	0.45	2.23	1.26±0.05	0.83	1.92	1.50±0.08	0.67	2.50	1.73±0.09	0.56	2.71
Max. projection acceleration (m s ⁻²)	314±38	53	677	559±41	100	1190	527±53	86	1620	626±46	155	1200	842±71	158	2110
Max. tongue mass-specific projection power (W kg ⁻¹)	22.1±35	10	560	52.1±60	24	1750	430±63	102	1900	587±61	91	1600	958±108	73	2760
Max. muscle mass-specific projection power (W kg ⁻¹)	49.2±78	21	1248	116.2±134	53	3900	95.8±139	227	4234	130.9±136	203	3565	213.4±241	163	6150
Projection energy (mJ)	4E-02±5E-03	3E-03	1E-01	8E-02±7E-03	8E-03	2E-01	7E-02±6E-03	3E-02	1E-01	1E-01±1E-02	2E-02	2E-01	1E-01±1E-02	1E-02	3E-01
Tongue mass-specific projection energy (J kg ⁻¹)	0.52±0.07	0.04	1.41	1.00±0.10	0.10	2.49	0.84±0.07	0.34	1.84	1.23±0.12	0.22	3.13	1.65±0.15	0.16	3.67
Muscle mass-specific projection energy (J kg ⁻¹)	1.15±0.15	0.08	3.14	2.22±0.21	0.22	5.54	1.88±0.16	0.76	4.11	2.75±0.28	0.49	6.96	3.67±0.34	0.35	8.18
Retraction duration (ms)	99.0±7.6	48.8	193.2	93.8±6.5	29.6	198.9	78.8±7.7	22.9	302.5	54.9±3.3	16.3	91.7	52.1±4.2	20.8	132.3
Average retraction velocity (m s ⁻¹)	0.07±0.01	0.03	0.15	0.08±0.01	0.03	0.25	0.10±0.01	0.02	0.42	0.16±0.01	0.05	0.36	0.18±0.01	0.04	0.48
Max. retraction velocity (m s ⁻¹)	0.14±0.01	0.08	0.34	0.23±0.03	0.07	0.96	0.27±0.03	0.07	0.70	0.43±0.05	0.10	1.13	0.49±0.04	0.08	1.09
Max. retraction acceleration (m s ⁻²)	8.3±1.5	2.2	33.2	13.8±2.3	1.1	82.0	16.7±2.2	2.4	60.7	34.3±5.2	5.0	139.0	62.0±8.8	0.3	257.0
Max. tongue mass-specific retraction power (W kg ⁻¹)	1.2±0.4	0.1	8.5	2.1±0.6	0.03	23.0	3.3±0.8	0.1	24.4	9.8±2.8	0.3	91.3	22.5±4.2	0.4	115.0
Max. muscle mass-specific retraction power (W kg ⁻¹)	1.5±0.5	0.2	11.3	2.7±0.8	0.05	30.4	4.3±1.1	0.1	32.2	13.0±3.7	0.4	120.7	29.7±5.5	0.5	152.0

Mean values are given ±s.e.m.

Plethodon: N=30, 27, 29, 30 and 25°C, respectively. *Ensatina*: N=26, 39, 36, 35 and 38 for 5, 10, 15, 20 and 25°C, respectively.

mouth towards the prey (Fig. 2; Movie 1). Upon contact with the prey, the tongue was retracted into the mouth along with the prey. A lunge of the whole body towards the prey occasionally accompanied tongue projection and retraction, and this occurred more often in *Plethodon* than in *Ensatina*.

Prey capture in *Plethodon*

A total of 138 feedings of *Plethodon* were recorded from six individuals across the entire range of experimental temperatures.

Tongue projection duration ranged from 17.9 to 132 ms (Table 2, Fig. 3) with average projection velocity as low as 0.02 m s^{-1} (at 5°C) and as high as 0.25 m s^{-1} (25°C). These durations and velocities were achieved over tongue projection distances that ranged from 1.1 mm (10°C) to a maximum of 8.7 mm (5°C). *Plethodon* achieved a maximum instantaneous muscle mass-specific power of 54 W kg^{-1} at the warmest temperature, 25°C . Tongue retraction duration and velocity were similar to the duration and velocity of tongue projection in *Plethodon*. Muscle

