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Comparative jumping mechanics in plethodontid salamanders

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Keywords

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Abstract

Plethodontid salamanders have a myriad of defense mechanisms with which to escape predation. One of the most poorly understood mechanisms is jumping, which is driven by lateral body bending; rapid straightening of the body propels the salamander into the air. One previous examination of jumping in one plethodontid salamander found little evidence of ontogenetic changes in the mechanics of jumping. Here, we investigate jumping in six species of plethodontid salamanders, across a wide array of body sizes. Comparing morphological and kinematic variables, jump height scaled with body size and forelimb length. Species differed in morphology, and when comparing kinematic variables across species, only bending duration differed. The lack of scaling patterns leads to several hypotheses, and further investigation of the morphology of the axial musculature is required.

Introduction

Given the link between morphology, performance and evolutionary fitness (Arnold, 1983), it comes as no surprise that escape behaviors are heavily studied (e.g. Schall & Pianka, 1980; Brodie, 1983; Beneski, 1989; Garcia-Paris & Deban, 1995; Williams *et al.*, 1996; Domenici & Blake, 1997), as the fitness consequences are clear: individuals that are eaten do not contribute to future generations. Defensive behaviors vary greatly; individuals employing a multitude of behaviors may be observed. Plethodontid salamanders are no exception to this rule, possessing a variety of defense mechanisms, including aposematic coloration, toxins, autotomy of limbs and tails, and behaviors such as biting, lashing, rolling down hills and playing dead (Brodie, 1977, 1983; Beneski, 1989; Whiteman & Wissinger, 1991; Garcia-Paris & Deban, 1995). Jumping as an escape behavior is well known in many animals, and while jumping salamanders have been known for many years (Cochran, 1911; Murphy, 1917; O'Reilly, Summers & Ritter, 2000), only recently have the mechanics of jumping been described (Ryerson, 2013; Hessel, 2014).

In most jumping terrestrial vertebrates, hind limbs generate high forces that propel the animal from the substrate (Alexander & Vernon, 1975; Marsh, 1994; Peplowski & Marsh, 1997). Plethodontid salamanders are long bodied with short limbs. Instead of relying on these smaller limbs to jump, the plethodontid *Desmognathus ocoee* uses its axial musculature to generate the forces required for a jump (Ryerson, 2013). The

pelvic girdle and hind limbs serve as the anchor point for the jump, and the forces generated by the axial musculature are applied to the substrate via the intervening pelvic limbs and girdle. Superficially, this resembles the C-start escape mechanism in fishes and aquatic salamanders (Domenici & Blake, 1997; Azizi & Landberg, 2002; Landberg & Azizi, 2010). The comparison is not unfounded, as some fully aquatic and semi-aquatic fish use a similar motion when locomoting terrestrially (Hsieh, 2010; Gibb *et al.*, 2011; Gibb, Ashley-Ross & Hsieh, 2013). Both salamanders and aquatic fishes show a bending stage, with a short pause, followed by rapid unbending of the torso resulting in the individual becoming airborne (Gibb *et al.*, 2013; Ryerson, 2013).

As important as escape behaviors are to fitness, our understanding of the scaling of these behaviors is lacking. Much of the focus has been on ontogenetic allometries within a species, and the selection pressures that drive juvenile and adult performance (reviewed by Herrel & Gibb, 2006). Even within escape responses in salamanders, studies have focused on the effects of ontogeny (Azizi & Landberg, 2002; Landberg & Azizi, 2010; Ryerson, 2013). Given the diversity of defensive behaviors in Plethodontidae (Brodie, 1977, 1983; Whiteman & Wissinger, 1991), it is plausible that variation in performance across taxa resulted in differential responses to predation. For example, the plethodontid *Pseudotriton ruber* secretes a potent toxin from dorsal skin glands (Brandon & Huheey, 1981) and is brightly colored to warn potential predators. *Plethodon glutinosus*, the slimy salamander, secretes thick mucus from similar

glands, making the salamander difficult to grasp (Brodie, 1983). Both of these salamanders, well known for their primary defense mechanisms, may not rely on alternative defense strategies such as jumping.

