Movements of vastly different performance have similar underlying muscle physiology

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ABSTRACT

Many animals use elastic recoil mechanisms to power extreme movements, achieving levels of performance that would not be possible using muscle power alone. Contractile performance of vertebrate muscle depends strongly on temperature, but the release of energy from elastic structures is far less thermally dependent, thus elastic recoil confers thermal robustness to whole-animal performance. Here we explore the role that muscle contractile properties play in the differences in performance and thermal robustness between elastic and non-elastic systems by examining muscle from two species of plethodontid salamanders that use elastically powered tongue projection to capture prey and one that uses non-elastic tongue projection. In species with elastic mechanisms, tongue projection is characterized by higher mechanical power output and thermal robustness compared with tongue projection of closely related genera with non-elastic mechanisms. *In vitro* and *in situ* muscle experiments reveal that species differ in their muscle contractile properties, but these patterns do not predict the performance differences between elastic and non-elastic tongue projection. Overall, salamander tongue muscles are similar to other vertebrate muscles in contractile performance and thermal sensitivity. We conclude that changes in the tongue-projection mechanism, specifically the elaboration of elastic structures, are responsible for high performance and thermal robustness in species with elastic tongue projection. This suggests that the evolution of high-performance and thermally robust elastic recoil mechanisms can occur via relatively simple changes to morphology, while muscle contractile properties remain relatively unchanged.

KEY WORDS: Elastic recoil, Salamander, Feeding

INTRODUCTION

Movements powered by elastic recoil rely on the storage of muscle work as energy in stretched elastic structures prior to the initiation of movement (Alexander and Bennet-Clark, 1977; Deban et al., 2007; Roberts and Azizi, 2011). Muscle power can be effectively amplified when muscles do work at relatively low power to stretch an elastic structure. The energy is subsequently released at a relatively high power when the elastic structure recoils (Alexander

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and Bennet-Clark, 1977; Roberts and Azizi, 2011) and this recoil is not expected to change substantially with temperature (Rigby et al., 1959; Alexander, 1966; Denny and Miller, 2006). Because of this temporal decoupling of movement from muscle contraction, muscle properties such as the rate of force development and contractile velocity, and thus the effects of temperature on these properties (reviewed in Bennett, 1984; James, 2013), are not expected to influence performance directly (Anderson and Deban, 2010). Therefore, elastic recoil can allow animals to achieve levels of performance that would be impossible using muscle alone and can also allow them to maintain high performance at varying temperatures.

Many animal behaviors take advantage of elastic recoil to achieve high-performance movements (Patek et al., 2011; Roberts and Azizi, 2011; Higham and Irschick, 2013). For example, elastically powered jumping in bushbabies is characterized by power output 15 times greater than what could be achieved by muscle (Aerts, 1998), and trap-jaw ants can close their jaws at speeds up to 64 m s⁻¹ for prey capture, defence and even to propel the body (Patek et al., 2006). Elastic recoil is thought to explain not only high performance, but also thermal robustness in the feeding mechanisms of several groups of ectothermic vertebrates. In these species, high-performance tongue projection exceeds what is possible from muscle power. Required muscle mass-specific power output in these species with elastic recoil reaches $9600\ \text{W kg}^{-1}$ in toads (Lappin et al., 2006), $14{,}000\ \text{W kg}^{-1}$ in chameleons (de Groot and van Leeuwen, 2004; Anderson, 2016), and $18{,}000\ \text{W kg}^{-1}$ in salamanders (de Groot and van Leeuwen, 2004; Lappin et al., 2006; Deban et al., 2007). Tongue projection in these animals is more robust to changes in temperature than would be expected based on the properties of vertebrate muscle, with temperature coefficients (Q_{10} values) of tongue projection velocity, acceleration and power of only 1.1–1.3 in chameleons (Anderson and Deban, 2010), 0.99–1.25 in toads (Deban and Lappin, 2011), and 0.94–1.04 in salamanders (Anderson and Deban, 2010; Deban and Lappin, 2011; Deban and Richardson, 2011; Anderson and Deban, 2012; Anderson et al., 2014; Deban and Scales, 2016; Scales et al., 2016).

Elastic recoil mechanisms can substantially change performance and thermal robustness by temporally decoupling movements from muscle contraction; however, it is unclear if underlying muscle contractile properties differ between elastic and non-elastic systems. Although muscle contractile velocity and power are largely inconsequential for final performance when a muscle is storing energy in an elastic structure, other properties, specifically the force of contraction and the mechanical work performed, will influence the total energy stored. Additionally, the rate of force development together with the timing of muscle activation could influence the amount of energy stored if high force is not reached before the elastic structure recoils. Because these muscle properties are affected by temperature (reviewed in Bennett, 1984; James,

2013), movement performance may still be thermally sensitive when elastic recoil is used in the absence of specialized physiology.

Here we use salamander tongue projection to examine three hypotheses in which muscle contractile properties may affect performance in elastic versus non-elastic systems. In the first hypothesis (H1), muscle contractile properties contribute to differences in performance and thermal robustness between elastic and non-elastic systems. We therefore expect muscle in elastic systems to reach higher forces more rapidly during contraction compared with muscle in non-elastic systems; we also expect temperature to have a reduced effect on force and rates of force generation in species with elastic tongue projection versus species with non-elastic tongue projection. In the second hypothesis (H2), elastic recoil solely determines performance and thermal robustness in elastic systems. In this case, we expect muscle properties to be similar in all elastic and non-elastic systems. In the third hypothesis (H3), elastic recoil again solely determines performance and thermal robustness in elastic systems, but relaxed selection on muscle contraction rates in elastic systems may have resulted in lower rates of force generation and possibly greater thermal sensitivity in those muscles compared with the non-elastic systems.

Lungless salamanders in Plethodontidae are a useful system for examining the role of muscle contractile properties in high-performance elastic recoil systems, because high-performance elastically powered tongue projection has evolved convergently. In the most extreme cases of elastic projection, the tongue is launched up to 80% of body length with accelerations up to 600 *g* and projection performance is robust to changing temperatures (Deban et al., 2007; Deban and Richardson, 2011; Anderson et al., 2014; Deban and Scales, 2016; Scales et al., 2016). In contrast, the tongue travels short distances and performance is thermally dependent in species with non-elastic tongue projection (Deban and Scales, 2016; Scales et al., 2016).

The muscles and skeletal elements of the tongue apparatus of plethodontid salamanders are fundamentally similar in species with elastic and non-elastic projection. The tongue skeleton includes paired, elongated epibranchial cartilages surrounded by cylindrical paired subarcualis rectus (SAR) muscles that power tongue projection (Lombard and Wake, 1977; Wake and Deban, 2000). The anterior ends of the epibranchials articulate with paired ceratobranchials, which are attached anteriorly to the unpaired basibranchial bearing the tongue pad. Contraction of the SAR (i.e. projector) muscles pushes the epibranchials rostrally, projecting the entire tongue skeleton and tongue pad. Tongue retraction is achieved through contraction of paired rectus cervicis profundus (RCP) muscles that originate on the pelvis and insert onto the tongue pad. In species with elastically powered tongue projection, the SAR includes well-developed aponeuroses in which elastic energy is stored prior to tongue projection; the tongue skeleton leaves the lumen of the SAR when fully projected and travels under its own momentum towards prey. In contrast, species with muscle-powered tongue projection lack sufficient elastic structures to store energy and the tongue skeleton does not function as a ballistic projectile. In both cases, the RCP retracts the tongue using muscle power alone and is not associated with stored elastic energy.

Here we examine whether isometric muscle contractile properties and the temperature effects on these muscle properties differ between elastic and non-elastic systems. We compare contractile properties across a range of temperatures of the tongue projector and retractor muscles of two species with independently evolved elastic tongue projection, *Ensatina eschscholtzii* Gray 1850 and *Eurycea guttolineata* (Holbrook 1838), and one species with non-elastic

tongue projection, *Desmognathus quadramaculatus* (Holbrook 1840). For each comparison, we examine several variables from isometric contractions, but seek holistic patterns of similarity rather than predict absolute quantitative differences across species or tongue types for each variable. Examining two elastic and one non-elastic species allows us to ask if species with elastic energy storage are more similar to each other than to the non-elastic species.