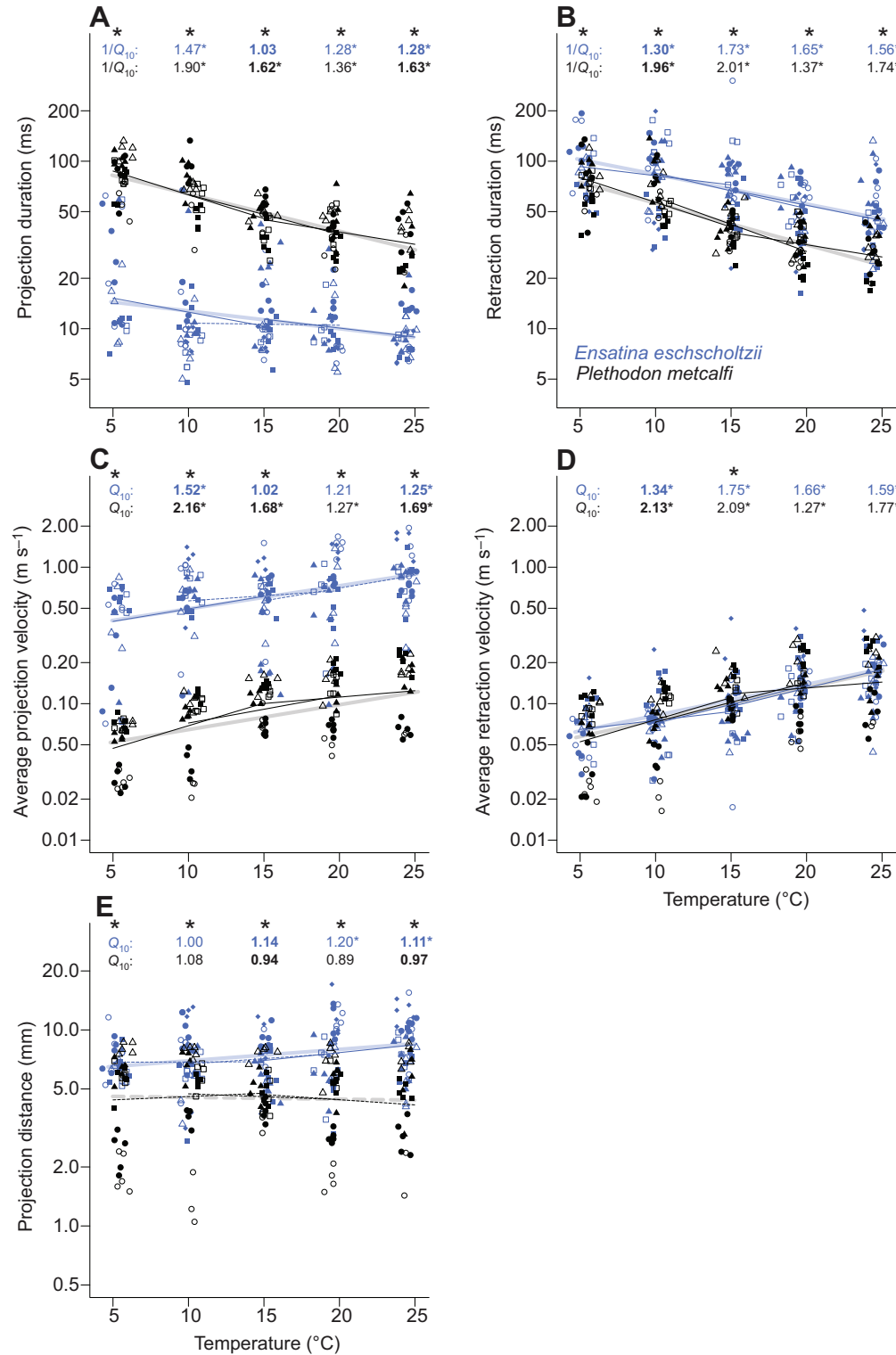


Fig. 3. Scatterplots of kinematic parameters versus body temperature including all feedings of *Plethodon* (black) and *Ensatina* (blue). Y-axes have log₁₀ scales and are the same for a given parameter to facilitate comparison between projection and retraction. Note the significantly higher performance of tongue projection in *Ensatina*. Individual salamanders are shown as different symbols. 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. Q₁₀ values are shown for each temperature interval (5–15, 10–20, 15–25 and 5–25°C from left to right), with asterisks on Q₁₀ values indicating a significant temperature effect. Bold Q₁₀ values indicate significantly different thermal sensitivity between species across each temperature interval.

mass-specific power, in contrast, was lower on average during tongue retraction than projection, but still showed significant overlap in values with a range of 0.03 W kg⁻¹ (5°C) to 34 W kg⁻¹ (25°C) (Table 2, Fig. 4).