In this study, we explore jumping performance in six sympatric species of plethodontid salamander to better understand the variation inherent in jumping as well as potentially elucidate the mechanisms responsible for this variation. We define performance as behaviors that allow the individual to quickly leave the site of potential predation (i.e. jump height, velocities). The specific goals of this project were: (1) Examine plethodontids other than *D. ocoee* for the ability to jump as a means to escape predation; (2) Test for differences in jumping kinematics among species; and (3) Determine if differences in morphology among species are correlated with differences in jumping.

Materials and methods

Desmognathus fuscus ($n = 9$, SVL = 3.6–5.5 cm), *Desmognathus ochrophaeus* ($n = 9$, SVL = 2.8–4.2 cm), *Plethodon cinereus* ($n = 9$, SVL = 1.9–3.9 cm), *P. glutinosus* ($n = 4$, SVL = 2.7–6.3 cm), *Eurycea bislineata* ($n = 6$, SVL = 3.6–4.0 cm) and *E. longicauda* ($n = 1$, SVL = 4.7 cm) were collected from the wild populations in Meadville, Pennsylvania. The sizes represented in our study reflect the full range of adult sizes encountered in the field. Individuals were housed in plastic containers with moist paper towels at 15°C and a 14:10 light:dark cycle, and kept for approximately one month over the course of this experiment. All individuals were fed several *Drosophila melanogaster* twice weekly. For jumping trials, salamanders were given at least one hour to acclimate to room temperature (20°C), after which each individual was placed on a platform with a narrow corridor and encouraged to jump over a 5 cm gap with gentle prodding, as described in Ryerson (2013). In this study, jumps were filmed at 500 frames s⁻¹ using a Fastec Inline 1000 high-speed camera system (Fastec Imaging, San Diego, CA, USA) and a mirror placed above the platform at a 45° angle to obtain simultaneous dorsal and lateral views. Five jumps per individual were recorded.

Kinematics & morphology

Kinematic variables were measured using MaxTraQ software (Innovision Systems Inc., Columbiaville, MI, USA) and Didge (v2.3, Cullum, 2012). Three digital points were added to the videos, one at the location of the pectoral girdle, one at the pelvic girdle and one at the point halfway between the two girdles. Using these points and time data, the following variables were measured, following Ryerson (2013): (1) duration of bending (time to bend from initiation of bending to cessation, ms), (2) duration of unbending (from initiation of straightening until the body left the substrate, ms), (3) angle of bending (taken at bending cessation and measured from the dorsal midline of pectoral girdle to vertex of angle made by the body to the midpoint of the pelvic girdle, deg) and (4) angle of unbending (taken at body straightening, when the salamander

leaves the substrate, deg). Two additional kinematic variables were calculated from the previous variables: bending angular velocity (the change in the angle of bending divided by duration of bending, deg/ms) and unbending angular velocity (the change in the angle of unbending divided by duration of unbending, deg/ms). These final two variables have been previously examined in the kinematics of jumping in another salamander, *D. ocoee* (Ryerson, 2013).

The center of mass (CoM) was tracked throughout the salamander jump using a custom-built program that incorporated Excel (Microsoft Corporation, Redmond, WA, USA) and Didge (Alistair Cullum, Creighton University). After jumping trials, individuals were killed in 0.2% tricaine methanesulfonate (Fisher Scientific International Inc., Hampton, NH, USA). Salamanders were disarticulated into 10 segments: complete limbs (4); torso (pectoral girdle to pelvic girdle); anterior end of the first caudal vertebra to the end of the tail; pelvis; cervical vertebra (head segment); the tail segment was dissected into two sections of equal length. Each segment's CoM was then located using the following assumptions. All segments were assumed to be cylindrical, and the CoM (of those segments) were placed at the halfway point along the segments' longest axis (Hessel, 2014).

