

RESEARCH ARTICLE

Functional morphology of terrestrial prey capture in salamandrid salamanders

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ABSTRACT

Salamanders use the hyobranchial apparatus and its associated musculature for tongue projection on land and for suction feeding in water. Hyobranchial apparatus composition and morphology vary across species, and different morphologies are better suited for feeding in aquatic versus terrestrial environments. We hypothesize that differences in hyobranchial morphology result in functional trade-offs in feeding performance. We predict that semi-aquatic and aquatic salamandrids with hyobranchial morphology suited for aquatic feeding will have lower performance, in terms of tongue-projection distance, velocity, acceleration and power, compared with terrestrial salamandrids when feeding in a terrestrial environment. We found that semi-aquatic and aquatic newts had lower velocity, acceleration and muscle-mass-specific power of tongue projection when compared with the terrestrial salamanders *Chioglossa lusitanica* and *Salamandra salamandra*. The fully aquatic newt, *Paramesotriton labiatus*, has a robust, heavily mineralized hyobranchial apparatus and was unable to project its tongue during terrestrial feeding, and instead exhibited suction-feeding movements better suited for aquatic feeding. Conversely, terrestrial species have slender, cartilaginous hyobranchial apparatus and enlarged tongue pads that coincided with greater tongue-projection distance, velocity, acceleration and power. *Chioglossa lusitanica* exhibited extreme tongue-projection performance, similar to that seen in elastically projecting plethodontid salamanders; muscle-mass-specific power of tongue projection exceeded 2200 W kg^{-1} , more than 350 times that of the next highest performer, *S. salamandra*, which reached 6.3 W kg^{-1} . These findings reveal that two fully terrestrial salamandrids have morphological specializations that yield greater tongue-projection performance compared with species that naturally feed in both aquatic and terrestrial environments.

KEY WORDS: Newt, Feeding, Tongue projection, Trade-offs, Salamandridae

INTRODUCTION

Most salamanders rely on rapid tongue projection to capture prey during terrestrial feeding (Wake and Deban, 2000). The hyobranchial apparatus enables tongue projection, and has diverse morphologies across salamander species (Lombard and Wake, 1977; Özeti and Wake, 1969; Wake and Deban, 2000). Studies of aquatic feeding morphology and performance have found that

salamanders with more robust and mineralized hyobranchial apparatus produce greater fluid velocity during suction-feeding events (Özeti and Wake, 1969; Miller and Larsen, 1989; Stinson and Deban, 2017). Our understanding of the direct effects of morphology on terrestrial feeding performance, and the trade-offs that may accompany feeding across aquatic and terrestrial environments, however, is limited (Beneski et al., 1995; Larsen et al., 1996, 1989; Miller and Larsen, 1990). In this study, we compared the morphology and tongue-projection performance of terrestrial, semi-aquatic and aquatic salamandrids to assess functional trade-offs during terrestrial-feeding events.

Salamanders that utilize tongue projection have morphological specializations, including flexible tongue skeletons (i.e. hyobranchial apparatus) and a tongue pad that is loosely attached to the floor of the mouth (Lombard and Wake, 1977). Some plethodontid salamanders have further specializations allowing for thermally robust, high-power tongue projection through elastic-recoil mechanisms (Anderson et al., 2014; Deban et al., 2007; Deban and Richardson, 2011; Deban and Scales, 2016; Scales et al., 2017, 2016). In an extreme case, *Hydromantes platycephalus* can project its tongue up to 80% its body length with muscle-mass-specific power reaching 4992 W kg^{-1} (Deban and Richardson, 2011). Most salamanders using tongue projection have lower tongue-projection velocity, acceleration and mass-specific power than those of elastically projecting plethodontids, and use a lunging motion to increase strike distance and presumably strike force (Larsen et al., 1996). The skeletal foundation of tongue projection, the paired ceratohyal (CH), has a more active role in salamandrid, ambystomatid and hynobiid salamanders that lunge during prey capture, moving forward as the tongue is projected, whereas in other salamanders it is relatively immobile (Findeis and Bemis, 1990; Larsen et al., 1996; Miller and Larsen, 1990; Reilly and Lauder, 1989). Maximum tongue extension is also shorter in species that lunge compared with plethodontids with elastic tongue projection, often only reaching 6–20% of snout–vent length (SVL) beyond the mandible (Beneski et al., 1995; Findeis and Bemis, 1990; Larsen et al., 1996; Miller and Larsen, 1990).

Tongue projection in salamanders is accomplished through the forward protrusion of the hyobranchial apparatus. The central axis of the hyobranchial apparatus is the medial basibranchial (BB), which connects posteriorly to the paired ceratobranchial I (CB I) and ceratobranchial II (CB II) (Wake and Deban, 2000). In most species, paired structures known as the epibranchials (EB) attach to the posterior aspects of CB I and CB II. In some salamanders, such as *Chioglossa*, *Salamandra* and *Salamandrina*, the EB is not present, and instead CB I extends caudally beyond its articulation with CB II (Özeti and Wake, 1969; Wake and Deban, 2000). The subarcualis rectus (SAR) muscles are wrapped around the posterior-most element (CB I or EB) and extend anteriorly to their origin on the paired, blade-like CH, which lies dorsal to the remainder of the hyobranchial apparatus. A sticky tongue pad sits at the rostral tip of

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List of abbreviations

BB	basibranchial
CB I	ceratobranchial I
CB II	ceratobranchial II
CB I+CB II	ceratobranchial I+ceratobranchial II complex
CH	ceratohyal
CSA	cross-sectional area
EB	epibranchial
RC	rectus cervicis
RI	robustness index
SAR	subarcualis rectus
SVL	snout–vent length
TL	total length

the BB and is carried out of the mouth during tongue projection. The SAR muscles power this projection, contracting around the EB, pushing the tongue skeleton rostrally relative to the CH and propelling the tongue out of the mouth. The rectus cervicis (RC) muscles attach anteriorly to the hyobranchial apparatus, and retract the hyobranchial apparatus and tongue into the mouth (Deban, 2003; Wake and Deban, 2000).

Based on the morphology of the hyobranchial apparatus and our understanding of feeding biomechanics, functional trade-offs resulting in lower tongue-projection velocity, acceleration and power are expected in salamanders that are proficient suction feeders compared with species that are specialized to feed on land (Deban, 2003; Özeti and Wake, 1969). Performance differences may occur during aquatic and terrestrial feeding because the hyobranchial apparatus has competing functions in semi-aquatic and aquatic newts. These salamanders rely on suction feeding in water, rapidly expanding the oropharynx via hyobranchial depression (Deban and Wake, 2000). Morphological and kinematic specializations, such as greater mineralization of the hyobranchial apparatus and faster hyobranchial depression, produce higher fluid velocity in aquatically feeding newts (Miller and Larsen, 1989; Stinson and Deban, 2017). A robust hyobranchial apparatus better resists flexion as the oropharynx is expanded, permitting faster and more forceful hyobranchial depression (Stinson and Deban, 2017). Additionally, the semi-aquatic newts *Lissotriton vulgaris* and *Ichthyosaura alpestris* modulate their feeding during seasonal shifts, using jaw prehension to feed on land during their aquatic phase and tongue projection during their terrestrial phase (Heiss et al., 2013, 2015). Differences in tongue-projection kinematics among salamandrids have also shown that semi-aquatic and aquatic newts have lower tongue-projection length compared with terrestrial species, or entirely lack the ability to project the tongue (Miller and Larsen, 1990).

This study examines the interplay of morphology and feeding performance, in terms of tongue-projection distance, velocity, acceleration and power, of two terrestrial (*Chioglossa lusitanica* and *Salamandra salamandra*), four semi-aquatic (*Pleurodeles waltl*, *Notophthalmus viridescens*, *Triturus dobrogicus* and *Cynops cyanurus*) and one fully aquatic (*Paramesotriton labiatus*) species of salamander in the Family Salamandridae. Salamandrids are an ideal focal group because species within the family are morphologically and ecologically diverse, allowing us to assess possible trade-offs in feeding performance. We hypothesize that differences in feeding morphology will yield differences in tongue-projection performance. Specifically, we predict that semi-aquatic and aquatic species will exhibit morphological and functional compromises in the hyobranchial apparatus, resulting in lower

tongue-projection velocity, acceleration and muscle-mass-specific power during terrestrial prey capture than species that feed only on land.

MATERIALS AND METHODS**Specimens**

To represent the broad life-history strategies within the Salamandridae, seven species that fed readily on land were used to study tongue-projection performance: *Chioglossa lusitanica* Bocage 1864, *Salamandra salamandra* (Linnaeus 1758), *Pleurodeles waltl* Michahelles 1830, *Notophthalmus viridescens* (Rafinesque 1820), *Triturus dobrogicus* (Kiritzescu 1903), *Cynops cyanurus* Liu, Hu and Yang 1962, and *Paramesotriton labiatus* (Unterstein 1930). All salamanders were obtained from commercial suppliers, except *C. lusitanica*, which were collected from wild populations (Oia, Spain). Salamanders were individually housed in 33.0×18.5×11.5 cm plastic containers placed on an incline with a lining of moist paper towels. Containers were filled partially with water, providing the salamanders with access to both water and land. Individuals were maintained on a diet of crickets, fruit flies, earthworms and bloodworms, and housed at 16–21°C. All procedures in this study were approved by the Institutional Animal Care and Use Committee of the University of South Florida, FL, USA.