To test the three alternative hypotheses above, we compare SAR properties between elastic and non-elastic tongue projectors to determine if the presence of elastic energy storage is correlated with particular muscle contractile properties. If the presence of elastic energy storage determines the contractile properties of the SAR, then we would expect values from the two independently evolved elastically powered tongue-projecting species, *Ensatina* and *Eurycea*, to be more similar to each other than the muscle-powered tongue projector, *Desmognathus*. We also compare contractile properties of the SAR with the RCP within a species to see if the differences in function – tongue projection versus retraction – between these muscles are accompanied by differences in contractile properties. If SAR contractile properties are correlated with an elastic energy-storage mechanism, then the differences between the SAR and RCP should be greater in *Ensatina* and *Eurycea* than in *Desmognathus*. Finally, we compare properties of both muscles from all three species with values from other taxa. Again, we would expect the SAR contractile properties to differ most from other vertebrate muscle in *Ensatina* and *Eurycea* if the presence of elastic energy storage is correlated with muscle contractile physiology.

MATERIALS AND METHODS
Retractor muscles

Data for both muscles from *E. guttolineata* were raw values used in Anderson et al. (2014). Similar methods were used to collect muscle contractile data from doubly pithed *E. eschscholtzii* and *D. quadramaculatus*. We dissected retractor (RCP) muscles from five *E. eschscholtzii* (body mass, 6.3–10.4 g) and eight *D. quadramaculatus* (body mass, 7.4–10.2 g). Prior to excision, the tongue was extended to maximum-projection distance by suspending the body mass of the individual by the tongue, which lengthened the RCP muscle to a position approximating the start of tongue retraction following maximal projection *in vivo*. The entire RCP muscle was then removed and a section approximately 30% of the length of an individual RCP muscle in the mid-abdominal region was tied off with Kevlar thread (The Thread Exchange, Weaverville, NC, USA). The length of this section under the condition of full tongue extension was then measured using digital calipers (±0.1 mm accuracy; Mitutoyo 700-126, Kawasaki-shi, Kanagawa, Japan), excised from the RCP muscle, and attached to a dual servomotor force lever (model 305C-LR, Aurora Scientific, Aurora, ON, Canada). The muscle was stimulated using a bipolar pulse stimulator (model 701B, Aurora Scientific) controlled by a custom instrument in LabVIEW software (version 9.0, National Instruments, Austin, TX, USA). The muscles were positioned between platinum-coated electrodes and submerged in a tissue–organ bath (model 805A, Aurora Scientific) filled with oxygenated amphibian Ringer's solution (Fischmeister and Hartzell, 1987). Temperature of the Ringer's solution was controlled using a temperature-controlled water circulator (IsoTemp 1013S, Fisher Scientific, Waltham, MA, USA).

Prior to stimulation, the section of RCP muscle was stretched to its *in situ* extended length using a micropositioner in order to measure contractions under functional situations analogous to

in vivo conditions. These conditions may not place each muscle at an optimal length for force production as the length–tension relationship of the RCP may differ among species, which may influence comparisons of peak contractile force. However, muscle length would not influence the effects of temperature on contractile force, nor comparisons of temperature effects among species and muscles.

Each muscle was allowed to rest in the muscle bath for 20 min before contractile data were collected at each temperature. Stimulus conditions resulting in maximum tetanic contraction were determined from preliminary experiments prior to data collection. Muscles were stimulated with 1 ms pulses at 10 V with a frequency ranging from 100 to 140 pulses s^{-1} to result in tetanic contraction for a duration of 300 to 400 ms depending on temperature to avoid overstimulation that would fatigue the muscle. The muscles rested for 5 min between each stimulation to avoid acute effects of fatigue. Forces from isometric contractions were recorded for each muscle at several temperatures in either of the following sequences: 25–20–15–10–5–10–15–20–25°C or 5–10–15–20–25–20–15–10–5°C. Two contractions were recorded at each temperature except the middle 5 or 25°C at which four contractions were measured to balance the dataset. This balanced sequence was chosen to eliminate the long-term effects of fatigue on the muscle across the entire experiment. By including both the initial and final measurements from each temperature, the effect of fatigue at each temperature was the same on average for muscles with complete series. If the attachment of the muscle to the lever failed before a complete series had been collected, the contralateral RCP muscle from the same individual was used to record contractions from the complementary sequence of increasing or decreasing temperatures. For some *D. quadramaculatus* RCP muscles, contractions at 25°C were unreliable, so contractions were recorded starting at 20°C to verify proper muscle function before proceeding through the temperature sequence.

Projector muscles

An *in situ* preparation was used to measure contractions from projector (SAR) muscles in six *E. eschscholtzii* (body mass, 5.0–10.4 g) and six *D. quadramaculatus* (body mass, 7.4–9.9 g). After retractor muscles were removed and the tongue was positioned at rest inside the mouth, a small, silver chain was attached to the paired ceratobranchials using two bespoke metal hooks fashioned from insect pins through an incision in the intermandibular skin and superficial muscles. A patch electrode was inserted subcutaneously over each of the paired SAR muscles to stimulate bilateral contraction. The salamander was secured in position in the muscle bath by tying its jaws to the bottom of the bath with Kevlar thread. The chain connected to the ceratobranchials was attached to the muscle lever so that stimulation of the SAR muscles resulted in the tongue skeleton being pushed forward, putting tension on the chain, which registered on the muscle lever (Anderson et al., 2014). Because this method measures the force of tongue projection rather than SAR force directly, comparisons of absolute muscle force among species would be confounded by morphological and functional differences in the tongue projection mechanism. However, force of projection must be proportional to muscle force because no other muscles are involved in the projection mechanism. The effect of temperature on tongue projection force is therefore directly representative of the effect of temperature on SAR force and comparisons of temperature sensitivity among species are valid. Values of SAR force were recorded for each species in order to describe interspecific differences in the temperature sensitivity of

this property, but we do not interpret interspecific differences in contractile force at any one temperature.

The SAR muscles were stimulated using 1 ms pulses at 20 V with frequencies ranging from 100 to 120 pulses s^{-1} to result in tetanic contractions for a stimulus duration of 300 ms. The muscles rested for 5 min between stimulations and two contractions were measured at each temperature starting at 15°C and following either an increasing (5–10–20–25–15°C) or decreasing (25–20–10–5–15°C) sequence. By including data from an equal number of individuals with increasing and decreasing temperature sequences, the effects of long-term fatigue are the same on average.

Direct measurement of force, velocity, power and work of concentric contractions would be most relevant for understanding *in vivo* function during tongue projection. However, it was not possible to measure concentric contractions due to the morphology of the SAR muscle and the tongue apparatus. Instead we measured the effects of temperature on several isometric contractile properties with the expectation that interspecific differences in temperature effects on isometric properties are correlated with interspecific differences in temperature effects on concentric contractions. Temperature effects on force production are correlated with the effects of temperature on muscle work, and in part, muscle power during concentric contractions (Olberding and Deban, 2017). In muscles where both have been measured, temperature effects on concentric contractile properties are often stronger than those on isometric properties (for example Renaud and Stevens, 1984; Rome and Sosnicki, 1990; James et al., 2012). Therefore, detecting significant effects of temperature on isometric properties would imply significant effects of temperature on concentric properties in the same muscle.

Analyses

All analyses were completed using custom scripts in R (www.r-project.org). For all muscle contractions, passive tension on the system was subtracted from total force measurements before analyses. Electromechanical delay was calculated as the elapsed time from the start of stimulation to the time at which force rose for six consecutive milliseconds. For each RCP muscle contraction, peak isometric force (P_0) was determined to be the maximum force reached during the contraction. Because P_0 for *E. guttolineata* RCP muscles was measured using two lengths of the muscle in parallel (Anderson et al., 2014), the values of P_0 were doubled for *E. eschscholtzii* and *D. quadramaculatus* to allow for comparison. The peak force measured from the SAR muscles is referred to as projection force, F_{proj}, because it is not a direct measurement of the P_0 of those muscles. The average and peak rate of force generation to 50 and 90% F_{proj} or P_0 was determined from the first time derivative of force using a quintic spline using the pspline package in R. The peak and average rates of muscle relaxation were measured from the end of stimulation to the time at which the force declined to 50% F_{proj} or P_0. For the RCP muscle, specific tension of the muscle was calculated by dividing the average cross-sectional area of the muscle using the formula: muscle mass/(1.06 g cm^{-3}×muscle length) (Pasi and Carrier, 2003). Specific tension could not be calculated for the SAR muscles because the complex architecture of these muscles prevents measurements of cross-sectional area.