On each video frame, all segment locations (relative to an external reference point) and masses were used to calculate the position of the CoM, (x , y , z), based on the following equations:

$$x_{\text{cm}} = \frac{m_1x_1 + m_2x_2 + \dots + m_nx_n}{M} \quad (1)$$

$$y_{\text{cm}} = \frac{m_1y_1 + m_2y_2 + \dots + m_ny_n}{M} \quad (2)$$

$$z_{\text{cm}} = \frac{m_1z_1 + m_2z_2 + \dots + m_nz_n}{M} \quad (3)$$

where segmented mass is denoted by (m_n) in grams, relative segment location is denoted by (x_n , y_n , z_n) in centimeters and salamander mass is denoted by (M) in grams (Özkaya *et al.*, 2012). Coordinates (x , y , z) for each segment were calculated for each jump using Didge at the five time points described above. Using the CoM, we measured (1) takeoff angle (TOA, angle created between the horizon at takeoff and a line which intersected both the girdles, deg), (2) takeoff velocity (TOV, linear velocity of the CoM at takeoff, cm s⁻¹) and (3) jump height (maximum vertical height attained by the CoM of the salamander while airborne, cm). We defined takeoff as the frame at which the last hind limb left the ground. Jump height was included in this analysis to examine the role of the limbs during jumping among individuals, as it is assumed that the limbs contribute to propelling the individual off the substrate (Toro *et al.*, 2003). Previously, Ryerson (2013) found high amounts of variation in jump distance with no relationship to any morphological variable, and suggests that jump distance may not have a significant impact on the individual's ability to escape predation.

To explore the effects of morphology on jump kinematics, mass (g), tail length (cm), SVL (cm), total length (cm), torso length (the distance between the pectoral and pelvic girdles,

cm), torso width (cm) and dorsoventral height (taken approximately at the middle of the length of the torso, cm), and limb lengths (to longest digit, cm) were recorded for each salamander. Limb length allows us to examine potential scaling patterns of jump height and limb length. Measuring torso and tail length allows us to understand how parts of the axial skeleton and musculature contribute to the mechanics of jumping. Mass was recorded on each day that jump trials were run. One ratio was calculated to explore the relationship of body proportions with jump kinematics: tail length to SVL. The ratio of tail length to SVL allows us to examine how distributing mass anterior and posterior to the pivot point of the jump (the pelvic girdle) affects the kinematics of the behavior, and the potential role that the tail plays in jumping.

Statistical analyses

Statistical analyses were performed on data from all species except for *E. longicauda*, as only one individual of this species is included in this study. To determine whether species differed in morphology, seven morphological variables (mass, tail length, total length, SVL, torso length, limb lengths and tail length:SVL) were tested with a MANOVA, with species as the factor. We also tested for species differences among the seven kinematic variables (bending and unbending durations, bending and unbending angular velocities, TOA, TOV and jump height) using MANOVAs. We were unable to accurately obtain CoM data for two of the four *P. glutinosus* individuals; given the small sample size for this species, we did not feel that the traditional methods of 'correcting' for missing data (deletion of cases with missing data or replacing missing data with group means) were appropriate. Therefore, we chose to run the CoM-derived variables (TOA, TOV, jump height) in one MANOVA, and the remaining kinematic variables (bending and unbending durations and angular velocities) were included in a second separate MANOVA. We ran an additional MANOVA on SVL-scaled CoM-derived variables; linear distances for jump height and TOV were converted from cm to SVLs for this analysis. For all MANOVAs, we used an alpha level of 0.05. One-way ANOVA was used as an initial post-hoc test on any significant MANOVAs,

followed by a Tukey post-hoc test on statistically significant ANOVAs to identify species differences. Multiple tests in this manner increase the likelihood of a Type I error, so to counteract, we applied a sequential Bonferroni correction (Holm, 1979).

To assess the relationship between morphology and jump kinematics, we performed Pearson correlations with sequential Bonferroni correction on the aforementioned kinematic and log-transformed morphological variables (including the SVL-scaled variables), pooled across species. Using a Pearson's correlation allows us to simply examine relationships between morphological and kinematic variables for significant patterns. Variables with significant correlations were then further analyzed with ordinary least-squares regressions to determine scaling relationships. If the expected isometric slope for each relationship fell within the 95% confidence interval around the observed slope, the relationship was considered isometric. Pearson correlations, MANOVAs, ANOVAs and linear regressions were performed in SPSS version 11 (IBM, New York, NY, USA).