Morphology

Three individuals from each of the seven species were euthanized by immersion in a 3 g l⁻¹ buffered aqueous solution of MS-222 (tricaine methanesulfonate; Sigma–Aldrich, St Louis, MO, USA), followed by exsanguination. SVL, from the tip of the rostrum to the posterior extent of the vent, and total length (TL), from the tip of the rostrum to the tip of the tail, were measured using Neiko 01408A digital calipers, and animals were weighed on a digital scale (Virtual Measurements and Control model VB-302A, Santa Rosa, CA, USA; ±0.001 g accuracy). Buccal area was calculated as half the area of an ellipse. The vertex of the ellipse was measured as the buccal length, from the anterior, inner edge of the mandible to the level of the jaw joint in ventral view, and the covertex was measured as one-half the buccal width, distance between the jaw joints in ventral view. The tongue and hyobranchial apparatus were then manipulated to simulate tongue projection. The range of tongue extension *in vivo* and maximum extension length in specimens were quantified as the distance from the tip of the BB to the rostral tip of the lower jaw. Salamanders were photographed in dorsal and ventral views using a digital camera (Canon PowerShot S70, Tokyo, Japan) attached to a dissecting microscope (Leica MZ6, Wetzlar, Germany). The curvature of the mandible of each animal was calculated in ImageJ software (National Institutes of Health, Bethesda, MD, USA) as the ratio of the arc length to the chord length of the mandible. The arc length was measured as the distance along the curve of the lower jaw from the posterior end of one mandible to the posterior end of the contralateral mandible, and chord length was measured as the distance across the floor of the mouth at the level of the jaw joint.

After external morphological measurements were taken, the SAR, or projector, muscles were peeled anteriorly from their origin on the CH, removed from the hyobranchial apparatus and weighed. The length of the RC, or retractor, muscles was measured from the anterior insertion on the tongue pad to the pectoral girdle, where the RC and rectus abdominis muscles are no longer distinct, in *P. waltl*, *N. viridescens*, *T. dobrogicus*, *C. cyanurus* and *P. labiatus*. Unlike in the semi-aquatic and aquatic newts, the RC muscles of

C. lusitanica and *S. salamandra* originate on the pelvis, and length of the RC muscles was instead measured from the anterior insertion on the tongue pad to the origin on the pelvis (Özeti and Wake, 1969). The remaining hyobranchial apparatus and musculature were excised by cutting the RC muscles at the level of the EB tip, and freeing the tongue pad and hyobranchial apparatus from the buccal mucosa and severing the genioglossus muscle at the origin on the mandible. The tongue (tongue skeleton, anterior RC muscles and tongue pad) was weighed prior to further dissection and independent massing of each component. To account for the remaining mass of the RC muscles in *C. lusitanica* and *S. salamandra*, the posterior portions were also removed and weighed. The cross-sectional area (CSA) of the RC muscles was calculated as the total mass of the RC muscles divided by the product of the density of muscle and the previously measured length. The ratio of the tongue-to-SAR muscle and tongue-to-RC muscle masses were calculated to determine mass-specific power of tongue projection and retraction, respectively, in later analyses.

Following dissection, the hyobranchial apparatus of three individuals per species was cleared and doubly stained (Hanken and Wassersug, 1981). The stained hyobranchial apparatus was photographed with a digital camera attached to a dissecting microscope. Percent mineralization and aspect ratio, ratio of length-to-width, were calculated in ImageJ software for each of the following elements: BB, CB I, CB II, ceratobranchial I + ceratobranchial II complex (CB I+CB II), EB and CH. Percent mineralization was calculated as the mineralized area of each hyobranchial element divided by the total area of the element. The percent mineralization of the entire tongue skeleton was also calculated. A robustness index (RI) for each specimen was calculated by dividing the total area of the hyobranchial apparatus by the buccal area. Calculations of percent mineralization, in conjunction with the calculated aspect ratios and RI, were used to quantitatively assess hyobranchial apparatus morphology for each species.

Videography and kinematic and inverse dynamic analyses

Six individuals of *C. lusitanica* and five individuals of *S. salamandra*, *P. waltl*, *N. viridescens*, *T. dobrogicus*, *C. cyanurus* and *P. labiatus* were imaged in lateral view at 3 kHz with a Photron Fastcam high-speed camera (1024 PCI, Photron USA Inc., San Diego, CA, USA) under white light-emitting diode illumination while feeding on prey placed at varying distances from the salamander. Prey type was varied for terrestrial feeding trials to elicit maximal feeding performance and included 0.5 cm pieces of earthworm, fruit flies, termites and crickets. Imaging occurred at 17–21°C against a 0.5 cm×0.5 cm grid for scale. Five feeding sequences from each of the five individuals per species were used for kinematic analyses in all but *C. lusitanica*, in which one to five recordings were obtained per individual.

Feeding sequences were digitized in ImageJ software. The position (*x,y* coordinates) of the tips of the upper and lower jaws, the nape (i.e. external point of flexion during rotation of the head about the atlanto-occipital joint), the ventral-most point of the oropharynx and the leading edge of the tongue were recorded for each frame of the feeding event. To standardize start time across all feeding trials, digitizing began 15 frames prior to mouth opening and ended the frame after the mouth was closed. Using a custom R script (R statistical software version 3.2.3, www.r-project.org), the *x,y* coordinates were used to calculate maximum gape distance (i.e. distance between the upper and lower jaw tips), hyobranchial depression (i.e. difference between maximum hyobranchial

depression distance and hyobranchial depression distance at the start of a feeding sequence), and tongue projection and retraction (i.e. distance between the tip of the tongue and lower jaw tip). The duration of mouth opening and closing, gape cycle, maximum hyobranchial depression duration and maximum tongue projection duration were also calculated.

Maximum velocity and acceleration for mouth opening, hyobranchial depression and tongue projection and retraction were calculated by taking the first and second derivatives, respectively, of a quintic spline fit to the position versus time data in R using the *pspline* package. Smoothing parameters of the quintic spline were adjusted to remove secondary oscillations from the acceleration data for each species. Inverse dynamics were used to calculate the maximum muscle-mass-specific power of tongue projection and retraction during each feeding event. Tongue-mass-specific power was calculated as the product of velocity and acceleration, which was multiplied by the ratio of the tongue-to-SAR masses for projection power and by the ratio of the tongue-to-RC masses for retraction power. Tongue-projection performance was measured as the distance, velocity, acceleration and muscle-mass-specific power of tongue projection in each species.

Statistical analysis

Custom R scripts were used to detect statistical differences in all morphological, kinematic and inverse dynamics data among the seven species. Analyses accounted for size by including SVL in the statistical model as a covariate. The data set was also tested for phylogenetic signal with the *Phytools* package in R, using the two most current phylogenies for Family Salamandridae (Pyrone and Weins, 2011; Zhang et al., 2008). No significant phylogenetic signal was found (Blomberg's $K < 1$, Pagel's $\lambda < 1$); therefore, standard statistical tests were conducted (Blomberg et al., 2003; Pagel, 1999). For all measured variables a nested two-way ANOVA, accounting for SVL and individual nested within species, was conducted. To meet parametric assumptions, the following variables were \log_{10} transformed during statistical analyses: SAR muscle and tongue skeleton masses; RC muscle mass and CSA; BB aspect ratio; maximum gape; maximum tongue projection; duration of mouth opening and closing; gape cycle; maximum projection duration; maximum mouth opening and hyobranchial depression acceleration; and maximum projection and retraction power. To determine statistical differences between species, Tukey's *post hoc* analyses were conducted. Additionally, statistical tests with multiple comparisons were corrected for false discovery rate (Benjamini and Hochberg, 1995). Species means and standard error of the mean (s.e.m.) were calculated for all morphological, kinematic and inverse dynamics variables.

RESULTS

Morphology

Extended tongue lengths were highest in the terrestrial species, *C. lusitanica* and *S. salamandra*, with the tongue extending past the mandible by 7% and 6% of the SVL, respectively. *Chioglossa lusitanica* and *S. salamandra* had SAR muscles that weighed less than the mass of the projectile unit of the tongue, tongue skeleton and anterior RC muscles (Table 1, Table S1). Both of the species had RC muscles that remained distinct from the surrounding trunk muscles along their entire course from tongue to pelvis, whereas the posterior region of the RC muscles was not distinct from the rectus abdominis muscles in the semi-aquatic and aquatic newts, *P. waltl*, *N. viridescens*, *T. dobrogicus*, *C. cyanurus* and *P. labiatus*. The RC muscles of *C. lusitanica* also had a greater CSA relative to their SVL

Table 1. Morphological measurements across salamander species and tests for species differences