We used analysis of covariance with temperature as a continuous variable, species as a categorical variable, and individual as a random factor to examine the interactive effects of species and temperature on contractile variables. Separate analyses were performed for the total 5–25°C temperature range and for three

overlapping temperature intervals: 5–15, 10–20 and 15–25°C. Because the relationship between the variables of interest and temperature is expected to be exponential, all dependent variables were $\log_{10}$ transformed prior to analyses. When the interaction between species and temperature was significant, similar analyses

were conducted for each pair of species to determine significant pairwise comparisons. Separate analyses for each species including temperature as a continuous variable and individual as a random factor were conducted for the total temperature range and for each temperature interval. The partial regression coefficients for
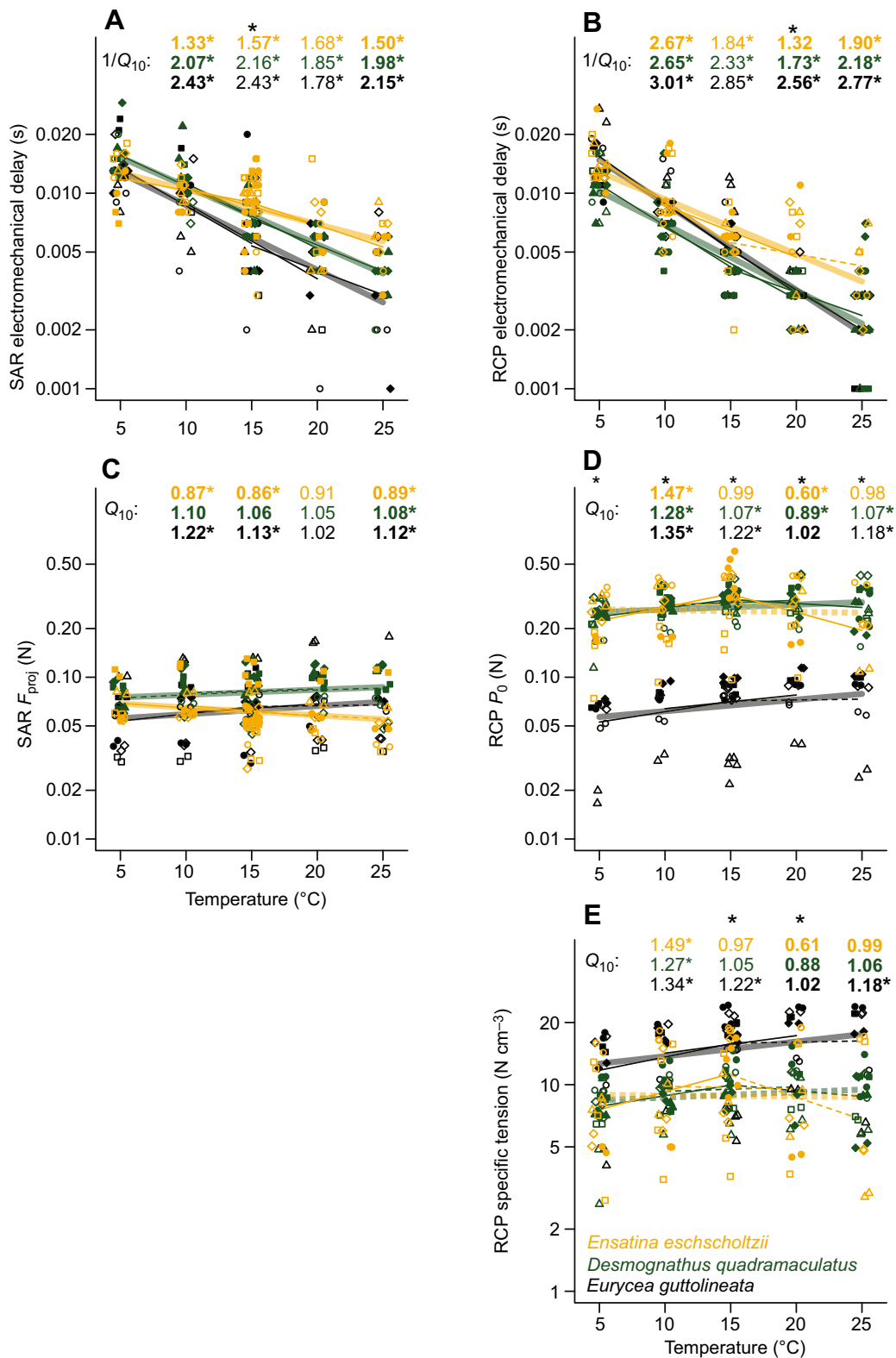

Fig. 1. See next page for legend.

Fig. 1. Electromechanical delay and peak force versus temperature. Data are plotted with introduced jitter in temperature values to make individuals plotted as symbols discernible at each temperature. Lines are colored by species and represent temperature effects from regressions with temperature as a continuous variable and individual as a random factor. Separate analyses were conducted for 5–15, 10–20 and 15–25°C temperature intervals (thin lines) the total 5–25°C range (thick lines). At the top, Q_{10} values are colored for each species for the 5–15, 10–20 and 15–25°C intervals from left to right, with the 5–25°C range on the far right. These values are proportional to the slopes of the plotted regression lines. Asterisks following Q_{10} values and continuous regression lines indicate significant temperature effects. Bold values indicate significant interactions between species and temperature at that temperature interval. Asterisks above each temperature indicate significant differences between species at that temperature. Electromechanical delay decreases significantly with increasing temperature for all species at all temperature intervals for the SAR muscle (A) and for all except *Ensatina eschscholtzii* at the 15–25°C interval for RCP muscle (B). For some species, F_{proj} of the SAR muscle (C) and P_0 of the RCP muscle (D) increase or decrease significantly with increasing temperature, but the magnitude of these effects is small and results in small differences between 5 and 25°C. Forces of the RCP expressed as specific tension (E) show a similar trend.

temperature from these analyses were used to calculate the temperature coefficient (Q_{10}) with the following equation: $Q_{10}=10^{(\text{partial regression coefficient}\times10)}$ (Deban and Lappin, 2011). Temperature coefficients were considered significantly different from 1.0 if the *P*-value of the regression coefficient was less than α=0.05. To examine interspecific differences at each experimental temperature, regression analyses including species as a fixed factor and individual as a random factor were conducted for each untransformed variable. When the species term was significant, similar analyses were conducted including each pair of species to determine significant pairwise comparison. Significance values of all tests were adjusted using the Benjamini–Hochberg procedure to control false discovery rate (Benjamini and Hochberg, 1995).

RESULTS
Muscle force followed an expected trajectory through time at most temperatures for both the SAR and RCP in all species (for descriptive statistics, see Table S1 and Table S2). After an initial electromechanical delay following stimulation, force increased until it reached a plateau where it remained until stimulation ended. For *D. quadramaculatus* muscle at 20 and 25°C, muscle force sometimes reached an early 'shoulder' where the rate of force development suddenly dropped, after which force rose slowly over time. In some cases of RCP muscle contraction for all species at 20 and 25°C, force dropped after reaching a peak value even though stimulation continued.

The electromechanical delay between the onset of stimulation and the start of force generation significantly decreased with increasing temperature across nearly all temperature intervals and the complete temperature range for all species in both the SAR and RCP muscles (Fig. 1A,B). However, the effect of temperature on the electrochemical delay of the RCP muscle in *E. eschscholtzii* was not significant at the 15–25°C interval (Fig. 1B). The Q_{10} values for electromechanical delay in *E. eschscholtzii* were significantly lower than the other two species for both the SAR (Table 1) and RCP (Table 2) muscles at most temperature intervals. Additionally, electromechanical delay of the SAR and RCP muscles was significantly longer for *E. eschscholtzii* compared with the other species at 15°C (Table 3).

The peak force of the SAR muscle, F_{proj}, was significantly affected by temperature in all species, but the effects in *E. guttolineata* were more similar to *D. quadramaculatus* than to *E. eschscholtzii*, the other species with elastic tongue projection (Fig. 1C, Table 1). In general, F_{proj} in *E. eschscholtzii* decreased with increasing temperature while F_{proj} in *D. quadramaculatus* and *E. guttolineata* increased with increasing temperature (Fig. 1C). Despite differing temperature effects, values of F_{proj} were not significantly different among all three species at all temperatures (Fig. 1C).

Peak forces for the RCP muscle were significantly lower for *E. guttolineata* compared with the other species (Table 4) and temperature effects were significantly different between species at the 5–15 and 15–25°C intervals (Table 2). The P_0 of *E. eschscholtzii* increased significantly with increasing temperature across the 5–15°C interval and decreased significantly with increasing temperature across the 15–25°C interval (Fig. 1D). The P_0 of *D. quadramaculatus* increased significantly with temperature over the 5–25°C range and the 5–15 and 10–20°C intervals, but this change was relatively small (Fig. 1D). For *E. guttolineata*, P_0 increased significantly with increasing temperature for the 5–25°C range and the 5–15 and 10–20°C intervals.