Results

All plethodontids tested perform the same jump as described in Ryerson (2013) (Fig. 1). The body bends into a U-shape, the salamander pauses, and then the body rapidly straightens, launching into a jump. Jump kinematics differ among species (Table 1, Wilk's lambda = 0.231, $P < 0.001$), but only for bending duration ($F_{4,32} = 8.068$, $P < 0.001$; Table 1); bending duration is longer in *D. ochrophaeus* as compared to *D. fuscus*, *P. cinereus*, *E. longicauda* and *P. glutinosus*. No other kinematic variables differed among species, including the variables derived from the center of mass (CoM scaled to SVL: Wilk's lambda = 0.590, $P = 0.227$; non-scaled CoM: Wilk's lambda = 0.668, $P = 0.472$).

Species also differ in morphology (Wilk's lambda = 0.177, $P = 0.004$) (Table 2). *P. cinereus* has less mass than *D. fuscus*, and *P. glutinosus* has more mass than *P. cinereus*, *E. bislineata*, and *D. ochrophaeus* ($F_{4,32} = 7.379$, $P < 0.001$). Torso length in *P. cinereus* is shorter than that of *D. fuscus* or *P. glutinosus* ($F_{4,32} = 4.933$, $P = 0.003$). Hind limb length in *P. cinereus* is

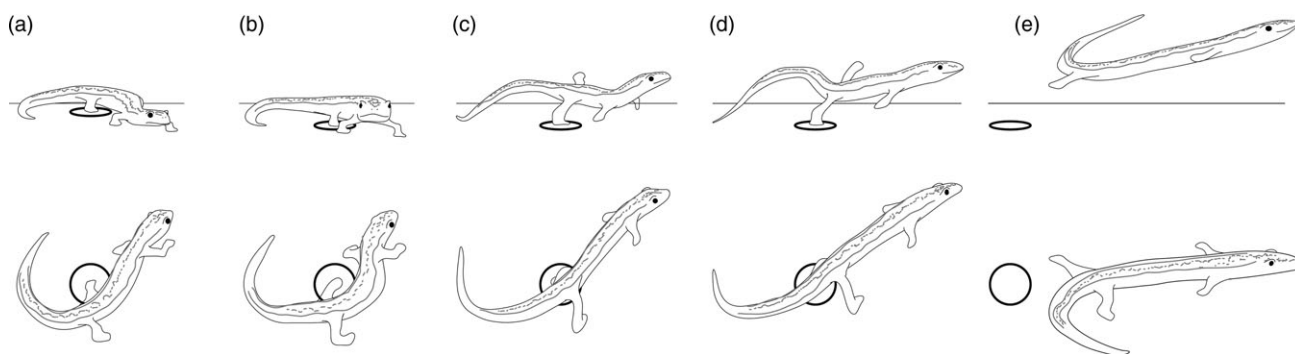


Figure 1 Line drawing of representative jump by *D. ochrophaeus*, simultaneously recorded in two planes with the aid of a mirror. Top row: dorsal view, Bottom row: lateral view. (a) Onset of bending, (b) Maximum bending, (c) End of unbending, (d) Last foot leaving the ground, (e) All limbs off the ground.

Table 1 Means and standard deviations for kinematic variables

Species	t_{bend} (ms)	t_{unbend} (ms)	α_{bend} (deg/ms)	α_{unbend} (deg/ms)	Jump height (cm)	Jump height (SVL)	TOA (deg)	TOV (cm/s)	TOV (SVL/s)
<i>D. fuscus</i>	8.5 ± 1.7	8.5 ± 1.7	5.0 ± 1.9	9.8 ± 3.4	7.6 ± 6.4	1.8 ± 1.5	13.0 ± 3.9	94.8 ± 13.1	22.7 ± 4.4
<i>D. ochrophaeus</i>	17.2 ± 6.2	6.2 ± 2.4	4.2 ± 0.8	12.8 ± 2.3	6.7 ± 4.5	1.8 ± 1.1	11.6 ± 4.4	98.6 ± 15.8	24.8 ± 3.9
<i>E. bislineata</i>	12.42 ± 2.1	6.6 ± 0.5	5.9 ± 2.6	10.2 ± 2.1	1.1 ± 0.3	0.3 ± 0.1	9.8 ± 5.8	94.3 ± 18.2	22.5 ± 4.5
<i>P. cinereus</i>	12.0 ± 3.0	7.1 ± 0.9	6.5 ± 1.5	11.7 ± 2.5	4.1 ± 2.8	1.2 ± 0.7	11.5 ± 2.7	89.8 ± 20.1	26.8 ± 6.5
<i>P. glutinosus</i>	7.6 ± 1.3	7.6 ± 1.3	4.9 ± 1.0	8.2 ± 1.0	2.1 ± 0.5	0.5 ± 0.1	10.3 ± 0.1	84.5 ± 17.7	19.2 ± 4.8
<i>E. longicauda</i>	13.6	7.9	4.0	7.6	X	X	X	X	X