Tongue projection kinematics and dynamics were generally temperature sensitive in *Plethodon*. There was no significant effect of temperature on tongue projection distance in any temperature interval (Table S1). However, at the 5–15 and 10–20°C temperature

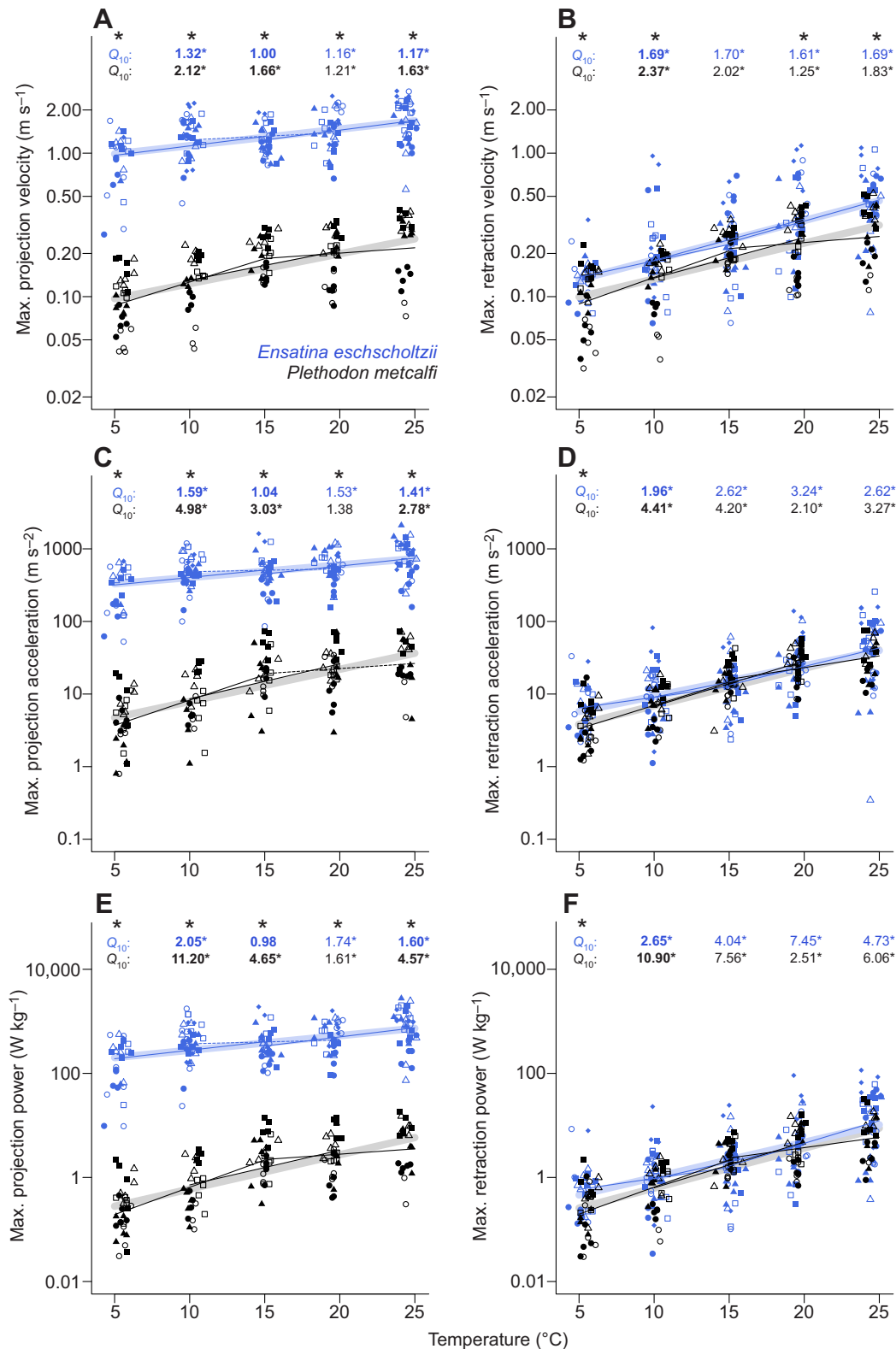


Fig. 4. Scatterplots of dynamic parameters versus body temperature including all feedings of *Plethodon* (black) and *Ensatina* (blue). For description, see Fig. 3.

intervals, all other projection variables were significantly influenced by temperature, and the greatest reduction in performance occurred below 15°C. Tongue retraction dynamics showed a similar relationship with temperature, being significantly affected by temperature over the 5–25°C range, with the greatest decline in performance below 15°C (Table S1).