Values and temperature effects of specific tension of the RCP were significantly different for *E. guttolineata* compared with the other two species (Fig. 1E). Specific tension was significantly higher for *E. guttolineata* than *E. eschscholtzii* and *D. quadramaculatus* at 15 and 20°C (Table 4). Specific tension increased significantly with increasing temperature over the 5–25, 5–15 and 10–20°C intervals in *E. guttolineata*, but only increased signficantly with temperature over the 5–15°C interval in *E. eschscholtzii* and *D. quadramaculatus* (Fig. 1E).

Rates of force development and temperature effects on these rates were similar for both muscles among all three species at different

Table 1. Pairwise comparisons between species from models of SAR muscle contractile variables with significant species effects

Variable	E. eschscholtzii vs D. quadramaculatus			E. eschscholtzii vs E. guttolineata			D. quadramaculatus vs E. guttolineata		
	F	*P*	Adj. α	*F*	*P*	Adj. α	*F*	*P*	Adj. α
5°C									
Time to 50% F_{proj}	0.086	0.777	0.050	23.481	**0.001**	0.017	17.638	**0.002**	0.017
Time to 90% F_{proj}	3.411	0.098	0.033	8.093	**0.016**	0.033	14.263	**0.004**	0.033
Time to relax to 50%	28.884	**<0.001**	0.017	0.167	0.691	0.050	12.949	**0.005**	0.050
15°C									
Electromechanical delay	8.052	**0.019**	0.050	13.041	**0.004**	0.050	1.706	0.221	0.050
Time to 50% F_{proj}	8.835	**0.016**	0.025	19.791	**0.001**	0.025	2.881	0.120	0.025
20°C									
Time to 50% F_{proj}	4.585	0.061	0.050	16.488	**0.002**	0.050	2.550	0.141	0.050

Species effects were not significant for any variables at 10 or 25°C. Bold values indicate statistical significance after controlling for false discovery rate.

Table 2. Pairwise comparisons between species from models of RCP contractile variables with significant species effects

Variable	*E. eschscholtzii* vs *D. quadramaculatus*			*E. eschscholtzii* vs *E. guttolineata*			*D. quadramaculatus* vs *E. guttolineata*		
	F	P	Adj. α	F	P	Adj. α	F	P	Adj. α
5°C									
P_0	0.002	0.968	0.050	27.751	**0.001**	0.030	106.292	**<0.001**	0.010
Peak relax 50%	6.381	0.028	0.020	6.500	**0.031**	0.040	0.119	0.736	0.050
Time to 50% P_0	1.518	0.244	0.030	58.541	**<0.001**	0.010	14.845	**0.002**	0.040
Time to 90% P_0	0.111	0.745	0.040	35.718	**<0.001**	0.020	15.092	**0.002**	0.030
Time to relax to 50%	12.521	**0.005**	0.010	2.682	0.136	0.050	19.316	**0.001**	0.020
10°C									
P_0	0.072	0.793	0.050	23.571	**0.001**	0.038	85.262	**<0.001**	0.013
Time to 50% P_0	0.887	0.366	0.025	48.243	**<0.001**	0.013	19.172	**0.001**	0.050
Time to 90% P_0	0.074	0.790	0.038	43.619	**<0.001**	0.025	21.553	**0.001**	0.038
Time to relax to 50%	26.756	**<0.001**	0.013	3.820	0.082	0.050	43.958	**<0.001**	0.025
15°C									
P_0	0.824	0.383	0.040	34.022	**<0.001**	0.030	78.596	**<0.001**	0.010
Specific tension	2.381	0.151	0.030	2.302	0.163	0.050	9.822	**0.009**	0.050
Time to 50% P_0	4.284	0.063	0.020	37.723	**<0.001**	0.020	17.506	**0.001**	0.040
Time to 90% P_0	0.541	0.477	0.050	56.450	**<0.001**	0.010	22.310	**<0.001**	0.030
Time to relax to 50%	12.908	**0.004**	0.010	16.752	**0.003**	0.040	48.769	**<0.001**	0.020
20°C									
Electromechanical delay	16.757	**0.002**	0.008	10.222	**0.011**	0.033	0.443	0.518	0.050
P_0	0.394	0.543	0.042	15.320	**0.004**	0.025	57.075	**<0.001**	0.008
Specific tension	0.006	0.941	0.050	6.088	0.036	0.042	14.600	**0.002**	0.033
Peak relax 50%	2.393	0.150	0.033	2.731	0.133	0.050	9.472	**0.010**	0.042
Time to 50% P_0	6.273	0.029	0.025	64.448	**<0.001**	0.008	17.593	**0.001**	0.025
Time to relax to 50%	6.518	0.027	0.017	22.237	**0.001**	0.017	26.822	**<0.001**	0.017
25°C									
P_0	0.863	0.375	0.038	10.453	**0.012**	0.025	40.044	**<0.001**	0.025
Peak relax 50%	0.004	0.954	0.050	4.708	0.062	0.050	10.229	**0.008**	0.038
Time to 90% P_0	5.251	0.045	0.013	6.694	**0.032**	0.038	0.498	0.494	0.050
Time to relax to 50%	4.932	0.051	0.025	55.610	**<0.001**	0.013	41.920	**<0.001**	0.013

'Peak relax 50%' is the peak rate of force decline measured from the end of stimulation to the time where force had declined to 50% of P_0. Bold values indicate statistical significance after controlling for false discovery rate.

temperatures ranges (Fig. 2; Tables 3 and 4). Both the peak and average rates of force development measured to 50% P_0 (or F_{proj}) increased significantly with increasing temperature for both muscles in all species at most temperature intervals (Fig. 2A–D). The only exception was the RCP muscle in *E. eschscholtzii*; both peak and average rates of force development did not change significantly with temperature at the 15–25°C interval (Fig. 1B,D).

Species differed in the effect of temperature on peak and average rate of force development for SAR muscle, with significantly lower Q_{10} values for *E. eschscholtzii* at most temperature intervals (Table 1). For the RCP muscle, Q_{10} values were significantly different between species at most temperature intervals, but this followed no clear pattern (Fig. 2; Table 2). The time to reach 50% of P_0 (F_{proj}) decreased significantly with increasing temperature for both muscles in all species at all temperature intervals (Fig. 1E,F). Time to reach 50% of peak force was significantly shorter in *E. guttolineata* at 5, 15 and 20°C for the SAR muscle (Table 3) and 5, 10, 15 and 20°C for the RCP muscle (Table 4).

Average rates of force development to 90% P_0 (F_{proj}) showed similar patterns to those when calculated to 50% P_0 with the exception of *D. quadramaculatus* (Fig. 3A,B). For *D. quadramaculatus*, the average rate of force development to 90% P_0 decreased significantly with increasing temperature at the 15–25°C interval for both the SAR and RCP muscles (Fig. 3A,B) and likewise the time taken to reach 90% P_0 increased significantly with increasing temperature for this species at that interval (Fig. 3C,D). This resulted in a non-significant temperature effect for both rate of force development and time to 90% P_0 across the total 5–25°C range

for *D. quadramaculatus* RCP muscle (Fig. 3B,D). Peak rates of force development to 90% of P_0 (or F_{proj}) were identical to those calculated to 50% P_0 because peak rate occurred early in force development.

Rates of relaxation following stimulation were similar for both muscles in all three species (Fig. 4A,B) but species differed significantly in the temperature effects on rates of relaxation for both muscles at all temperatures, with *E. eschscholtzii* having lower Q_{10} values in most cases (Tables 1 and 2). For SAR muscles, the peak rate of relaxation increased significantly with increasing temperature for all three species at all temperature intervals except for *E. eschscholtzii* at the 15–25°C interval (Fig. 4A). The average rate of relaxation for the SAR muscle increased significantly with increasing temperature for all species at all temperature intervals (Fig. 4C). Peak rate of relaxation of the RCP muscle increased significantly with increasing temperature over the entire temperature range (5–25°C) and the 5–15°C interval in *E. eschscholtzii*. For *D. quadramaculatus*, peak rate of relaxation of the RCP increased significantly with increasing temperature for all intervals except 15–25°C. Peak rate of relaxation of the RCP increased significantly with increasing temperature for all temperature intervals for *E. guttolineata* (Fig. 4B). The average rate of relaxation of the RCP muscle increased significantly with increasing temperature at all intervals for all species except 15–25°C in *E. eschscholtzii* (Fig. 4D).