Mean and standard deviation is not given for *E. longicauda*, as only one jump was recorded for one individual. For jump height, TOA, and TOV, $n = 2$ for *P. glutinosus*.

t_{bend} , bending time; t_{unbend} , unbending time; α_{load} , angular acceleration of bending; α_{unbend} , angular acceleration of unbending; TOA, takeoff angle; TOV, takeoff velocity; X, unable to measure.

Table 2 Means and standard deviations for morphological variables

Species	Mass (g)	L_{tail} (cm)	TL (cm)	SVL (cm)	L_{torso} (cm)	L_{fore} (cm)	L_{hind} (cm)	$L_{\text{tail}}/\text{SVL}$
<i>D. fuscus</i>	1.5 ± 0.6	4.0 ± 0.7	8.2 ± 0.8	4.2 ± 0.6	2.8 ± 0.5	0.9 ± 0.3	1.3 ± 0.2	1.0 ± 0.2
<i>D. ochrophaeus</i>	0.8 ± 0.4	3.0 ± 1.0	6.6 ± 1.3	3.5 ± 0.5	2.2 ± 0.4	0.8 ± 0.2	1.3 ± 0.2	0.9 ± 0.3
<i>E. bislineata</i>	0.8 ± 0.1	3.8 ± 0.9	7.6 ± 0.8	3.8 ± 0.2	2.4 ± 0.1	0.8 ± 0.2	1.2 ± 0.2	1.0 ± 0.2
<i>P. cinereus</i>	0.5 ± 0.2	3.0 ± 0.7	6.2 ± 1.2	3.2 ± 0.6	2.0 ± 0.3	0.7 ± 0.2	1.0 ± 0.2	1.0 ± 0.1
<i>P. glutinosus</i>	2.6 ± 1.9	4.8 ± 2.2	9.3 ± 3.6	4.5 ± 1.5	2.9 ± 1.0	1.1 ± 0.5	0.9 ± 0.2	1.0 ± 0.2
<i>E. longicauda</i>	1.7	6.0	10.7	4.7	2.9	0.9	1.1	1.3

Mean and standard deviation is not given for *E. longicauda*, as only individual was included in this study.

L_{tail} , tail length; TL, total length; SVL, snout-vent length; L_{torso} , torso length; L_{fore} , fore limb length; L_{hind} , hind limb length; $L_{\text{tail}}/\text{SVL}$, ratio of tail length to snout-vent length.

longer than that of *P. glutinosus*, but shorter than both *D. fuscus* and *D. ochrophaeus* ($F_{4,32} = 4.766$, $P = 0.004$). No other morphological variables differed among species.

When species means were pooled to look for broader trends using the Pearson's correlations and log-transformed morphological variables, TOV scaled to SVL decreased with SVL ($r = -0.576$, $P < 0.001$), torso length ($r = -0.587$, $P < 0.001$). Both significant relationships were negatively allometric (SVL: $b = -0.63302$, $\text{CI} = -1.0775$, -0.403 ; torso: $b = -0.56935$, $\text{CI} = -0.943$, -0.333) (Fig. 2), indicating that longer salamanders have slower TOV when measured in SVL/s. No other kinematic variables exhibited significant correlations with any other morphological variables, including TOV measured in cm s^{-1} .

Discussion

This study is the first to examine jumping kinematics in multiple species of plethodontids. As was the case in *D. ocoee* (Ryerson, 2013), we found that all salamanders tested performed a jump in the context of escaping a potential predator (the experimenter) as previously described (Fig. 1; Ryerson, 2013). Salamanders laterally bent their body, bringing the head toward the tail, paused, and then rapidly straightened the torso. This rapid straightening generated sufficient forces from the body to propel a salamander forward and into the air, most likely applied through the hind limbs. The initial height of the jump, which would affect the ballistic motion of the behavior, was not found to differ among species, suggesting initial jump height is not strongly selected for.