	<i>Chioglossa lusitana</i>		<i>Salamandra salamandra</i>		<i>Pleurodeles waltl</i>		<i>Notophthalmus viridescens</i>		<i>Triturus dobrogicus</i>		<i>Cynops cyanurus</i>		<i>Paramesotriton labiatus</i>		Species comparison	
	Means±s.e.m.	N	Means±s.e.m.	N	Means±s.e.m.	N	Means±s.e.m.	N	Means±s.e.m.	N	Means±s.e.m.	N	Means±s.e.m.	N	F-value	P-value
Snout–vent length (mm)	47.4±1.7	3	100.4±3.7	3	73.4±2.3	3	46.1±0.9	3	73.0±3.2	3	57.7±1.4	3	74.2±1.9	3	65.12	<0.0001*
Head width (mm)	4.6±0.3	3	15.8±1.0	3	11.2±0.6	3	6.0±0.1	3	7.5±0.4	3	8.4±0.3	3	8.5±0.6	3	11.28	0.0002*
Tongue extension length (mm)	3.5±0.3	3	6.4±0.9	3	3.1±0.0	3	2.5±0.2	3	2.0±0.2	3	1.7±0.2	3	0.0±0.0	3	31.91	<0.0001*
Mandible curvature	3.1±0.2	3	2.2±0.0	3	2.4±0.1	3	3.1±0.2	3	2.3±0.2	3	2.8±0.3	3	3.2±0.1	3	3.51	0.0274*
Subarcualis rectus mass (g)	0.004±0.001	3	0.027±0.004	3	0.023±0.002	3	0.005±0.001	3	0.009±0.000	3	0.012±0.002	3	0.084±0.009	3	32.95	<0.0001*
Tongue skeleton mass (g)	0.005±0.001	3	0.032±0.008	3	0.022±0.002	3	0.004±0.001	3	0.013±0.001	3	0.013±0.001	3	0.034±0.002	3	14.78	<0.0001*
Anterior rectus cervicis mass (g)	0.003±0.000	3	0.042±0.016	3	0.012±0.004	3	0.002±0.000	3	0.010±0.002	3	0.009±0.000	3	0.044±0.004	3	6.58	0.0023*
Rectus cervicis cross-sectional area (cm ²)	0.004±0.001	3	0.018±0.001	3	0.011±0.000	3	0.003±0.001	3	0.012±0.003	3	0.011±0.000	3	0.037±0.004	3	20.91	<0.0001*
Tongue pad mass (g)	0.005±0.000	3	0.072±0.009	3	0.009±0.002	3	0.003±0.001	3	0.007±0.001	3	0.006±0.000	3	0.004±0.001	3	25.15	<0.0001*
Tongue-to-SAR mass ratio	3.3±0.3	3	5.4±0.5	3	1.9±0.2	3	2.0±0.4	3	3.5±0.1	3	2.6±0.6	3	1.0±0.1	3	13.10	<0.0001*
Mineralization basibranchial (%)	77±3	3	0±0	3	0±0	3	45±8	3	55±9	3	68±3	3	70±7	3	25.98	<0.0001*
Mineralization ceratobranchial I (%)	0±0	3	0±0	3	75±3	3	79±2	3	83±2	3	86±0	3	85±1	3	548.30	<0.0001*
Mineralization ceratobranchial II (%)	0±0	3	0±0	3	0±0	3	9±9	3	39±20	3	70±5	3	70±2	3	17.34	<0.0001*
Mineralization ceratobranchial I+II complex (%)	0±0	3	0±0	3	50±2	3	62±4	3	71±6	3	81±1	3	81±2	3	160.05	<0.0001*
Mineralization epibranchial (%)	0±0	3	0±0	3	69±4	3	79±2	3	79±1	3	87±0	3	86±2	3	364.20	<0.0001*
Mineralization ceratohyal (%)	0±0	3	0±0	3	28±3	3	41±3	3	43±3	3	40±1	3	54±3	3	118.30	<0.0001*
Mineralization tongue skeleton (%)	1±0	3	0±0	3	41±3	3	51±1	3	59±4	3	59±1	3	69±1	3	289.05	<0.0001*
Robustness index	0.69±0.06	3	0.45±0.01	3	0.55±0.09	3	0.74±0.02	3	0.51±0.07	3	0.67±0.09	3	0.78±0.09	3	1.640	0.2128
Aspect ratio basibranchial	17.5±1.7	3	4.1±0.3	3	3.2±0.4	3	8.1±0.5	3	5.2±0.6	3	6.5±0.5	3	7.6±0.1	3	19.37	<0.0001*
Aspect ratio ceratobranchial I	7.3±0.8	3	8.0±0.7	3	7.6±0.8	3	6.4±0.9	3	5.3±0.9	3	8.5±0.6	3	5.5±0.5	3	2.78	0.0580
Aspect ratio ceratobranchial II	14.6±4.1	3	17.6±0.3	3	12.5±1.0	3	15.3±0.5	3	14.9±1.9	3	20.1±0.8	3	14.8±0.7	3	1.74	0.1890
Aspect ratio ceratobranchial I+II complex	2.2±0.03	3	3.3±0.1	3	2.4±0.1	3	1.9±0.0	3	2.3±0.2	3	2.3±0.1	3	1.6±0.2	3	17.44	<0.0001*
Aspect ratio epibranchial	12.5±1.4	3	5.0±0.5	3	10.7±0.5	3	11.7±1.9	3	8.3±0.6	3	11.6±0.6	3	5.2±0.5	3	2.57	0.0723
Aspect ratio ceratohyal	5.1±0.8	3	5.4±0.4	3	6.0±0.5	3	7.0±0.1	3	7.6±0.1	3	6.1±0.5	3	5.3±0.1	3	4.10	0.0158*

*Significant effect at α of 0.05 after adjusting for false discovery rate. Significant species differences corrected for snout–vent length.

compared with all other salamandrids, except *P. labiatus*. Both *C. lusitanica* and *S. salamandra* had significantly lighter hyobranchial apparatus at 0.005 ± 0.001 g and 0.032 ± 0.008 g, respectively, than either *C. cyanurus* or *P. labiatus* (Table 1, Table S1). The BB in *C. lusitanica* was more mineralized at 77% and had a high aspect ratio compared with *S. salamandra* and most newts. The remainder of the hyobranchial apparatus in the terrestrial species had no mineralization (Table 1, Fig. 1).

Extended tongue length was lowest in *P. labiatus*, which was entirely unable to project the tongue. Also, the mandible was relatively more curved, elongated and tapered in *P. labiatus* than in *T. dobrogicus* (Table 1, Table S1). Labial lobes were more pronounced in the fully aquatic newt than in the other salamandrids examined. The SAR muscles were heavier in *P. labiatus* at 0.084 ± 0.009 g, and more than three times the mass of the SAR muscles of other salamandrids (Table 1, Table S1). Additionally, the tongue-to-SAR muscle-mass ratio was relatively low in this species, with a ratio of 1.0, because the SAR muscles and the tongue projectile (the hyobranchial apparatus, tongue pad and anterior RC muscles) had similar masses. The RC muscles were heavier in this species than in *C. lusitanica*, *S. salamandra*, *P. waltl*, *N. viridescens* and *T. dobrogicus*, and had the greatest CSA (Table 1, Table S1). Overall, the hyobranchial apparatus was heavier at 0.034 ± 0.002 g and was highly mineralized in the fully aquatic newt. Mineralization was greatest in the hyobranchial apparatus of *P. labiatus*, with 69% of the total apparatus mineralized. The CB I+CB II was also relatively wide in this species with an aspect ratio of 1.6, whereas all other salamanders examined reached values of 2 or greater (Table 1, Table S1, Fig. 1).

The semi-aquatic salamandrids examined had hyobranchial and tongue morphology that was intermediate between terrestrial and aquatic species (Fig. S1). Extended tongue lengths were less than

those of *C. lusitanica* and *S. salamandra*, but more than that of *P. labiatus*. The mass of the SAR muscles in *P. waltl* and *T. dobrogicus* was between the SAR muscle masses of *P. labiatus* and *C. lusitanica*, at 0.023 ± 0.002 g and 0.009 ± 0.000 g, respectively, with *P. waltl* having the lowest and *T. dobrogicus* having the greatest SAR muscle mass of the semi-aquatic newts. The CSA of the RC muscles in *C. cyanurus* was also intermediate between fully terrestrial and fully aquatic salamandrids, but was greater than the other semi-aquatic newts (Table 1, Table S1). Similarly, the mineralization of the hyobranchial apparatus and each of the hyobranchial elements was intermediate between those of fully terrestrial or fully aquatic salamanders. *Cynops cyanurus* and *T. dobrogicus* had more mineralized BB than other semi-aquatic newts, whereas a relatively more mineralized CB I was present in *C. cyanurus* and *P. waltl*. The remainder of the tongue skeleton was less mineralized than in *P. labiatus* and more mineralized than in *C. lusitanica* and *S. salamandra*, ranging from 41% to 59% mineralized in the semi-aquatic newts (Table 1, Table S1, Fig. 1).