Prey capture in *Ensatina*

A total of 179 feedings from seven individuals were recorded across the entire temperature range for *Ensatina*. Tongue projection duration had a range of 4.8 ms (at 10°C) to 67.2 ms (10°C) (Table 2, Fig. 3). Average tongue projection velocity ranged from 0.07 m s⁻¹ (5°C) to 1.94 m s⁻¹ (25°C). Projection covered distances ranging from 2.7 mm (10°C) to 17.1 mm (20°C). *Ensatina* were capable of high power output during projection as maximum muscle mass-specific power reached 6150 W kg⁻¹ at the warmest temperature, 25°C. Tongue retraction performance was greatly reduced compared with tongue projection, with lower performance at all experimental temperatures (Figs 3, 4). Notably, muscle mass-specific power reached a maximum of only 152 W kg⁻¹ (at 25°C).

The thermal dependence of kinematic and dynamic variables varied greatly in *Ensatina*, but tongue projection was generally less temperature sensitive than retraction. Tongue projection distance was not significantly affected by temperature in the 5–15 and 10–20°C intervals (Table S2, Fig. 3). In the 15–25°C interval and over the entire 5–25°C temperature range, projection distance was significantly influenced by temperature, but with low Q_{10} values. All other projection variables also showed a significant temperature effect across the full 5–25°C range, but again had relatively low Q_{10} values. Temperature significantly influenced all projection variables in the 5–15°C interval, but did not affect any variable in the 10–20°C interval. However, most projection variables were significantly affected by temperature across the 15–25°C range. Tongue retraction performance was also temperature dependent over the full 5–25°C temperature range (Table S2) and at every interval, and the Q_{10} values were generally higher than those for tongue projection.

Comparison of *Plethodon* and *Ensatina*

Ensatina exhibited significantly greater tongue projection performance than *Plethodon*. At all temperatures, *Ensatina* tongue projection had shorter durations, but covered greater distances (Table S3, Fig. 3). Tongue projection velocity (and hence kinetic energy), acceleration and power were all significantly higher in *Ensatina* across all temperatures. Differences were especially large between the two species for projection acceleration, kinetic energy and power.

Ensatina tongue projection was also less thermally dependent than that of *Plethodon*. The Q_{10} values of *Ensatina* projection variables were significantly lower when calculated over the entire range of temperatures (5–25°C) examined in this study (Table S4). Furthermore, Q_{10} values for projection velocity, acceleration, power and kinetic energy were significantly lower in *Ensatina* at both 5–15 and 10–20°C intervals.

In contrast to the divergent performance of tongue projection in *Plethodon* and *Ensatina*, tongue retraction kinematics of the two species were similar. Only tongue retraction duration differed between the species at all temperatures, with *Plethodon* having a significantly shorter duration (Table S3, Fig. 3). Average retraction velocity only differed between the species at 15°C; therefore, the reduced retraction duration of *Plethodon* was likely the result of

significantly shorter projection distance. *Ensatina* had significantly higher maximum retraction velocity at all temperatures except 15°C (Table S3, Fig. 4).

Tongue retraction was also similar between the two species with regard to temperature dependence. Across the 5–15°C range, *Ensatina* tongue retraction was less temperature sensitive than that of *Plethodon*, having significantly lower Q_{10} for all variables ($1/Q_{10}$ for durations) (Table S4). However, the two species did not differ in tongue retraction Q_{10} or $1/Q_{10}$ values for intervals above 15°C, or across the entire 5–25°C temperature range. Tongue retraction in the two species showed similar and relatively high temperature sensitivity.