We found that all species included in this study have similar jumping kinematics, with the exception of bending duration. Only *D. ochrophaeus* had a longer bending duration than the other species tested; all other species were not significantly different from each other. *D. ochrophaeus* did not consistently differ in morphology compared to the other species; on average *D. ochrophaeus* weighed less than *P. glutinosus* and had longer hind limbs than *P. cinereus*. Additionally, we did not find a significant correlation between bending duration and any size variable, and we therefore cannot link the kinematic difference to any particular morphological attribute measured in this study. The lack of correlation between bending duration and size is contrary to the results of Ryerson (2013). In *D. ocoee*, bending duration increased with size, and was hypothesized to allow for contralateral activation of the axial muscles, in turn enabling larger salamanders to maintain higher unbending velocities at the onset of jumping (Ryerson, 2013). However, the *D. ocoee* were an ontogenetic series, while the individuals in this study were confined to several adults from multiple species. Differences in ontogenetic versus among species scaling relationships have been found in other taxa as well (Herrel & Gibb, 2006). For example, across *Anolis* species, many of the scaling relationships become non-significant when the largest taxa are included, and Toro *et al.* (2003) suggest that this is the result of differential selective pressures and scaling issues of muscle size.

Quantifying the effect of size, and changes in size, has long been a goal of research in biomechanics. Traditionally, researchers have relied on Hill (1950) to serve as the baseline for how we expect anatomy, physiology and behavior to change as size changes. Hill's model makes several predictions