For the SAR muscle, *D. quadramaculatus* had a significantly longer relaxation time to reach 50% F_{proj} compared with the other species, but only at 5°C. The relaxion time to 50% P_0 (F_{proj}) decreased significantly with increasing temperature for both

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Table 3. Pairwise comparisons of temperature effects between genera from models of SAR muscle contractile variables with significant temperature–species interactions for each interval

Variable	E. eschscholtzii vs D. quadramaculatus			E. eschscholtzii vs E. guttolineata			D. quadramaculatus vs E. guttolineata		
	F	P	Adj. α	F	P	Adj. α	F	P	Adj. α
5–25°C									
Electromechanical delay	13.738	**<0.001**	0.033	17.264	**<0.001**	0.022	0.922	0.339	0.033
Average rate 50%	23.215	**<0.001**	0.022	9.768	**0.002**	0.033	2.074	0.152	0.017
Average relax 50%	34.807	**<0.001**	0.011	37.247	**<0.001**	0.006	0.330	0.567	0.044
F_{proj}	22.932	**<0.001**	0.028	35.928	**<0.001**	0.017	1.562	0.214	0.028
Peak rate 50%	32.627	**<0.001**	0.017	16.450	**<0.001**	0.028	1.725	0.191	0.022
Peak relax 50%	41.125	**<0.001**	0.006	36.586	**<0.001**	0.011	0.022	0.883	0.050
Time to 50% F_{proj}	1.300	0.256	0.050	2.371	0.126	0.050	5.814	0.017	0.006
Time to 90% F_{proj}	8.563	**0.004**	0.044	3.064	0.082	0.044	2.378	0.125	0.011
Time to relax to 50%	9.109	**0.003**	0.039	5.747	**0.018**	0.039	0.911	0.342	0.039
5–15°C									
Electromechanical delay	9.965	**0.002**	0.040	17.711	**<0.001**	0.020	1.414	0.238	0.040
Average rate 50%	47.443	**<0.001**	0.005	3.944	0.050	0.045	16.229	**<0.001**	0.010
Average rate 90%	17.154	**<0.001**	0.025	1.794	0.184	0.050	7.011	**0.010**	0.025
Average relax 50%	18.029	**<0.001**	0.020	28.755	**<0.001**	0.005	1.535	0.219	0.035
F_{proj}	8.488	**0.004**	0.045	19.274	**<0.001**	0.015	2.024	0.159	0.030
Peak rate 50%	35.624	**<0.001**	0.010	4.230	0.042	0.040	8.229	**0.005**	0.020
Peak relax 50%	11.979	**0.001**	0.035	20.909	**<0.001**	0.010	0.490	0.486	0.050
Time to 50% F_{proj}	19.320	**<0.001**	0.015	6.705	**0.011**	0.035	42.106	**<0.001**	0.005
Time to 90% F_{proj}	2.172	0.144	0.050	10.122	**0.002**	0.030	16.149	**<0.001**	0.015
Time to relax to 50%	12.491	**0.001**	0.030	12.439	**0.001**	0.025	1.251	0.267	0.045
10–20°C									
Average rate 50%	11.893	**0.001**	0.020	4.346	**0.040**	0.050	0.538	0.465	0.040
Average relax 50%	10.712	**0.001**	0.030	14.666	**<0.001**	0.010	0.636	0.427	0.020
F_{proj}	5.358	**0.023**	0.050	9.768	**0.002**	0.030	0.642	0.425	0.010
Peak rate 50%	13.792	**<0.001**	0.010	8.010	**0.006**	0.040	0.014	0.907	0.050
Peak relax 50%	9.028	**0.003**	0.040	12.982	**0.001**	0.020	0.565	0.454	0.030
15–25°C									
Average rate 90%	12.089	**0.001**	0.050	2.233	0.139	0.033	15.916	**<0.001**	0.033
Peak relax 50%	24.542	**<0.001**	0.033	8.198	**0.005**	0.017	2.370	0.128	0.050
Time to 90% F_{proj}	25.049	**<0.001**	0.017	0.000	0.991	0.050	22.493	**<0.001**	0.017

'Average rate 90%' and 'Average rate 50%' are the average rate of force development measured from start of stimulation to time of 90 and 50% of F_{proj}, respectively. 'Peak rate 50%' is the peak rate of force development measured from start of stimulation to time of 50% F_{proj}. 'Peak relax 50%' and 'Average relax 50%' are the peak and average rates of force decline measured from the end of stimulation to the time where force had declined to 50% of F_{proj}. Bold values indicate statistical significance after controlling for false discovery rate.

muscles for all species at all temperature intervals (Fig. 4E,F). Species differed significantly in the relaxation time to 50% P_0 for the RCP muscle at all temperatures with *D. quadramaculatus* tending to be higher than the other two species at most temperatures and *E. guttolineata* tending to be lower than the other two species at most temperatures (Table 4).

DISCUSSION

Plethodontid salamanders with elastic tongue projection, such as species of the genera *Ensatina* and *Eurycea*, have tongue-projection velocities, accelerations and power that are not only higher but are also more robust to changing temperature than species with non-elastic tongue projection, such as those of the genus *Desmognathus* (Anderson et al., 2014; Scales et al., 2016). Even considering the thermal robustness afforded by elastic recoil mechanisms, these performance differences might be expected to result in part from the projector muscles (SAR) of species with elastic projection reaching higher forces more rapidly during activation than those of species with non-elastic tongue projection while also being less affected by changing temperature (H1). Alternatively, relaxed selection may be expected to produce decreased muscle contractile rates in species with elastic tongue projection (H3). Contrary to these hypotheses, however, several lines of evidence below suggest that muscle contractile properties are unrelated to the presence or absence of elastic recoil (H2).

Despite differences in both performance and thermal robustness between elastic and non-elastic tongue projection (Anderson et al., 2014; Deban and Scales, 2016; Scales et al., 2016), the thermal dependence of rates of force development and relaxation in both the SAR and RCP muscles are similar to those in other vertebrates (reviewed in Bennett, 1984; James, 2013). As in other vertebrate muscles, the highest Q_{10} values of isometric force production at any temperature in the tongue muscles of *Desmognathus* (SAR: 1.10; RCP: 1.47) and *Eurycea* (SAR: 1.22; RCP: 1.35) are small compared with Q_{10} values for rate properties like maximum shortening velocity (1.5–4.2), and power output (1.37–6.86) (Ranatunga, 1982, 1998; Bennett, 1984; Johnston and Gleeson, 1984, 1987; Renaud and Stevens, 1984; Johnston and Altringham, 1985; Rome and Sosnicki, 1990; Coughlin et al., 1996; De Ruiter and De Haan, 2000; James, 2013; Olberding and Deban, 2017). For *Ensatina*, isometric force production decreases with increasing temperature at all temperature ranges for the SAR muscle, a pattern that is not found in the other genera. Additionally, isometric force is highest at 15°C for the RCP muscle in *Ensatina* (Fig. 1). This decrease in force at warmer temperatures may indicate that muscles in *Ensatina* are adapted for functioning at colder temperatures compared with the other genera. Other than the possible adaptation to different thermal optima, the tongue muscles in these three species function similarly to other vertebrate muscle and

Table 4. Pairwise comparisons of temperature effects between species from models of RCP contractile variables with significant temperature–species interactions for each interval