DISCUSSION

Morphology

Plethodon and *Ensatina* both have large, fleshy tongue pads and a genioglossus muscle attaching the tongue pad to the lower jaw. This morphology is unlike the ballistic tongues of other plethodontid salamanders such as *Hydromantes*, *Bolitoglossa* and *Eurycea* in which the tongue pad is compact and lacks a genioglossus muscle (Lombard and Wake, 1977; Wake and Deban, 2000). *Ensatina* has an absolutely and relatively larger tongue and SAR muscle than *Plethodon* (Table 1), yet it is capable of ballistic projection as shown by the absence of inner SAR muscle fibers inserting on the epibranchials (Fig. 1). The lack of inner myofibers permits the epibranchial to completely evacuate the SAR muscle lumen upon projection (Fig. 1C), a mechanism not possible in *Plethodon*, which retains inner fibers. Freedom of the epibranchials from the SAR, combined with greater epibranchial length than for *Plethodon*, allows *Ensatina* to extend its tongue over twice as far as *Plethodon* of the same body length (Table 1). Projection ability in *Ensatina* is also improved by the elongated genioglossus muscle, which originates more caudally on the mandible than in *Plethodon* (Lombard and Wake, 1977).

Ensatina has a greater tongue and SAR mass than *Plethodon* (Table 1), indicating greater specialization for tongue projection. Our analysis also revealed a significant difference between the species in tongue mass when accounting for SAR mass, consistent with the pattern of reduced tongue mass relative to projector muscle mass seen in other ballistic-tongued plethodontids. With a ratio of tongue mass to SAR mass of 2.2, *Ensatina* lies between *Plethodon* (with a ratio of 3.0) and other ballistic taxa (*Hydromantes*, 1.04; *Bolitoglossa*, 0.79; and *Eurycea*, 1.3; Deban et al., 2007). A lower ratio of tongue mass to muscle mass contributes to higher velocity, acceleration and power of tongue projection, i.e. higher performance, whether the mechanism is powered by muscle contraction or elastic recoil.

Ensatina differs from other ballistic species in having a relatively large tongue mass for its body size; however, it shares with them an epibranchial shape that is round in cross-section (Deban et al., 2007), a shape unlike that of *Plethodon* (Fig. 1). This round shape may facilitate projection, given that myofibers inserting on the epibranchial are absent and muscle forces are only applied radially to the epibranchial via the collagen sheath.

Plethodon and *Ensatina* differ in the configuration of the collagenous aponeuroses within the SAR muscle. The aponeuroses are significantly better developed in *Ensatina*, forming spirals (Fig. 1) similar to those found in the SAR of *Hydromantes*, a species with an elastic recoil mechanism of tongue projection (Deban et al., 2007). The presence and orientation of these spiral aponeuroses indicate that *Ensatina*, unlike *Plethodon*, is capable of elastic energy storage and recoil like *Hydromantes*.

Prey capture in *Plethodon*

Our analyses of feeding kinematics provide evidence that tongue protraction is the result of muscle activity in *Plethodon*. Maximum muscle mass-specific power reached only 54 W kg^{-1} at 25°C , which is well below the maximum instantaneous power of 373 W kg^{-1} measured in amphibian muscle (Lutz and Rome, 1996). Tongue projection and retraction performance were markedly similar in *Plethodon*, with velocity, acceleration and power output largely overlapping (Table 2). Tongue retraction is a muscle-powered movement in salamanders (Wake and Deban, 2000; Deban and Richardson, 2011; Anderson et al., 2014) so this similarity in performance indicates that both projection and retraction are the result of direct muscle power rather than elastic recoil.

Plethodon achieved relatively low maximum velocity (0.4 m s^{-1}), acceleration (74.0 m s^{-2}) and projection distance (8.7 mm). These values are low compared with those of plethodontids with elastically powered tongue projection (Deban, 1997; Wake and Deban, 2000; Deban et al., 2007; Deban and Richardson, 2011; Anderson et al., 2014), but consistent with previous studies of *Plethodon* feeding (Maglia and Pyles, 1995) and similar to other species with attached tongues (Wake and Deban, 2000). The maximum tongue reach observed in *Plethodon* was 15.6% of SVL. Although this is a longer projection distance than for other species with attached tongues (e.g. 7% of SVL; Wake and Deban, 2000), it is much shorter than that of *Ensatina* (see below).