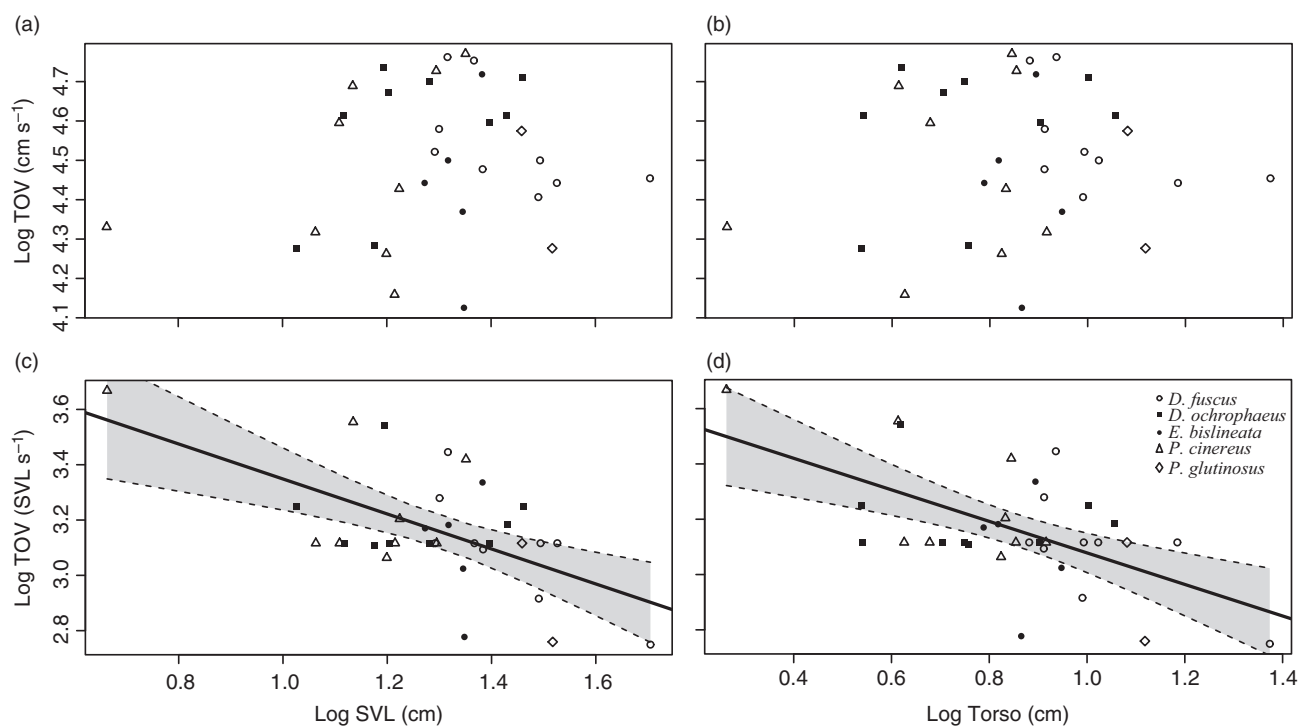


Figure 2 Scatter plots of takeoff velocity plotted against (a) SVL. (b) Torso length. (c) Size-corrected SVL. (d) Size-corrected Torso length. All variables have been log-transformed. Solid lines are the significant regressions. Dashed lines with shading represent the 95% confidence intervals for the slope of the regression.

for geometrically similar organisms, based primarily on the understanding of muscle physiology at the time. We should expect, given Hill's predictions, that absolute (not scaled to SVL) TOV should be same in all jumping animals. Our data indicate that absolute TOV has no correlation with any of our morphological variables, including standard measures of size such as SVL and mass (Tables 1 and 2). Many have suggested in both salamanders (Ryerson, 2013) and other taxa (Herrel & Gibb, 2006; Astley & Roberts, 2012; Gibb *et al.*, 2013) that deviations from expected muscle-powered movements can be explained by the use of power amplification (e.g. energy-storing tendons and ligaments). However, in the years since Hill's (1950) original predictions, larger samples of additional taxa have shed more light on the scaling patterns of muscle-powered behaviors (Emerson, 1978; Domenici, 2001; Toro *et al.*, 2003; Herrel & Gibb, 2006). In a large comparative study, Marsh (1994) examined jumping ability across anurans, finding patterns that differ from predictive models (i.e. Hill (1950)), both comparatively and ontogenetically. Jumping distance was correlated with the interaction of takeoff velocity and angle. Surprisingly, he found similar scaling patterns of jump performance across the phylogeny of frogs, where jump distance scaled with body length to a power of 0.6 (Emerson, 1978; Zug, 1978; Marsh, 1994). This was true across groups of frogs, and at different temperatures. Toads, as a group, did not perform as highly as the frogs, but also scaled similarly to frogs (Zug, 1978; Marsh, 1994). This sheds some doubt on the utility of scaling data to examine power amplification using elastic mechanisms, as toads do not use power amplification (Emerson, 1978; Marsh, 1994)

and frogs do (Emerson, 1978; Herrel & Gibb, 2006; Astley & Roberts, 2012). Regardless of the potential power amplification, there is a strong positive relationship between size and measures of jumping performance in anurans (Emerson, 1978; Marsh, 1994), a relationship that we do not see in plethodontids. All amphibian jumping studies have been focused on leg-powered jumping in anurans, with the exception of Ryerson (2013). While there is likely some contribution of the limbs in order for plethodontids to become airborne (even to just place the individual at an angle away from the substrate), this is where the similarity in jump mechanics ends. A more appropriate comparison may be to burst speed or aquatic escape of lateral undulators, both of which make extensive use of the trunk muscles. There are fundamental differences in muscle architecture, fiber length and fiber angle when comparing anuran leg muscles to salamander trunk muscles (Marsh, 1994; O'Reilly *et al.*, 2000; Azizi & Landberg, 2002; Gemballa & Ebmeyer, 2003; Toro *et al.*, 2003; Schilling & Deban, 2010). As such, comparing jumps of these two disparate organisms has limited utility and we should not be surprised to find a difference in scaling relationships between anuran and plethodontid jumps. However, it should be noted that the body size represented in this study is not as extensive as the previously mentioned frog (Emerson, 1978; Marsh, 1994) data. The limited adult size range of these salamanders potentially confounds our ability to detect a strong signal, and any scaling relationships observed should be taken with caution.