Terrestrial feeding

Individuals across the seven species successfully captured prey during terrestrial-feeding events (Fig. 2). *Chioglossa lusitanica*, *S. salamandra*, *P. waltl*, *N. viridescens* and *T. dobrogicus* fed using tongue projection in all trials, whereas *C. cyanurus* fed using tongue prehension in 68% of feeding trials, resorting to jaw prehension during the remaining feeding attempts. During tongue prehension, the tongue pad and hyobranchial apparatus moved forward out of the mouth until the sticky tongue pad contacted the prey. The tongue was then retracted into the mouth as the jaws closed. *Paramesotriton labiatus* did not feed using tongue prehension in any feeding trials and instead only used jaw prehension. During jaw prehension, *P. labiatus* rapidly expanded the oropharyngeal region, similar to

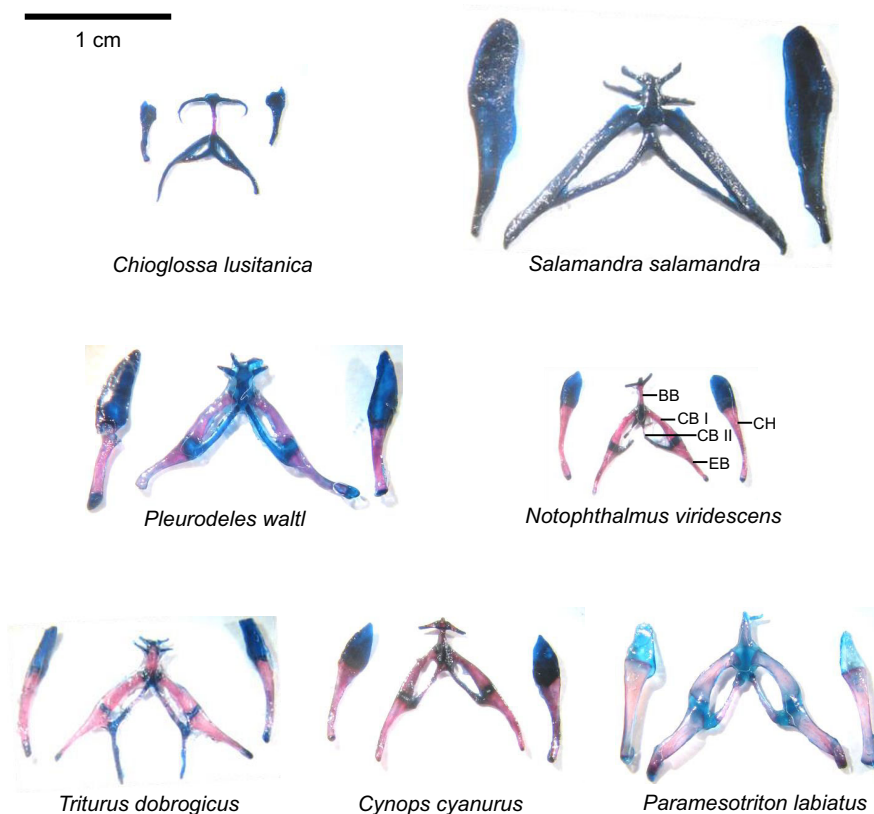


Fig. 1. Representative images of the cleared and stained hyobranchial apparatus of *Chioglossa lusitanica*, *Salamandra salamandra*, *Pleurodeles waltl*, *Notophthalmus viridescens*, *Triturus dobrogicus*, *Cynops cyanurus* and *Paramesotriton labiatus*. Cartilage appears blue whereas mineralization appears red. The hyobranchial apparatus of *N. viridescens* indicate the relative structures of the apparatus and include the basibranchial (BB), ceratobranchial I (CB I), ceratobranchial II (CB II), epibranchial (EB) and the ceratohyals (CH).

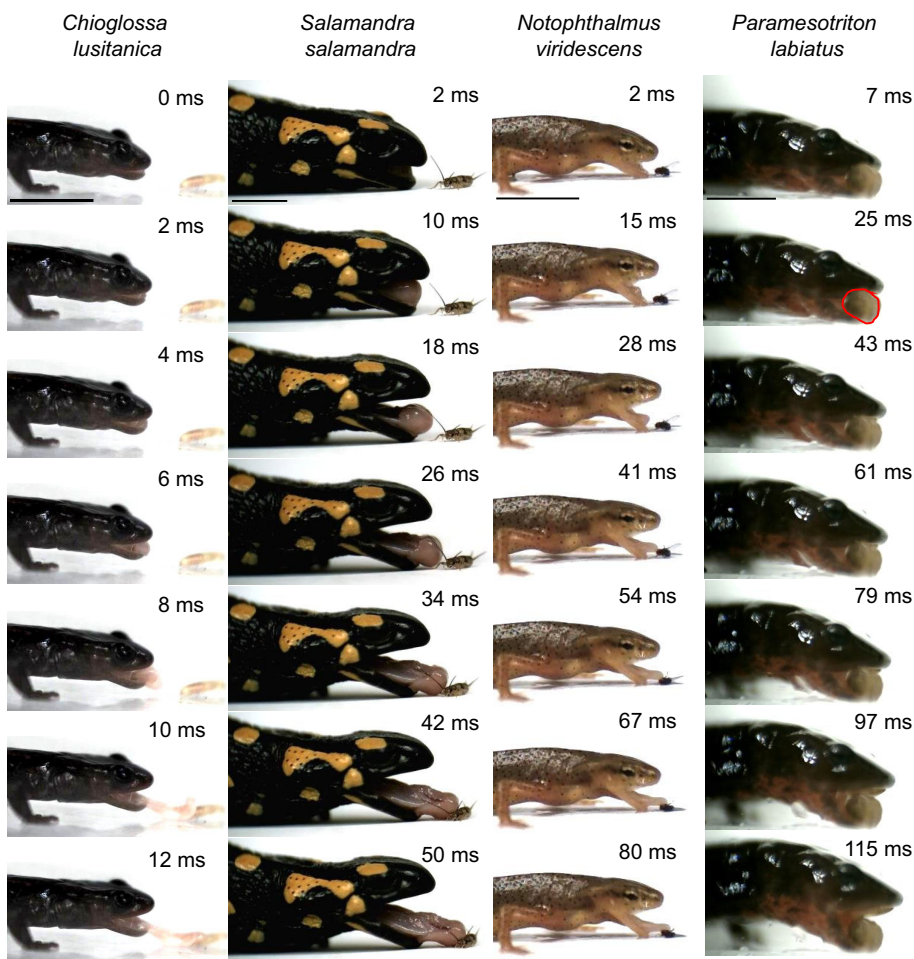


Fig. 2. Representative feeding sequences showing maximum tongue projection. Images show timing and maximum projection for *Chioglossa lusitanica*, *Salamandra salamandra*, *Notophthalmus viridescens* and *Paramesotriton labiatus*, from left to right. Scale bars beneath the first image of each column are 1 cm. Prey items in images are termites, crickets, fruit flies and earthworms. It is notable that in *P. labiatus* the tongue does not project; therefore, maximum gape is shown for this species. The earthworm (prey item) captured in this feeding sequence is highlighted in red in the second frame of the sequence.

that used during aquatic-suction feeding (Miller and Larsen, 1989; Stinson and Deban, 2017).

Prey capture kinematics and feeding performance

A total of 172 image sequences were captured from the seven species examined. For *S. salamandra*, *P. waltil*, *N. viridescens*, *T. dobrogicus*, *C. cyanurus* and *P. labiatus*, five image sequences from five individuals of each species were obtained. A total of 22 image sequences from six individuals of *C. lusitanica* were recorded. Maximum gape distance in *N. viridescens* and *P. labiatus* exceeded those of all other salamanders tested, except *S. salamandra*. Additionally, gape cycle duration was shortest in *C. lusitanica* and *P. labiatus*, and longest in *N. viridescens*. Hyobranchial depression was greatest in *P. labiatus*. *Cynops cyanurus* and *N. viridescens* had greater hyobranchial depression compared with *C. lusitanica* and *T. dobrogicus*. Maximum hyobranchial depression duration was greatest in *N. viridescens* and lowest in *C. lusitanica* (Table 2, Table S2). Tongue-projection distance was greatest in the terrestrial salamanders, *C. lusitanica* and *S. salamandra*, whereas the fully aquatic newt, *P. labiatus*, was unable to feed using tongue projection. *Chioglossa lusitanica* had the shortest tongue-projection duration. The semi-aquatic newts had lower projection distance and greater projection duration when compared with terrestrial salamandrids (Table 2, Table S2, Fig. 2).

Maximum mouth-opening velocity and acceleration were greatest in the fully aquatic newt, *P. labiatus*. Maximum mouth-opening velocity in *P. labiatus* was $0.30 \pm 0.02 \text{ m s}^{-1}$, whereas mouth-opening acceleration was $50.9 \pm 7.7 \text{ m s}^{-2}$ (Table 2,

Table S2, Fig. 3). Higher velocity and acceleration of mouth opening resulted from a relatively wide gape occurring over a shorter duration (Table 2, Fig. 2). Although the values of *C. lusitanica* and *S. salamandra* did not exceed those of *P. labiatus*, mouth-opening velocity was also greater in these species at $0.20 \pm 0.01 \text{ m s}^{-1}$ and $0.29 \pm 0.01 \text{ m s}^{-1}$, respectively; however, only mouth-opening acceleration was greater in *C. lusitanica* (Table 2, Table S2, Fig. 3). As with *P. labiatus*, *S. salamandra* opened its mouth relatively wide, but over a duration similar to those of other newts. *Chioglossa lusitanica* gape distance was comparable with that of semi-aquatic newts, but mouth-opening duration was relatively shorter than in semi-aquatic newts (Table 2, Table S2, Fig. 2). Overall, mouth-opening velocity and acceleration were lowest in semi-aquatic species (Table 2, Table S2, Fig. 3).