Variable	E. eschscholtzii vs D. quadramaculatus			E. eschscholtzii vs E. guttolineata			D. quadramaculatus vs E. guttolineata		
	F	P	Adj. α	F	P	Adj. α	F	P	Adj. α
5–25°C									
Electromechanical delay	2.018	0.158	0.030	3.258	0.074	0.040	17.360	**<0.001**	0.030
Average rate 50%	6.534	0.012	0.010	2.595	0.110	0.045	1.850	0.176	0.045
Average rate 90%	4.484	0.036	0.025	4.650	**0.033**	0.035	22.266	**<0.001**	0.025
Average relax 50%	0.828	0.364	0.040	28.931	**<0.001**	0.005	64.982	**<0.001**	0.010
Specific tension	0.002	0.963	0.050	5.984	**0.016**	0.030	28.278	**<0.001**	0.015
Peak rate 50%	4.936	0.028	0.015	6.306	**0.013**	0.025	0.404	0.526	0.050
Peak relax 50%	0.079	0.779	0.045	28.719	**<0.001**	0.010	87.622	**<0.001**	0.005
Time to 50% P_0	1.432	0.234	0.035	28.106	**<0.001**	0.015	27.511	**<0.001**	0.020
Time to 90% P_0	22.196	**<0.001**	0.005	20.089	**<0.001**	0.020	12.825	**<0.001**	0.035
Time to relax to 50%	4.805	0.030	0.020	0.086	0.769	0.050	6.728	**0.010**	0.040
5–15°C									
Electromechanical delay	2.816	0.097	0.029	0.447	0.506	0.050	9.591	**0.003**	0.043
Average rate 50%	0.007	0.933	0.043	4.344	0.040	0.029	14.058	**<0.001**	0.029
Average relax 50%	7.815	**0.006**	0.007	0.899	0.346	0.043	10.829	**0.001**	0.036
P_0	3.071	0.083	0.021	11.832	**0.001**	0.014	24.142	**<0.001**	0.021
Peak rate 50%	7.041	**0.010**	0.014	0.979	0.326	0.036	8.527	**0.004**	0.050
Time to 50% P_0	0.002	0.961	0.050	15.397	**<0.001**	0.007	29.819	**<0.001**	0.007
Time to 90% P_0	0.131	0.719	0.036	11.200	**0.001**	0.021	25.404	**0.000**	0.014
10–20°C									
Average relax 50%	0.769	0.383	0.025	12.203	**0.001**	0.025	25.974	**<0.001**	0.025
Peak relax 50%	0.428	0.515	0.038	12.761	**0.001**	0.013	33.297	**<0.001**	0.013
Time to 50% P_0	0.374	0.543	0.050	5.485	**0.022**	0.038	15.937	**<0.001**	0.038
Time to relax to 50%	5.052	0.027	0.013	0.760	0.386	0.050	3.889	0.052	0.050
15–25°C									
Electromechanical delay	0.231	0.632	0.050	4.977	**0.029**	0.050	7.539	**0.007**	0.025
Average rate 50%	7.619	**0.007**	0.030	15.491	**<0.001**	0.030	1.611	0.208	0.050
Average rate 90%	2.409	0.125	0.040	17.050	**<0.001**	0.020	46.651	**<0.001**	0.010
Average relax 50%	12.553	**0.001**	0.010	38.601	**<0.001**	0.010	34.783	**<0.001**	0.020
P_0	8.675	**0.004**	0.025	16.681	**<0.001**	0.025	4.715	**0.033**	0.040
Specific tension	1.958	0.166	0.045	6.352	**0.014**	0.040	6.376	**0.013**	0.030
Peak rate 50%	9.251	**0.003**	0.020	22.087	**<0.001**	0.015	5.061	**0.027**	0.035
Peak relax 50%	10.729	**0.002**	0.015	48.422	**<0.001**	0.005	57.527	**<0.001**	0.005
Time to 50% P_0	3.760	0.056	0.035	6.633	**0.012**	0.035	3.205	0.077	0.045
Time to 90% P_0	26.572	**<0.001**	0.005	5.441	**0.023**	0.045	36.978	**<0.001**	0.015

'Average rate 90%' and 'Average rate 50%' are the average rate of force development measured from start of stimulation to time of 90 and 50% of P_0, respectively. 'Peak rate 50%' is the peak rate of force development measured from start of stimulation to time of 50% P_0. 'Peak relax 50%' and 'Average relax 50%' are the peak and average rates of force decline measured from the end of stimulation to the time where force had declined to 50% of P_0. Bold values indicate statistical significance after controlling for false discovery rate.

show no obvious modifications that could produce the documented thermal robustness of tongue-projection performance without the contribution of elastic recoil.

The species examined differ in some contractile properties of the SAR muscle both in terms of performance and thermal robustness; however, these patterns are not consistent with tongue-projection performance differences in genera with elastic versus non-elastic tongue projection. The F_{proj} of *Eurycea* and *Desmognathus* SAR muscles increased with increasing temperature as expected from many previous studies of vertebrate muscle (Bennett, 1984; Lännergren and Westerblad, 1987; Rall and Woledge, 1990; Marsh, 1994; Syme, 2006; Herrel et al., 2007; James et al., 2012; Olberding and Deban, 2017), in contrast to the decline in force seen in *Ensatina* (Fig. 1). Temperature sensitivity of the rate of force development in *Ensatina*, however, was significantly lower than in *Desmognathus* and *Eurycea* (Figs 2 and 3). These differences in muscle properties may lead us to expect that tongue-projection performance would be more similar in *Desmognathus* and *Eurycea* compared with *Ensatina*. However, performance is more similar in *Ensatina* and *Eurycea* with thermally robust high-power elastic

projection, than muscle-powered, non-elastic tongue projection in *Desmognathus* (Anderson et al., 2014; Deban and Scales, 2016; Scales et al., 2016). Considering that the species also did not differ in the rate of SAR force development, these patterns of interspecific differences suggest that contractile properties are not apparently shaped either by selection for increased performance and thermal robustness or by relaxed selection in the presence of an elastic recoil mechanism that could compensate for lower muscle performance.