Alternatively, we can look to c-starts in fishes. In larval fishes, data on escape behavior among five species found that a single regression could describe the patterns observed within and among

species (Williams *et al.*, 1996). Larger species of fish had higher escape velocities than smaller species, whereas we were unable to detect major differences in the kinematics of jumping in our salamanders, similar to previous examination of scaling in *D. ocoee* (Ryerson, 2013). This may be the result of differences in the environment between our terrestrial salamanders and fish. In fishes (Hunt von Herbing & Keating, 2003; McHenry & Lauder, 2005; Danos & Lauder, 2012) and tadpoles (Ryerson & Deban, 2010), fluid density and viscosity can strongly impact an aquatic vertebrate's ability to move, but would not impact our salamanders. Conversely, overall velocity may be less important than the surprise nature and maneuverability of the jump, as has been suggested in fish (Domenici & Blake, 1997; Domenici, 2001). Additionally, variation in the muscle architecture driving the escape response may also be contributing to this variation. Westneat *et al.* (1998) determined that in two species of fish, *Amia calva* and *Polypterus palmas*, the combination of axial muscle morphology and intramuscular pressure drove the variation in these two species. Variation in the fiber angles, arrangement of myosepta, and how these morphological features interact has the potential to be vital for our system. However, for non-plethodontid salamanders, changes in the fiber architecture do not have a significant effect on the fiber strains produced during locomotion (Brainerd & Azizi, 2005; Azizi & Brainerd, 2007). This has not yet been explored in plethodontids.

While we have demonstrated that jumping in salamanders does not seem to be constrained by size of the organism, we cannot conclusively derive the mechanism that allows them to maintain these levels of performance across a range of sizes. The most obvious explanation would be differences in the mass of axial musculature available for jumping in different species, or in the muscle architecture as described above. While we did not measure the trunk musculature directly, we measured torso length (a potential proxy for total muscle length available for jumping) and did not find a similar pattern of scaling as in the *Anolis* (Toro *et al.*, 2003). In addition to the fiber orientation and the amount of muscle available for use in jumping, there is also the question of fiber type. In anurans, fiber-type distribution in the hind limb muscles is strongly correlated with jumping ability (Marsh, 1994). Species with the highest jump performance have a majority of glycolytic fibers with a much smaller amount of oxidative (Putnam & Bennett, 1983; Mendiola *et al.*, 1991; Marsh, 1994). Members of the genus *Anaxyrus* (formerly *Bufo*), known more for hopping than great jumps, possess more oxidative fibers (Putnam & Bennett, 1983; Mendiola *et al.*, 1991). In salamanders, much weaker correlations exist. In two species of ambystomatid, *Ambystoma tigrinum* and *Ambystoma maculatum*, Schilling & Deban (2010) found consistent trends of fiber-type distribution down the body axis. Oxidative fibers were dominant superficially, while intermediate fibers became the majority in the underlying muscles. Schilling & Deban (2010) argue that changes in fiber type in the different muscle layers reflect the muscle's activity during different functional tasks, swimming and walking. However, to our current knowledge, *Ambystoma* does not exhibit any jumping-like behavior. The fiber-type distribution of the axial muscles of *Plethodon* and *Desmognathus* is unknown, and may prove to be a valuable area of investigation.

A potential confounding variable for escape performance is the tail. In anoles, the tail plays an important role in maintaining stability during a jump; individuals who had autotomized their tails frequently spun out of control (Gillis, Bonvini & Irschick, 2009). For the aboreal *Anolis*, a coordinated landing is important to escape and moving through the environment. Plethodontids will autotomize the tail in the event of capture, and possess the ability to regrow that tail (Brodie, 1977, 1983; Beneski, 1989). We found no correlation with our kinematic variables with the length of the tail or the ratio of tail length to SVL, indicating that the length of an intact tail had no effect on the beginning stages of a jump. Dropping the tail in a previous escape attempt may still have complex implications for the salamander's ability to jump and escape another predation attempt.

We have documented in this study that despite differences in size and morphology in six species of plethodontid, the kinematics of jumping are remarkably similar across all species. We suggest that the morphology of the axial musculature, and its variation in these species, would be a fruitful area of investigation. The myosepta in the axial musculature are a strong possibility for the site of energy storage, and require further investigation, as do the limits of this behavior in salamanders. We are just beginning to understand jumping, mechanistically and ecologically, and remains a promising avenue for future research.

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