Hyobranchial depression velocity was greatest in *P. labiatus*, whereas acceleration varied across species. During terrestrial-feeding trials, *P. labiatus* had a mean maximum hyobranchial depression velocity of $0.189 \pm 0.015 \text{ m s}^{-1}$, more than double the velocity measured in the other salamanders (Table 2, Fig. 4A). *Paramesotriton labiatus* achieved this velocity with the greatest hyobranchial depression distance during terrestrial feeding and a shorter maximal hyobranchial depression duration (Table 2, Fig. 2). Hyobranchial depression velocity was also greater in *C. lusitanica* at $0.086 \pm 0.011 \text{ m s}^{-1}$; however, this was caused by a shorter duration to maximum hyobranchial depression rather than any significant increase in hyobranchial depression distance (Table 2, Table S2, Figs 2 and 3). Semi-aquatic species and *S. salamandra* had lower

Table 2. Terrestrial feeding kinematics and inverse dynamics across salamander species and tests for species differences

	Chioglossa		Salamandra		Pleurodeles		Notophthalmus		Triturus		Cynops		Paramesotriton		Species	
	Means±s.e.m.	N	Means±s.e.m.	N	Means±s.e.m.	N	Means±s.e.m.	N	Means±s.e.m.	N	Means±s.e.m.	N	Means±s.e.m.	N	F-value	P-value
Gape distance (mm)	3.5±0.1	6	13.1±0.3	5	6.9±0.8	5	4.3±0.1	5	5.6±0.2	5	4.0±0.5	5	7.9±0.3	5	8.27	<0.0001*
Hyobranchial depression (mm)	0.3±0.0	6	2.0±0.2	5	1.3±0.2	5	1.3±0.0	5	0.9±0.0	5	1.2±0.2	5	3.8±0.0	5	39.95	<0.0001*
Tongue projection (mm)	7.2±0.3	6	8.7±0.2	5	3.1±0.0	5	2.8±0.0	5	2.2±0.0	5	1.1±0.0	5	0.0±0.0	5	259.42	<0.0001*
Mouth-opening duration (s)	0.04±0.00	6	0.11±0.01	5	0.12±0.01	5	0.18±0.01	5	0.11±0.01	5	0.11±0.01	5	0.05±0.01	5	67.10	<0.0001*
Mouth-closing duration (s)	0.08±0.01	6	0.16±0.01	5	0.18±0.01	5	0.28±0.01	5	0.16±0.01	5	0.17±0.02	5	0.12±0.01	5	78.77	<0.0001*
Gape cycle (s)	0.11±0.01	6	0.27±0.02	5	0.31±0.01	5	0.46±0.02	5	0.27±0.02	5	0.27±0.03	5	0.16±0.01	5	84.59	<0.0001*
Maximum hyobranchial depression duration (s)	0.02±0.00	6	0.11±0.01	5	0.10±0.02	5	0.24±0.01	5	0.07±0.01	5	0.09±0.02	5	0.07±0.01	5	40.30	<0.0001*
Maximum tongue-projection duration (s)	0.020±0.001	6	0.072±0.005	5	0.092±0.006	5	0.118±0.005	5	0.090±0.007	5	0.062±0.014	5	0.000±0.000	5	62.24	<0.0001*
Maximum mouth-opening velocity (m s ⁻¹)	0.20±0.01	6	0.29±0.01	5	0.13±0.01	5	0.10±0.01	5	0.14±0.01	5	0.14±0.03	5	0.30±0.02	5	21.37	<0.0001*
Maximum mouth-opening acceleration (m s ⁻²)	34.2±5.2	6	15.6±2.6	5	5.8±1.0	5	1.5±0.2	5	9.7±1.6	5	16.3±6.4	5	50.9±7.7	5	40.15	<0.0001*
Maximum hyobranchial depression velocity (m s ⁻¹)	0.086±0.011	6	0.098±0.013	5	0.067±0.009	5	0.022±0.002	5	0.120±0.047	5	0.061±0.012	5	0.189±0.015	5	12.33	<0.0001*
Maximum hyobranchial depression acceleration (m s ⁻²)	31.0±8.1	6	6.8±1.9	5	6.6±1.4	5	0.9±0.4	5	98.8±84.4	5	8.1±2.9	5	21.9±3.7	5	20.60	<0.0001*
Maximum tongue-projection velocity (m s ⁻¹)	1.860±0.081	6	0.193±0.007	5	0.089±0.009	5	0.049±0.003	5	0.062±0.005	5	0.040±0.009	5	0.000±0.000	5	317.48	<0.0001*
Maximum tongue-projection acceleration (m s ⁻²)	533.3±35.9	6	11.7±0.8	5	10.8±2.3	5	2.0±0.2	5	5.1±0.7	5	3.6±1.1	5	0.0±0.0	5	214.98	<0.0001*
Maximum mass-specific tongue-projection power (W kg ⁻¹)	2244.17±209.61	6	6.33±0.69	5	0.92±0.33	5	0.16±0.03	5	0.86±0.19	5	0.67±0.30	5	0.00±0.00	5	265.52	<0.0001*
Maximum tongue-retraction velocity (m s ⁻¹)	0.078±0.015	6	0.056±0.007	5	0.035±0.010	5	0.011±0.0020	5	0.028±0.006	5	0.012±0.003	5	0.000±0.000	5	6.17	<0.0001*
Maximum tongue-retraction acceleration (m s ⁻²)	52.9±7.2	6	21.8±2.3	5	8.5±1.7	5	2.1±0.3	5	7.2±1.5	5	2.5±0.8	5	0.0±0.0	5	50.64	<0.0001*
Maximum mass-specific tongue-retraction power (W kg ⁻¹)	38.10±5.19	6	3.12±0.54	5	0.69±0.35	5	0.05±0.01	5	0.53±0.21	5	0.05±0.018	5	0.00±0.00	5	74.38	<0.0001*

*Significant effect at α of 0.05 after adjusting for false discovery rate. Significant species differences corrected for snout–vent length.

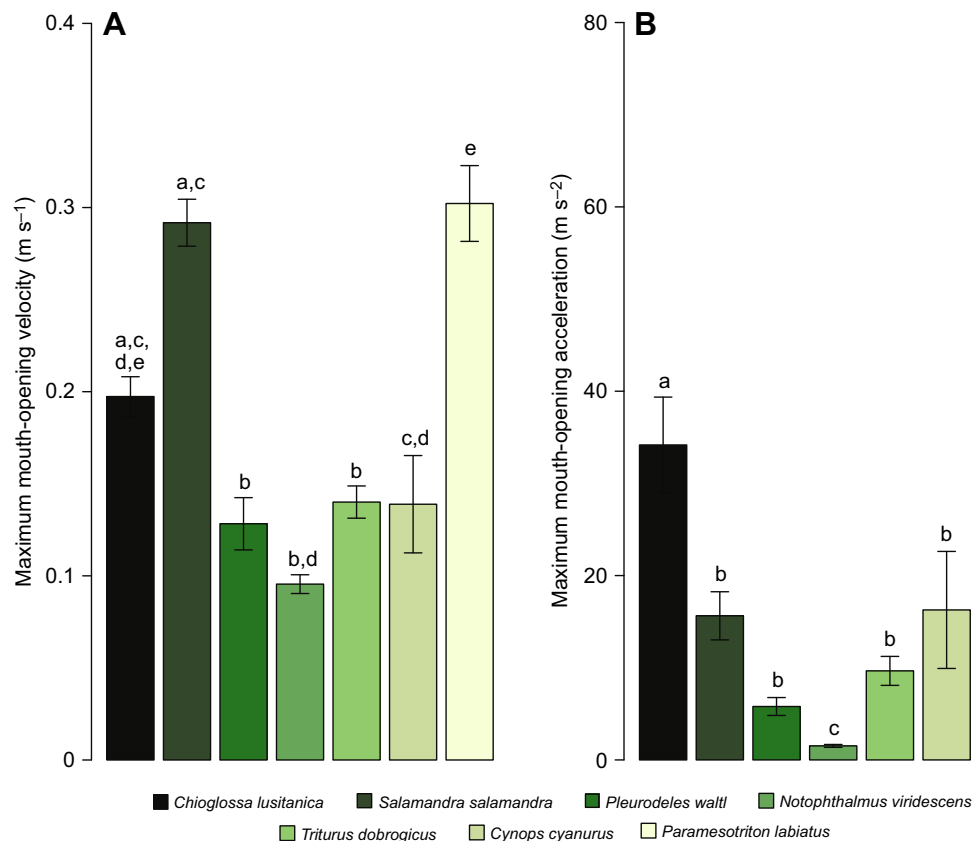


Fig. 3. Bar plots depicting mouth-opening velocity and acceleration. Graphs show mouth-opening (A) velocity and (B) acceleration for the seven experimental species (*Chioglossa lusitanica*, $N=6$; *Salamandra salamandra*, *Pleurodeles waltl*, *Notophthalmus viridescens*, *Triturus dobrogicus*, *Cynops cyanurus* and *Paramesotriton labiatus*, all $N=5$). Individual bars depict mean kinematics for each species with standard error. Shared letters over the bars indicate that means are not significantly different. Snout–vent length was accounted for during ANOVA analyses for statistical differences between taxa.

hyobranchial depression velocity during terrestrial feeding than *P. labiatus* and *C. lusitanica* (Table 2, Table S2, Fig. 3).