Tongue projection in *Plethodon* was achieved with little mechanical work from the SAR, based on the calculated kinetic energy of projection. The total muscle mass-specific energy of 0.24 J kg^{-1} (achieved at 25°C) is much lower than that of the SAR during ballistic tongue projection in *Eurycea* at $3.9\text{--}6.5 \text{ J kg}^{-1}$ (Deban et al., 2007; Anderson et al., 2014), *Bolitoglossa* at 19.5 J kg^{-1} (Deban et al., 2007), *Hydromantes* at 12.3 J kg^{-1} (Deban and Richardson, 2011), and of the depressor mandibulae during ballistic mouth opening in *Rana* at 6.3 J kg^{-1} (27°C ; Sandusky and Deban, 2012) and *Bufo* at 3.6 J kg^{-1} (24°C ; Deban and Lappin, 2011; Deban and Richardson, 2011; Sandusky and Deban, 2012; Anderson et al., 2014). Non-elastic projection of *Plethodon* falls short of these values from elastic systems, but all of these values are lower than the maximum theoretical muscle work of 67 J kg^{-1} for frog striated muscle (Peplowski and Marsh, 1997), indicating that these feeding muscles are operating at low stress, low strain, or both.

In *Plethodon*, maximum velocity, acceleration and muscle mass-specific power of tongue retraction (0.53 m s^{-1} , 75 m s^{-2} and 34 W kg^{-1} , respectively) are lower than those of the ballistic-tongued *Eurycea* (0.81 m s^{-1} , 171 m s^{-2} and 74 W kg^{-1}) (Anderson et al., 2014). The lower retraction performance of *Plethodon* relative to *Eurycea* may be due to its relatively more massive tongue. *Plethodon* achieves similar values to *Hydromantes* (0.91 m s^{-1} , 75 m s^{-2} and 36 W kg^{-1}) (Deban and Richardson, 2011), which also has a more massive tongue than does *Eurycea*.

Kinematic and dynamic analyses indicate that tongue projection and retraction in *Plethodon* are significantly and similarly influenced by temperature. At lower temperature ranges, Q_{10} values were all above 1.6 and frequently above 2.0, demonstrating high temperature dependence. However, in the $15\text{--}25^\circ\text{C}$ range, temperature effects diminish with Q_{10} values below 1.6 for nine of the 12 kinematic variables. This pattern of high thermal sensitivity (Q_{10} near 2) coupled with a plateau of optimal performance is consistent with the responses of other muscle-powered movements including feeding movements of other amphibians (Bennett, 1984,

1985; Deban and Lappin, 2011; Anderson et al., 2014), and provides further evidence that tongue projection and retraction are both the result of direct muscle power. Furthermore, temperature sensitivity of projection in *Plethodon* is greater than that of ballistic species with elastic projection – *Eurycea*, *Hydromantes* (Deban and Richardson, 2011; Anderson et al., 2014) and *Ensatina*.

Prey capture in *Ensatina*

The results from our dynamic analysis of feeding reveal that *Ensatina* is utilizing an elastic recoil mechanism to achieve high-performance tongue projection. The maximum muscle mass-specific power of tongue projection in *Ensatina* reached 6150 W kg^{-1} at a body temperature of 25°C , which is well in excess of the maximum instantaneous power measured in amphibian muscle (373 W kg^{-1} at 25°C) (Lutz and Rome, 1996). This performance is similar to that of *Eurycea*, another ballistic-tongued plethodontid with elastic recoil (Anderson et al., 2014). In contrast to tongue projection, tongue retraction in *Ensatina* is performed with a maximum muscle mass-specific power of 152 W kg^{-1} , which is well within the limits of muscle power.

Ensatina tongue projection achieved a maximum velocity of 2.7 m s^{-1} and acceleration up to 2110 m s^{-2} . These values vastly exceed those of non-ballistic projection in *Plethodon*, they overlap considerably with those of *Eurycea*, and they fall short of the maximum performance of *Hydromantes* and *Bolitoglossa*, which also possess an elastic mechanism (Deban et al., 2007). Similarly, the maximum tongue reach observed in *Ensatina* of 29% SVL is similar to that of *Eurycea* (33% SVL) and *Bolitoglossa* (31% SVL) (Deban et al., 2007) but is far short of that of the salamander with the longest tongue relative to body length, *Hydromantes* (80% SVL) (Deban et al., 1997).

In *Ensatina*, maximum velocity, acceleration and muscle mass-specific power of tongue retraction (1.09 m s^{-1} , 257 m s^{-2} and 152 W kg^{-1} , respectively) were also much lower than those of tongue projection. However, retraction performance values are higher than the corresponding values of *Eurycea*, *Hydromantes* (Deban and Richardson, 2011; Anderson et al., 2014) and *Plethodon*, indicating that greater emphasis is placed on tongue retraction by *Ensatina*. This higher performance retraction combined with our observation that *Ensatina* has a massive tongue apparatus and a large tongue pad, suggests that *Ensatina* may feed on relatively larger prey than the other species.