Overall, the temperature effects on muscle properties in *Ensatina* do not explain the temperature effects on performance. Unlike *Eurycea* and *Desmognathus*, SAR projection force decreased (albeit weakly) with increasing temperature in *Ensatina* (Fig. 1), contrasting with results from previous studies of vertebrate muscle at similar temperatures (Bennett, 1984; Lännergren and Westerblad, 1987; Rall and Woledge, 1990; Marsh, 1994; Syme, 2006; Herrel et al., 2007; James et al., 2012). Decreasing force with increasing temperature would reduce the work done by the SAR muscle at warmer temperatures, assuming constant load. However, tongue projection performance does not decrease with increasing temperature in *Ensatina* (Deban and Scales, 2016), possibly
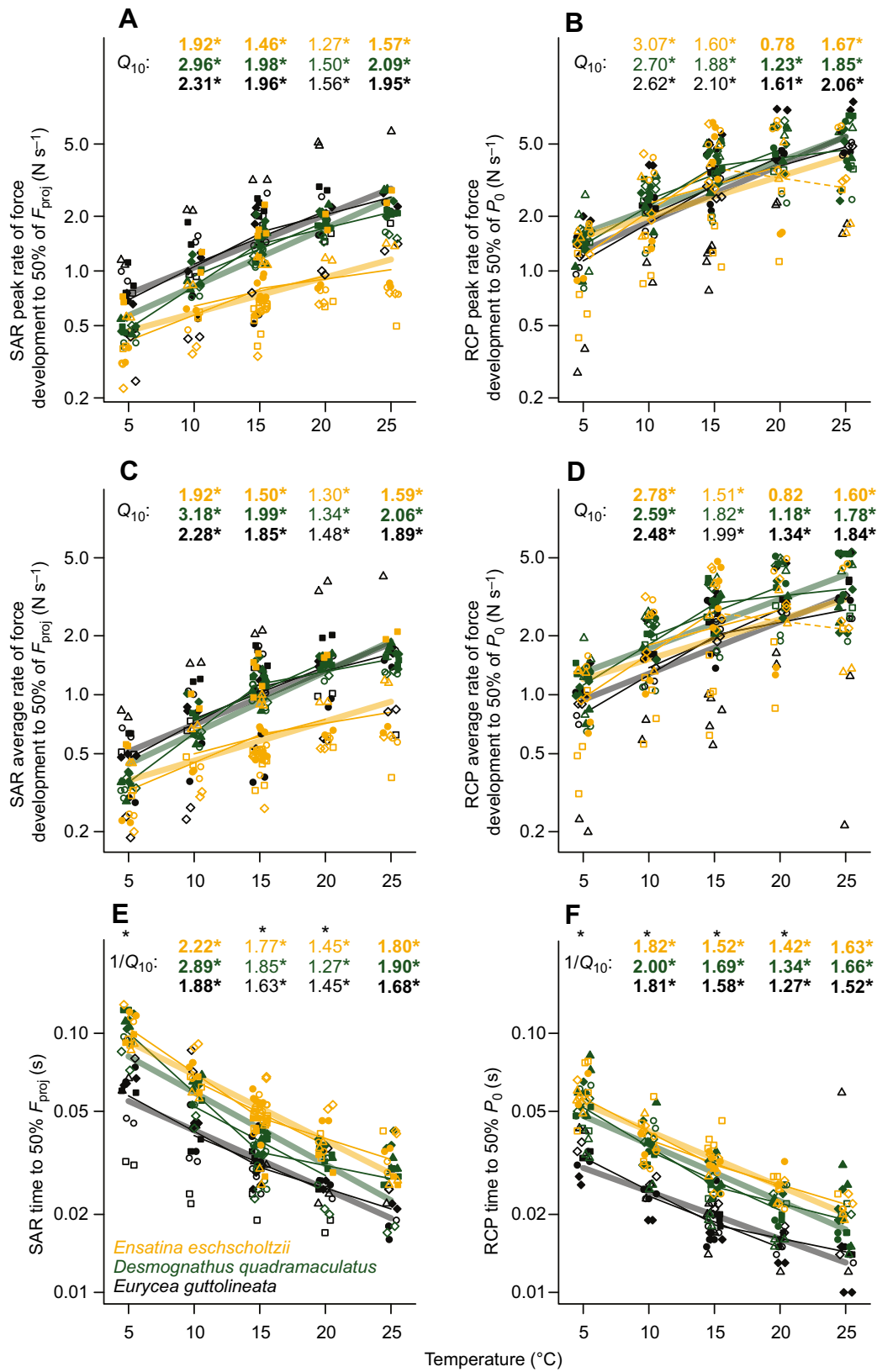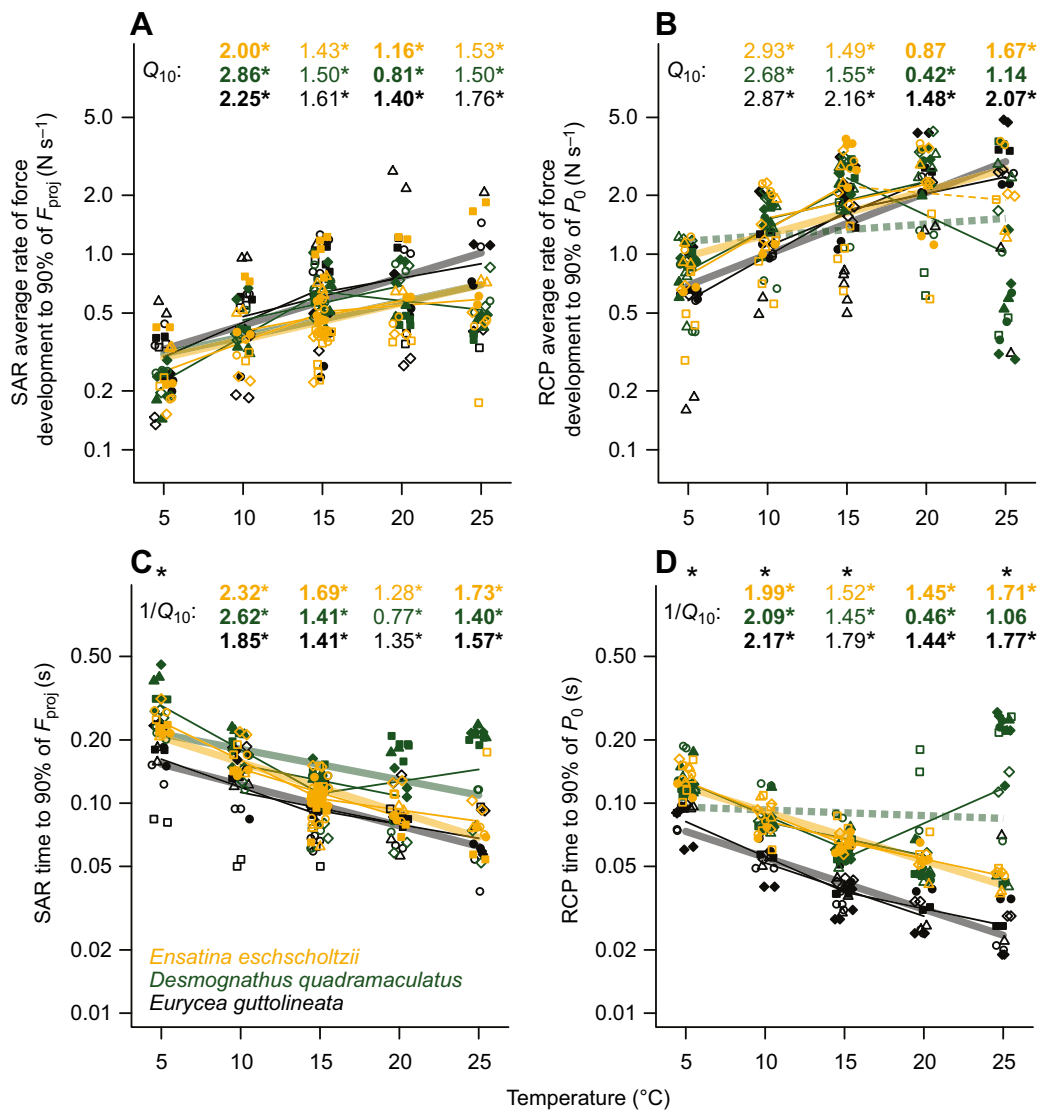
Fig. 2. Rate of force generation and time to 50% peak force. Indications are as in Fig. 1. Peak rates of force development for the SAR muscle (A) and RCP muscle (B) and average rates of force development for the SAR muscle (C) and RCP muscle (D) increase significantly with increasing temperature. Time to reach 50% F_{proj} for SAR muscle (E) and P_0 for RCP muscle (F) decrease significantly with increasing temperature for most species at most temperature intervals. Exceptions are no significant temperature effects on peak and average rate of force of RCP muscle for *Ensatina eschscholtzii* at 15–25°C (B,D).

Fig. 3. Rate of force generation and time to 90% peak force. Indications are as in Fig. 1. Peak rates of force development (not shown) are identical to those measured to 50% peak force (Fig. 2). Average rates of force development for the SAR muscle (A) and RCP muscle (B) increase significantly with increasing temperature and time to reach 50% F_{proj} for SAR muscle (C) and P_0 for RCP muscle (D) decrease significantly with increasing temperature for most species at most temperature intervals. Average rates of force development to 90% P_0 are not significantly affected by temperature at 15–25°C for *Ensatina eschscholtzii* (B). Notably, rate of force development decreases with increasing temperature and time to 90% peak force increases significantly with increasing temperature for *Desmognathus quadramaculatus* SAR muscle (A,C) and RCP muscle (B,D) at 15–25°C. Temperature effects on rate of force and time to 90% P_0 are not significant for *Desmognathus* for the total 5–25°C range (B,D).

because the muscle is operating with relatively low force, which minimizes the effects of temperature on muscle work (Olberding and Deban, 2017). The SAR muscle could be 'overbuilt' in *Ensatina* with only a fraction of the potential muscle work used to stretch elastic structures and achieve the observed tongue-projection performance. This is supported by relatively low values of SAR muscle mass-specific projection energy in *Ensatina* (0.08–8.18 J kg^{-1}) (Deban and Scales, 2016) compared with muscle mass-specific work from amphibian limb muscles (8.8–54.6 J kg^{-1}) (Peplowski and Marsh, 1997; Roberts et al., 2011; Olberding and Deban, 2017). *Eurycea* and *Bolitoglossa franklini*, both of which have elastic projection, also have low muscle mass-specific projection energy (0.06–3.85 and 0.18–5.62 J kg^{-1}, respectively) (Anderson et al., 2014; Scales et al., 2016). In these species, however, tongue-projection performance, while less thermally sensitive than muscle-powered tongue projection, is not

independent of temperature, indicating that other factors such as motor control are important in linking muscle properties with performance (Anderson et al., 2014; Scales et al., 2016, 2017).