Tongue-projection performance was greatest in *C. lusitanica*, with tongue-projection velocity and acceleration exceeding values of the other species by over four and 36 times, respectively. Mean maximum tongue-projection velocity was $1.860 \pm 0.081 \text{ m s}^{-1}$ and mean maximum acceleration was $533.3 \pm 35.9 \text{ m s}^{-2}$. Maximum muscle-mass-specific tongue-projection power was highest in this terrestrial salamandrid, reaching power output over 350 times that of other salamandrids at $2244.17 \pm 209.61 \text{ W kg}^{-1}$ (Table 2, Table S2, Fig. 5C). Whereas *S. salamandra* had lower tongue-projection velocity, acceleration and muscle-mass-specific power than *C. lusitanica* of $0.193 \pm 0.007 \text{ m s}^{-1}$, $11.7 \pm 0.8 \text{ m s}^{-2}$ and $6.33 \pm 0.69 \text{ W kg}^{-1}$, respectively, overall tongue-projection performance was higher than in semi-aquatic and aquatic newts (Table 2, Table S2, Fig. 5A–C). Tongue-retraction velocity, acceleration and power were greatest in *C. lusitanica*, with *S. salamandra* again exceeding the values of the other newts, but less than that of *C. lusitanica* (Table 2, Table S2, Fig. 5D, Fig. S2).

When comparing the semi-aquatic and aquatic newts, minor differences were evident in tongue-projection velocity, acceleration and power between these species (Fig. S2). Furthermore, the tongue-projection performance of some semi-aquatic newts did not differ from *P. labiatus*, which was unable to project its tongue (Table 2, Table S2, Fig. 5). Maximum projection velocity and acceleration were not significantly different in *P. waltl*, *T. dobrogicus* and *P. labiatus*. Additionally, muscle-mass-specific projection power was not significantly different in *T. dobrogicus*, *C. cyanurus* and *P. labiatus* (Table 2, Table S2, Figs 2 and 5A–C).

DISCUSSION Morphology

Terrestrial salamanders *Chioglossa* and *Salamandra* have larger tongue-to-SAR muscle-mass ratio and retractor muscles that are anatomically distinct from the surrounding musculature (Table 1, Table S1). Enlarged tongue pads and greater extended tongue lengths can be beneficial in capturing elusive prey or reaching food items at greater distances (Wake and Deban, 2000). Furthermore, the presence of distinct RC muscles throughout the length of the body resembles the musculature of plethodontid salamanders with high tongue-projection performance (Anderson et al., 2014; Deban et al., 2007; Deban and Scales, 2016; Lombard and Wake, 1977; Scales et al., 2016).

In *Chioglossa* and *Salamandra*, the hyobranchial apparatus is lighter and less mineralized compared with semi-aquatic and aquatic species (Table 1, Table S1, Fig. 1). As the tongue is projected, the SAR muscles contract, folding and bending the hyobranchial apparatus medially as it moves rostrally out of the mouth (Wake and Deban, 2000). Cartilaginous elements allow for greater flexibility, and enable medial folding of the tongue skeleton during this rostral movement. Similar hyobranchial apparatus morphology is seen in plethodontid salamanders, which have specialized and relatively elaborated tongue projection (Lombard and Wake, 1977). While both of these species have less mineralization compared with other newts, *Salamandra* is the only species in this study to have a fully cartilaginous hyobranchial apparatus (Table 1, Fig. 1). In *Chioglossa* the hyobranchial apparatus is primarily cartilage, except for the BB, which is mineralized. Mineralization may lower flexion in the BB as the radials are flipped during tongue projection and when the RC muscles, which insert at the anterior tip of the element, contract during tongue retraction.

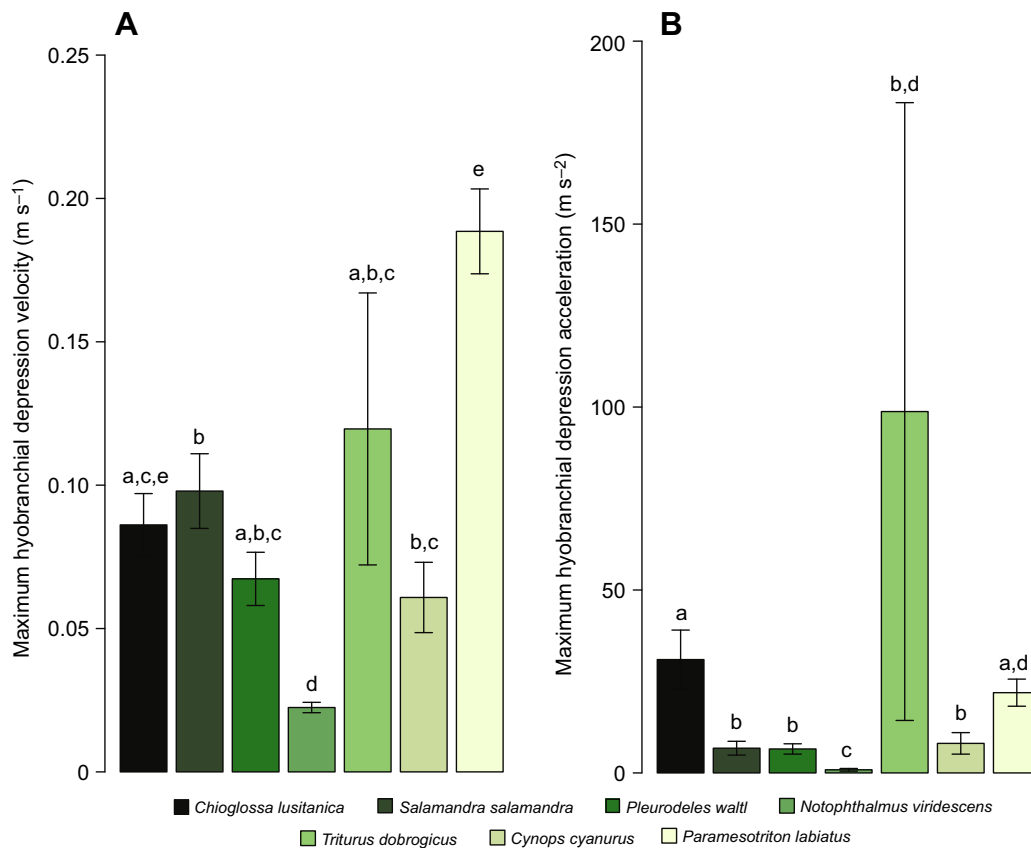


Fig. 4. Bar plots depicting hyobranchial depression velocity and acceleration. Graphs show the mean hyobranchial depression (A) velocity and (B) acceleration for *Chioglossa lusitanica*, $N=6$; *Salamandra salamandra*, *Pleurodeles waltl*, *Notophthalmus viridescens*, *Triturus dobrogicus*, *Cynops cyanurus* and *Paramesotriton labiatus*, all $N=5$. Indications as in Fig. 3.

The feeding musculature and hyobranchial apparatus morphology of the semi-aquatic newts, *Pleurodeles*, *Notophthalmus*, *Triturus* and *Cynops*, is intermediate between the morphology of terrestrial salamandrids and *Paramesotriton* (Table 1, Fig. 1, Fig. S2). The tongue pad is distinguished from the buccal mucosa and can be protruded forward; however, not at lengths comparable with those of *Chioglossa* and *Salamandra* (Özeti and Wake, 1969; Regal, 1966). The tongue pad is reduced in semi-aquatic species and the ratio of tongue-to-SAR muscle mass is lower than in the terrestrial species, with the exception of *Triturus*, which has a larger tongue-to-SAR muscle mass than *Chioglossa* (Table 1, Table S1). Although the SAR muscles are relatively heavier in semi-aquatic newts than in terrestrial salamandrids, tongue projection is restricted by robust muscular and ligamentous attachments of the tongue to the oropharynx and mandible. Additionally, the tongue skeletons are more mineralized in semi-aquatic species; however, not as mineralized as that of *Paramesotriton* (Table 1, Table S1, Fig. 1). Greater mineralization adds mass to the projectile unit and reduces flexibility of the hyobranchial apparatus, hindering medial folding and forward movement of the tongue during feeding.

In *Paramesotriton*, the tongue pad is not well differentiated from the buccal mucosa and the tongue is unable to leave the mouth. Similar tongue morphology has been described in hynobiids, ambystomatids, other salamandrids and larval plethodontids, suggesting trends away from specialized terrestrial feeding in semi-aquatic and aquatic salamanders (Deban and Wake, 2000; Özeti and Wake, 1969; Regal, 1966). During terrestrial feeding, rather than projecting outward, the tongue tended to move ventrally, expanding

the oropharyngeal cavity. This expansion is powered by the RC muscles, which were more massive and have a greater CSA in *Paramesotriton* (Table 1, Table S1). Greater CSA of the RC muscles allows for greater force generation to power hyobranchial depression (Powell et al., 1984). These morphological modifications, as well as greater mineralization and reduced flexibility of the hyobranchial apparatus in *Paramesotriton*, constrain tongue projection; however, this morphology is well suited for suction feeding (Table 1, Fig. 1). Furthermore, *Paramesotriton* have enlarged labial lobes and a tapered snout, which also enhance suction-feeding performance (Deban and Wake, 2000; Elwood and Cundall, 1994; Motta et al., 2002; Stinson and Deban, 2017; Van Wassenbergh and Heiss, 2016; Wilga and Motta, 1998).