Ensatina achieved moderately high SAR muscle work during tongue projection, peaking at 8.2 J kg^{-1} at 25°C and at 3.1 J kg^{-1} at 5°C . These values overlap with the range of values from other elastic feeding systems in frogs and salamanders ($3.6\text{--}19.5 \text{ J kg}^{-1}$) (Deban et al., 2007; Deban and Lappin, 2011; Deban and Richardson, 2011; Sandusky and Deban, 2012; Anderson et al., 2014). Muscle work of *Ensatina* is significantly higher than in non-elastic projection by *Plethodon*, but like *Plethodon* and the elastic species, the muscle work of *Ensatina* falls short of the maximum theoretical muscle work of 67 J kg^{-1} (Peplowski and Marsh, 1997), suggesting constraints on muscle strain or stress.

Comparison of kinematic and dynamic parameters across temperature intervals showed that tongue projection in *Ensatina* is significantly affected by temperature changes, but is nonetheless relatively thermally robust and maintains high performance across the $5\text{--}25^\circ\text{C}$ range. Twenty-one of the 28 Q_{10} values for projection performance fell below 1.5 (Table S2; Figs 3, 4). Tongue retraction, in contrast, showed greater thermal sensitivity, with all but one Q_{10} value above 1.6 and most values significantly greater than the corresponding Q_{10} values for projection. Considering the lower

thermal sensitivity of projection compared with retraction, we can conclude that the elastic recoil mechanism of projection confers its thermal robustness.

Thermal robustness of movement dynamics such as we found in *Ensatina* tongue projection has been demonstrated in other systems with elastic recoil such as the tongue projection of plethodontid salamanders *Eurycea* and *Hydromantes*, toads, frogs and chameleons (Anderson and Deban, 2010; Deban and Lappin, 2011; Deban and Richardson, 2011; Sandusky and Deban, 2012; Anderson et al., 2014). Projection performance in *Ensatina* declines at lower temperatures (5–10°C) but is more thermally robust at lower temperatures than that of *Eurycea*, which drops dramatically at 5°C (Anderson et al., 2014); perhaps the greater mass-specific muscle work of *Ensatina* compared with *Eurycea* provides a greater ‘reservoir’ of muscle energy that can be used at lower temperatures. Projection performance in *Ensatina* is not as thermally robust as that of *Hydromantes*, which maintains complete thermal independence across a temperature range of 2–24°C (Deban and Richardson, 2011) and has higher mass-specific muscle work than the other species.

Tongue projection mechanisms compared

The ballistic tongue of *Ensatina* displayed significantly greater projection performance than the non-ballistic tongue of *Plethodon* at all experimental temperatures. The greater tongue reach of *Ensatina* is attributable to the complete freedom of the tongue skeleton from the SAR muscle and the elongated genioglossus muscle. The higher dynamic performance of *Ensatina* is accomplished by a mechanism of stretching and subsequent recoil of the collagen aponeuroses in the SAR muscle that decouples muscle contractile rate from the rate at which projection is performed. This elastic recoil system allows the SAR muscle of *Ensatina* to put energy into elastic tissue prior to tongue projection and thereby produce higher total muscle work than that of *Plethodon* (e.g. 3.67 versus 0.117 J kg⁻¹ on average at 25°C). By extracting more work from the muscle, *Ensatina* is capable of projection with a velocity several times greater than *Plethodon* (1.73 versus 0.26 m s⁻¹; Table 2). During tongue projection the elastic tissue can release stored energy at a higher rate (i.e. with higher power) than the SAR muscle is capable of producing directly (2134 versus 18.4 W kg⁻¹), thus achieving higher kinetic energy in the limited excursion of tongue projection. The more massive tongue apparatus of *Ensatina* would be expected to yield lower accelerations than *Plethodon* if the systems were otherwise similar (Hill, 1950), but the mechanism of elastic recoil in the tongue of *Ensatina* allows it to far outperform the smaller tongue of *Plethodon* in this regard.