It is unlikely that differences in temperature effects on rates of force development could explain the performance differences between genera with elastic versus non-elastic tongue projection. Significantly lower temperature effects on the rates of force development in the SAR of *Ensatina* compared with other genera without significant interspecific differences in the rates themselves suggest that tongue-projection performance should be more thermally robust in *Ensatina* compared with the other genera (Fig. 2). However, *Ensatina* and *Eurycea* have similar, relatively thermally robust tongue projection performance, compared with *Desmognathus*, despite nearly identical rates of force development in *Eurycea* and *Desmognathus* (Anderson et al., 2014; Deban and Scales, 2016; Scales et al., 2016). Additionally, for all species, SAR
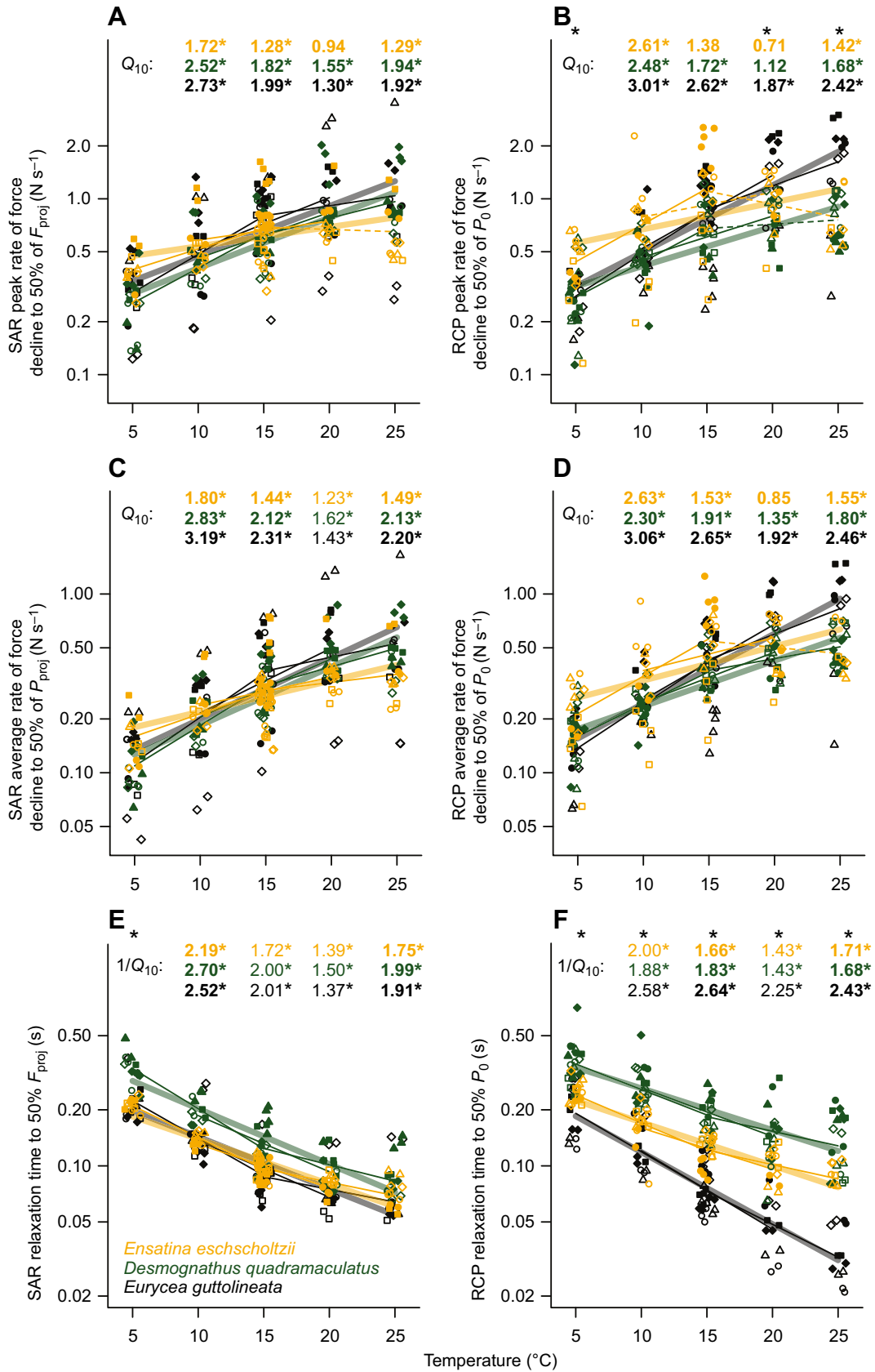
Fig. 4. See next page for legend.

Fig. 4. Rate of force decline and time to relax to 50% of peak force.
Indications are as in Fig. 1. Peak rates of force decline for the SAR muscle (A) and RCP muscle (B) and average rates of force decline for the SAR muscle (C) and RCP muscle (D) increase significantly with increasing temperature, and time to reach 50% F_{proj} for SAR muscle (E) and P_0 for RCP muscle (F) decrease significantly with increasing temperature for most species at most temperature intervals. Exceptions are no significant temperature effects on peak and average rate of force decline in SAR muscle in *Ensatina eschscholtzii* at 15–25°C (A), peak rate of RCP muscle at 10–20°C in *Ensatina* and 15–25°C in *Ensatina* and *Desmognathus quadramaculatus* (B), and average rate of RCP at 15–25°C in *Ensatina* (D).

muscle recruitment is lowest at the lowest temperature interval, but is commonly unaffected by temperature at warmer intervals (Anderson et al., 2014; Scales et al., 2017). This similarity in thermal sensitivity of muscle recruitment suggests that interspecific differences in temperature effects on muscle work (which may predict performance) could result from differences in the effects of temperature on rates of force development, if duration of activity is constant. However, *Eurycea*, with elastically powered elastic tongue projection and *Desmognathus*, with muscle-powered, non-elastic tongue projection, show identical temperature effects on rates of force development (Fig. 2).

The unusual behavior of *Desmognathus* muscles at 25°C could represent failure of *in vitro* muscle tissue at warmer temperatures in the absence of buffering from other physiological systems (Marsh and Bennett, 1986). At 20 and 25°C, the muscles from *Desmognathus* exhibit a 'shoulder' pattern during force development during which the rate of force development drops dramatically ∼50 ms after start of stimulation, yet force continues to climb slowly to P_0 (Fig. 3). Even though *Desmognathus quadramaculatus* will feed in a laboratory setting at 25°C, we observed that they do not thrive at this temperature. These muscle properties may lead us to predict that performance would decrease in *Desmognathus* at warmer temperatures (25°C), rather than increase. However, tongue projection and retraction performance in *Desmognathus* significantly increases with increasing temperature in both the 10–20 and 15–25°C intervals (Scales et al., 2016).

Temperature effects on contractile performance are similar in the RCP and SAR muscles, despite differences in the thermal robustness of tongue projection and retraction during feeding. Within each species examined, the RCP muscle has higher rates of force development and thus takes less time to reach peak force compared with the SAR muscle (Fig. 2). This may be related to the specialized functions of the muscles. The RCP muscle must be activated at the end of projection to decelerate the tongue projectile and pull it back into the mouth (Deban et al., 2007). Rapid force development may allow for the RCP muscle to be activated at the last possible moment and avoid stealing energy from tongue projection as force is developed for tongue retraction. The rates of relaxation for both muscles are similar, which may be expected given that relaxation rate of neither muscle should impact performance of a single feeding event (Fig. 4). Differences in values of P_0 (F_{proj}) between the muscles are harder to interpret due to differences in the anatomy and methods of force measurement. Because force from the SAR muscles was measured as the force of tongue projection, the magnitude of this force is certainly different from the magnitude of the force exerted by the muscle on the tongue skeleton. However, similar temperature sensitivity of both muscles indicates that the differences in thermal robustness of tongue projection compared with tongue retraction are not due to differences in contractile physiology between the SAR and RCP muscles.

Overall, muscle properties such as rate of force development and the temperature sensitivity of muscle performance among the species studied here do not vary in a manner consistent with variation in tongue-projection performance. Temperature effects on both muscles in these species are like those of other vertebrate muscles (reviewed in Bennett, 1984; James, 2013). *Ensatina* differs from the other two taxa in some contractile properties; however, tongue projection performance in *Ensatina* is more similar to *Eurycea* than to *Desgmognathus*, the latter of which has lower performance and reduced thermal robustness (Anderson et al., 2014; Deban and Scales, 2016; Scales et al., 2016). Within species, temperature effects on muscle contractile properties do not match temperature effects on tongue-projection performance (Anderson et al., 2014; Deban and Scales, 2016; Scales et al., 2016). Finally, the SAR and RCP muscles show similar temperature effects, despite the different functions of these muscles during feeding.

The high-performance and thermal robustness of tongue projection in *Ensatina* and *Eurycea* are likely to be the result solely of an elastic recoil mechanism, and we find no evidence of either enhanced muscle contractile rates in these taxa that could explain their high performance, or of the converse, i.e. reduced muscle contractile performance in genera with elastic tongue projection that might result from relaxed selection. Thus changes in muscle contractile physiology probably do not contribute to the evolution of high-performance movements that are robust to changing temperature. These findings contribute to a growing body of evidence indicating that morphological changes in muscle architecture and connective tissue can produce an elastic recoil mechanism that confers both high performance and thermal robustness to organismal movements in the absence of changes in muscle contractile properties.

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