Feeding kinematics and performance

Feeding kinematics differ across terrestrial, semi-aquatic and aquatic salamander species (Fig. S2). Maximum gape distance was greater and gape cycle was shorter in *Chioglossa* and *Paramesotriton*. Furthermore, mouth-opening velocity and acceleration were higher in both of these species, as well as *Salamandra* (Table 2, Table S2, Fig. 3). Previous research on feeding in *Salamandra* and *Salamandrina terdigitata* found larger gape angles during feeding when compared with semi-aquatic species, as well as earlier occurrence of peak gape (Miller and Larsen, 1990). Although these species have similar gape kinematics, their feeding modes differ. *Chioglossa*, *Salamandra* and *Salamandrina* rely on tongue projection to feed terrestrially, whereas *Paramesotriton* is unable to project its tongue and instead uses jaw prehension (Fig. 2) (Miller and Larsen, 1990). The rapid movements used during jaw prehension are

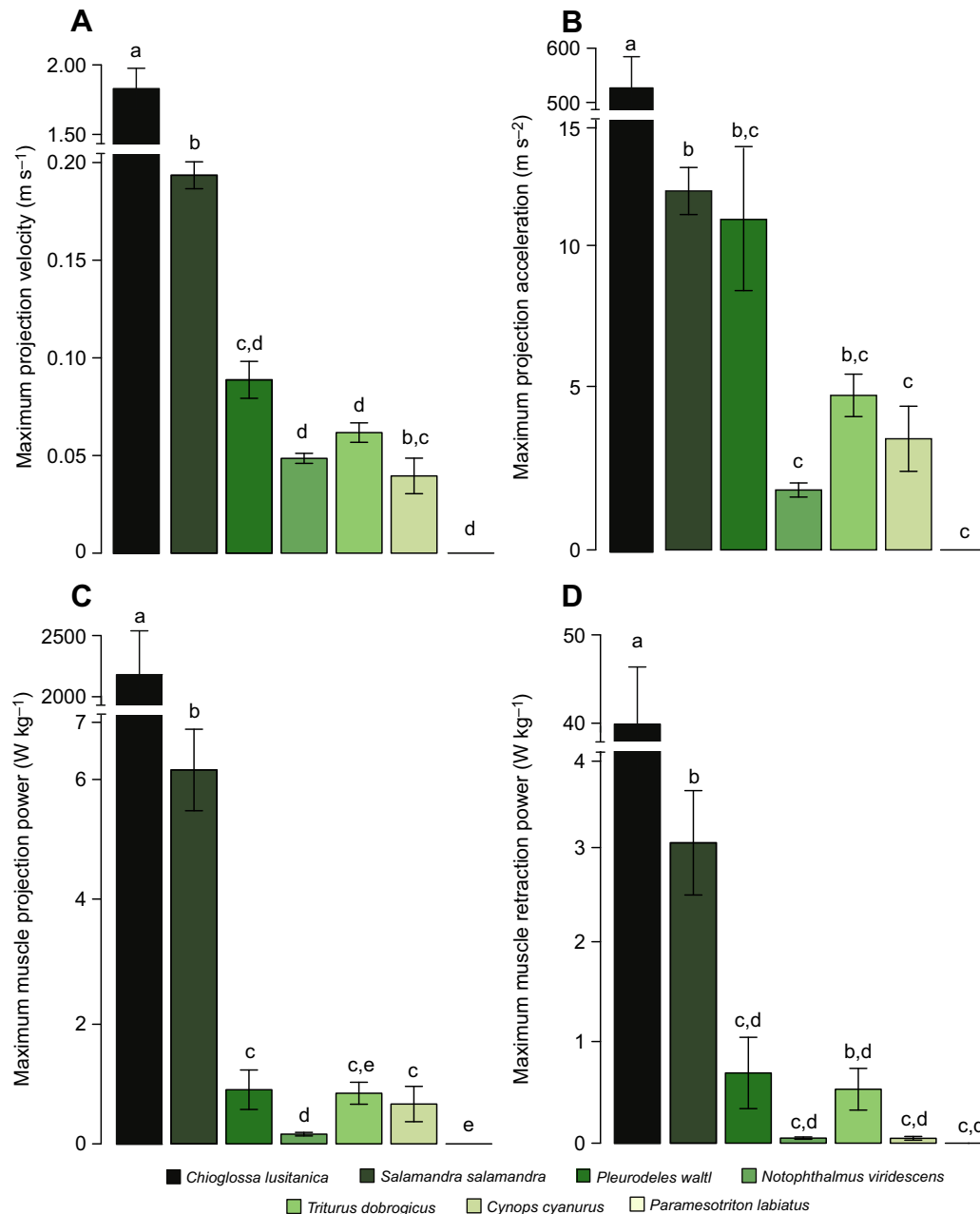


Fig. 5. Bar plots of tongue projection and retraction kinematics and dynamics. Graphs show mean (A) velocity, (B) acceleration and (C) mass-specific power for tongue projection and (D) mass-specific tongue-retraction power for each of the seven experimental species (*Chioglossa lusitanica*, $N=6$; *Salamandra salamandra*, *Pleurodeles waltl*, *Notophthalmus viridescens*, *Triturus dobrogicus*, *Cynops cyanurus* and *Paramesotriton labiatus*, all $N=5$). Note that the y-axis is broken in all graphs to better show the difference in the kinematics and dynamics of *C. lusitanica*. Indications as in Fig. 3.

similar to those used by *Paramesotriton* during aquatic prey capture and by semi-aquatic newts feeding terrestrially during their aquatic reproductive phase (Heiss et al., 2013; Heiss et al., 2015; Miller and Larsen, 1989; Stinson and Deban, 2017).

Maximum hyobranchial depression distance and velocity were greatest in *Paramesotriton* when feeding terrestrially (Table 2, Table S2, Figs 2 and 4). These results are similar to those seen in *P. labiatus*, *Paramesotriton hongkongensis* and *Pachytriton brevipes* when feeding in aquatic environments (Miller and Larsen, 1989; Stinson and Deban, 2017). Greater hyobranchial depression velocity enables the generation of faster flow during suction feeding; however, because air is less dense and viscous than water, suction feeding cannot be performed during terrestrial

feeding (Carroll et al., 2004; Deban and Wake, 2000; Herrel et al., 2012; Stinson and Deban, 2017; Svanbäck et al., 2002; Van Wassenbergh, 2013). Greater mouth opening and hyobranchial depression velocity and acceleration during terrestrial feeding suggest that *Paramesotriton* is attempting to suction feed on land. Mouth opening and hyobranchial depression kinematics, particularly with regards to distance and velocity, are comparable during terrestrial and aquatic feeding in this fully aquatic newt (Stinson and Deban, 2017).

Hyobranchial depression distance in terrestrial and semi-aquatic salamandrids was significantly lower than in *Paramesotriton* and was associated with tongue retraction (Table 2, Table S2). In tongue projection, the oropharyngeal region did not expand; however, during

the latter part of the feeding sequences, the hyobranchial apparatus was involved in prey transport and slightly expanded the oropharyngeal cavity. The measured hyobranchial depression in terrestrial and semi-aquatic species was, therefore, caused by retraction of the hyobranchial apparatus and was associated with intraoral transport (Deban and Wake, 2000; Gillis and Lauder, 1994).

Maximum tongue-projection velocity, acceleration and muscle-mass-specific power were higher in *Chioglossa* and *Salamandra* compared with semi-aquatic and aquatic newts. Maximum tongue-retraction velocity and muscle-mass-specific power were also higher in these two species, and maximum tongue-retraction acceleration was greatest in *Chioglossa* (Table 2, Table S2, Fig. 5A–C). These values are similar to those measured in plethodontid salamanders using ballistic tongue projection. Plethodontids exhibit projection velocity from 1.1 to 3.3 m s⁻¹ and acceleration ranging from 779 up to 1750 m s⁻² (Anderson et al., 2014; Deban and Richardson, 2011; Scales et al., 2016). Tongue-projection acceleration in *Chioglossa* was also similar to those measured during ballistic tongue projection in frogs and chameleons of 449 and 357 m s⁻², respectively (Anderson and Deban, 2010; Sandusky and Deban, 2012).

Functional trade-offs in salamander feeding

Feeding morphology and performance (i.e. tongue-projection distance, velocity, acceleration and power) are specialized for terrestrial feeding in *Chioglossa* and *Salamandra*, whereas *Paramesotriton* is specialized for aquatic feeding (Stinson and Deban, 2017). The tongue-projection performance of semi-aquatic species *Pleurodeles*, *Notophthalmus*, *Triturus* and *Cynops*, however, consistently fell between these two extremes. These differences, as well as those measured in aquatically feeding salamandrids, indicate that functional trade-offs occur in species that feed across environments (Miller and Larsen, 1989, 1990; Stinson and Deban, 2017). To facilitate feeding in multiple environments, the musculature and hyobranchial apparatus morphology must be suited for both suction feeding and tongue prehension. These feeding behaviors require opposing functions of the hyobranchial apparatus and place limitations on semi-aquatic species, resulting in overall lower feeding performance across aquatic and terrestrial environments (Table 2, Fig. 5) (Stinson and Deban, 2017).