In addition to enhancing performance of tongue projection at all temperatures, the elastic mechanism of *Ensatina* reduces its thermal dependence compared with *Plethodon*, as evidenced by the significantly lower Q_{10} values of projection parameters of *Ensatina* across the entire 5–25°C temperature range and in the 5–15°C and 10–20°C intervals. The higher thermal robustness of projection velocity in *Ensatina* indicates that the elastic recoil mechanism enhances the thermal robustness of muscle work, in addition to the relative amount of muscle work. The comparative temperature coefficients of these two species that are similar in many aspects of their biology including ecology, developmental mode and the general anatomy of their tongue apparatus (Lombard and Wake, 1977; Stebbins, 1985; Conant and Collins, 1991) highlights the benefits of elastic mechanisms with regard to the thermal robustness of muscle function and high-performance movements.

Comparison of the morphology and performance of feeding in *Plethodon* and *Ensatina* indicates that it is the freedom of the tongue skeleton from the SAR and the elaboration of the collagen aponeuroses in the SAR muscle that underpin the high performance and thermal robustness of tongue projection in salamanders. Elongation of an elastic element in series with muscle fibers can increase the capacity for the work performed by the muscle fibers to be stored as elastic energy and subsequently released more quickly, even the absence of other morphological or physiological changes (Alexander, 2002). Ballistic projection and elastic recoil are found in several species of plethodontid salamanders, and elongated spiral aponeuroses in the SAR like those we see in *Ensatina* are present in all species with high performance that have been examined, including *Hydromantes*, *Eurycea* and *Bolitoglossa* (Deban et al., 2007). These species also lack SAR myofibers that insert on the epibranchial. These results demonstrate that changes in morphology – loss of myofiber attachments and elaboration of connective tissue – can dramatically alter the mechanism, performance and robustness of a musculoskeletal system.

Our comparison of *Ensatina* and *Plethodon* additionally suggests that feeding in Plethodontidae is an exceptional model in which to study the evolution of elastic mechanisms and their impact on organismal performance. Within the plethodontid clade Plethodontinae, *Plethodon* is basal and has muscle-powered tongue protraction, while *Ensatina* and *Hydromantes*, with their ballistic tongue projection, are more deeply nested (Vieites et al., 2011). *Eurycea* and *Bolitoglossa* both use ballistic, elastic tongue projection and are members of the sister clade Hemidactyliinae (Vieites et al., 2011). These phylogenetic relationships suggest that ballistic tongue projection has evolved more than once independently within the Plethodontidae. Documenting the feeding performance and tongue apparatus morphology of more plethodontid taxa will provide valuable insights into how elastic systems and thermal robustness have evolved in salamanders.

Conclusions

Elastic recoil mechanisms have been shown to enhance the performance and thermal robustness of diverse feeding mechanisms in ectotherms, including ballistic tongue projection in chameleons and salamanders and ballistic mouth opening in frogs and toads (Anderson and Deban, 2010, 2012; Deban and Lappin, 2011; Deban and Richardson, 2011; Sandusky and Deban, 2012; Anderson et al., 2014). The divergent anatomy of these independently evolved systems has made it difficult to identify which specific morphological features contribute to high performance. Our comparison of elastic projection in *Ensatina* with non-elastic projection in *Plethodon* indicates that the elaboration of collagenous tissue in series with myofibers is a critical feature of these high-performance systems. Elastic tissue in series with muscle fibers has the potential to decouple muscle shortening from movement as the elastic tissue is stretched (Roberts and Azizi, 2011); this may permit the muscle to operate with a force and duration that enhance its work output, while the rapid recoil of elastic tissue at a wide range of temperatures increases thermal robustness of the movement.

Elastic recoil has been demonstrated in an array of high-performance movements, such as suction feeding in fish, jumping in frogs and bushbabies, and predatory and defensive movements of many arthropods (Aerts et al., 1987; Aerts, 1998; Roberts and Marsh, 2003; Patek et al., 2006; Burrows, 2006; Patek et al., 2007,

2011; Van Wassenbergh et al., 2008; Burrows, 2011; Astley and Roberts, 2014). Elastic mechanisms may also operate in systems without extraordinary performance; for example, in frogs, stretch and recoil of the plantaris tendon occurs even in short jumps (Astley and Roberts, 2012). In systems with modest performance, elastic recoil may still enhance muscle work output but would not be detected via analyses that seek high acceleration or power production. However, in all elastic systems we expect thermal robustness of movement dynamics to be enhanced, and propose that temperature-manipulation experiments can be used to detect such cryptic elastic recoil mechanisms.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Both authors designed the study, were involved in data collection, analyzed the data and wrote the manuscript.

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Supplementary information

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