Potential trade-offs in feeding performance are seen in the seasonal changes of semi-aquatic newts, with kinematics and morphology differing in *L. vulgaris* and *I. alpestris* between the breeding and non-breeding seasons. These semi-aquatic species exhibit morphological plasticity and can develop structures, such as labial lobes, during their aquatic phase to occlude the lateral gape and generate greater flow velocity during suction feeding (Heiss et al., 2013; Heiss et al., 2015; Van Wassenbergh and Heiss, 2016). Additionally, the semi-aquatic salamandrids *L. vulgaris* and *I. alpestris* can alter the surface of their tongue pad and mucous secretions in the mouth during seasonal breeding phases, developing slender lingual papillae and complex adhesive systems during the terrestrial phase to aid in tongue prehension (Heiss et al., 2017). Gape, hyobranchial and tongue movements vary between aquatic- and terrestrial-feeding events in terrestrial, semi-aquatic and aquatic salamandrids as well, further suggesting that whereas semi-aquatic and aquatic salamanders are able to feed in different environments, their kinematics and consequently their performance are less extreme compared with species that are specialized feeders for one environment (Heiss and De Vylder, 2016; Miller and Larsen, 1990; Stinson and Deban, 2017).

Convergence of high-powered feeding mechanisms

Specializations were found in both of the terrestrial species investigated, but tongue projection and retraction performance were consistently higher in *Chioglossa* than in *Salamandra*. Maximum muscle-mass-specific power of tongue projection in *Chioglossa* averaged over 2200 W kg⁻¹, exceeding 371 W kg⁻¹, the power that muscle alone is able to produce (Lutz and Rome, 1996) (Table 2, Fig. 5C). The plethodontid salamanders *Bolitoglossa*, *Eurycea*, *Hydromantes* and *Ensatina* are also capable of high muscle-mass-specific tongue-projection power ranging from 560 to 18,000 W kg⁻¹ (Deban et al., 2007; Deban and Scales, 2016; Scales et al., 2016). Elastic recoil is also seen in the high-powered tongue projection of chameleons, which achieve over 1800 W kg⁻¹ at similar experimental temperatures (Anderson and Deban, 2010). High-powered, ballistic tongue projection in plethodontids is achieved through an elastic-recoil mechanism, in which energy is stored within the collagen aponeuroses of SAR muscles (Anderson et al., 2014; Deban et al., 2007; Deban and Richardson, 2011; Deban and Scales, 2016; Scales et al., 2016). Studying the morphology of the SAR muscles of *Chioglossa* in greater detail would provide greater understanding of this high-powered mechanism.

High-powered projection indicates that morphological differences between *Chioglossa* and *Salamandra* enable elastic recoil in *Chioglossa*. The occurrence of high-powered tongue projection in the Family Salamandridae represents a novel, independent evolution of elastically powered feeding and convergence on a specialized feeding mechanism previously known only in plethodontid salamanders. To better understand the tongue-projection mechanism in *Chioglossa*, further examination is needed. By examining tongue projection across a range of temperatures, the thermal robustness of this high-powered system could be tested. Thermal robustness of performance has been observed in plethodontids with elastic tongue projection (Anderson et al., 2014; Deban and Richardson, 2011; Deban and Scales, 2016; Scales et al., 2016).

Conclusions

Differences in morphology are associated with differences in feeding kinematics and ultimately with differences in performance. Variations in tongue-projection velocity, acceleration and power across salamandrid salamanders are associated with functional differences in the hyobranchial apparatus during feeding events. Specifically, morphological specializations that increase tongue-projection performance, such as flexible, cartilaginous hyobranchial apparatus and well-developed tongue pads, occur in the terrestrial salamandrids *Salamandra* and *Chioglossa*. Tongue projection was greatest in *Chioglossa*, and greater in *Salamandra* than in the semi-aquatic and aquatic newts examined. Tongue-projection performance was lowest in *Paramesotriton*, which has morphological specializations, such as heavy mineralization of the hyobranchial apparatus and a small tongue pad that are better suited for aquatic-suction feeding. Semi-aquatic salamandrids have lower tongue-projection capabilities than terrestrially feeding specialists, and can be viewed as performance generalists that feed in different environments. Feeding performance in a given environment is constrained by the competing functions of the hyobranchial apparatus during tongue projection and suction feeding. Further studying these systems would improve our understanding of how feeding patterns evolved within the Family Salamandridae, as well as provide insight into how integrated systems meet the challenges of transitioning between different environments and functional demands.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: C.M.S., S.M.D.; Methodology: C.M.S., S.M.D.; Software: C.M.S., S.M.D.; Validation: C.M.S.; Formal analysis: C.M.S., S.M.D.; Investigation: C.M.S.; Resources: C.M.S., S.M.D.; Data curation: C.M.S.; Writing - original draft: C.M.S., S.M.D.; Writing - review & editing: C.M.S., S.M.D.; Visualization: C.M.S.; Supervision: C.M.S., S.M.D.; Project administration: C.M.S., S.M.D.; Funding acquisition: C.M.S., S.M.D.

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Supplementary information

Supplementary information available online at <http://jeb.biologists.org/lookup/doi/10.1242/jeb.164285.supplemental>

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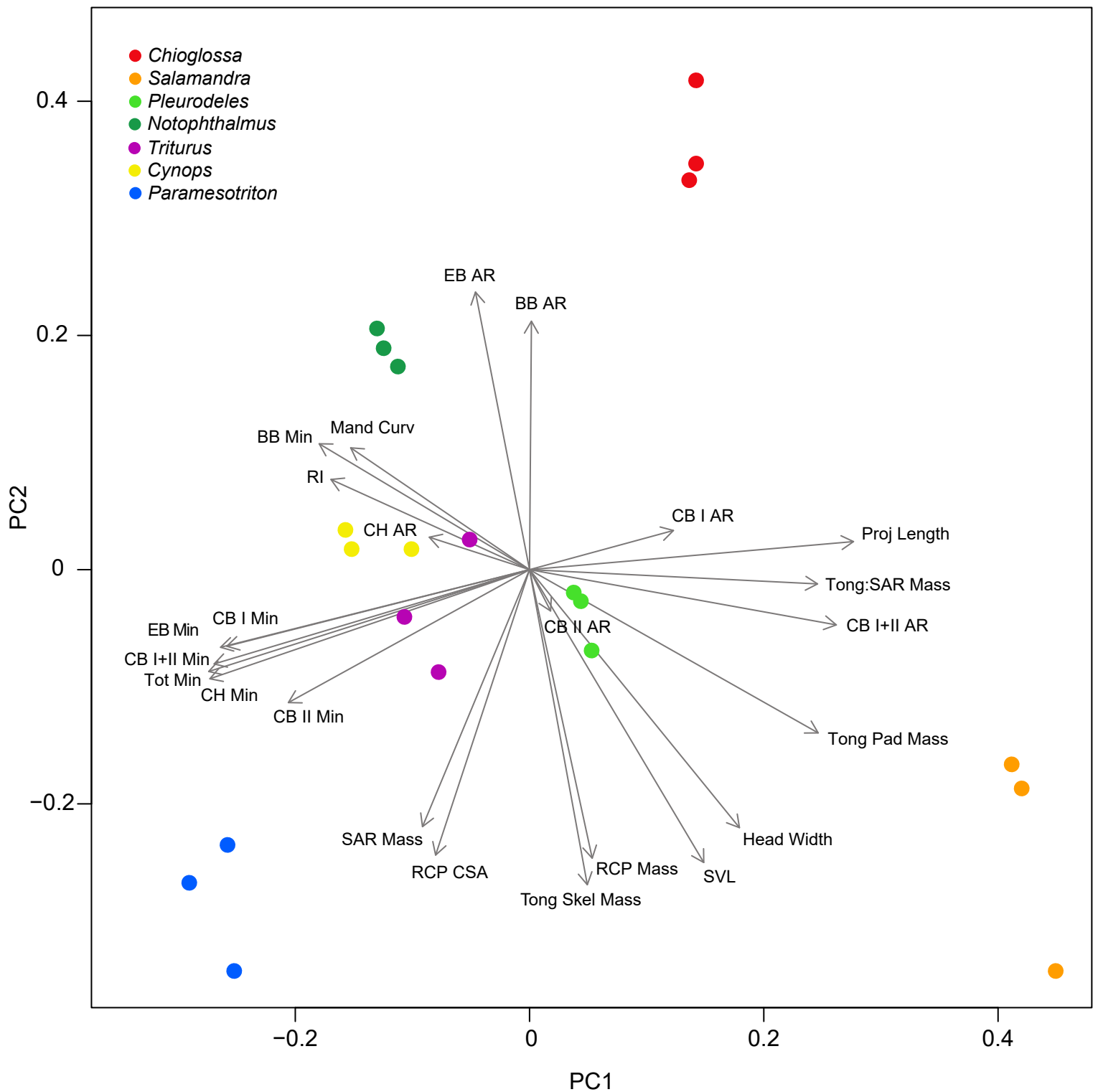


Figure S1. Principle components analysis of morphological data. Variable loadings represented by grey arrows, colored points show individual salamanders of each species (*Chioglossa*, *Salamandra*, *Pleurodeles*, *Notophthalmus*, *Triturus*, *Cynops*, and *Paramesotriton*, N=3). The x and y axes represent the loadings of the first and second principle components. Terrestrial species are depicted on the far right, the fully aquatic newt, *Paramesotriton*, is shown on the far left, and semi-aquatic newts cluster towards the center of the plot. The first principle component describes 40.7% of the variance in the data set, while the second describes 25.5%.

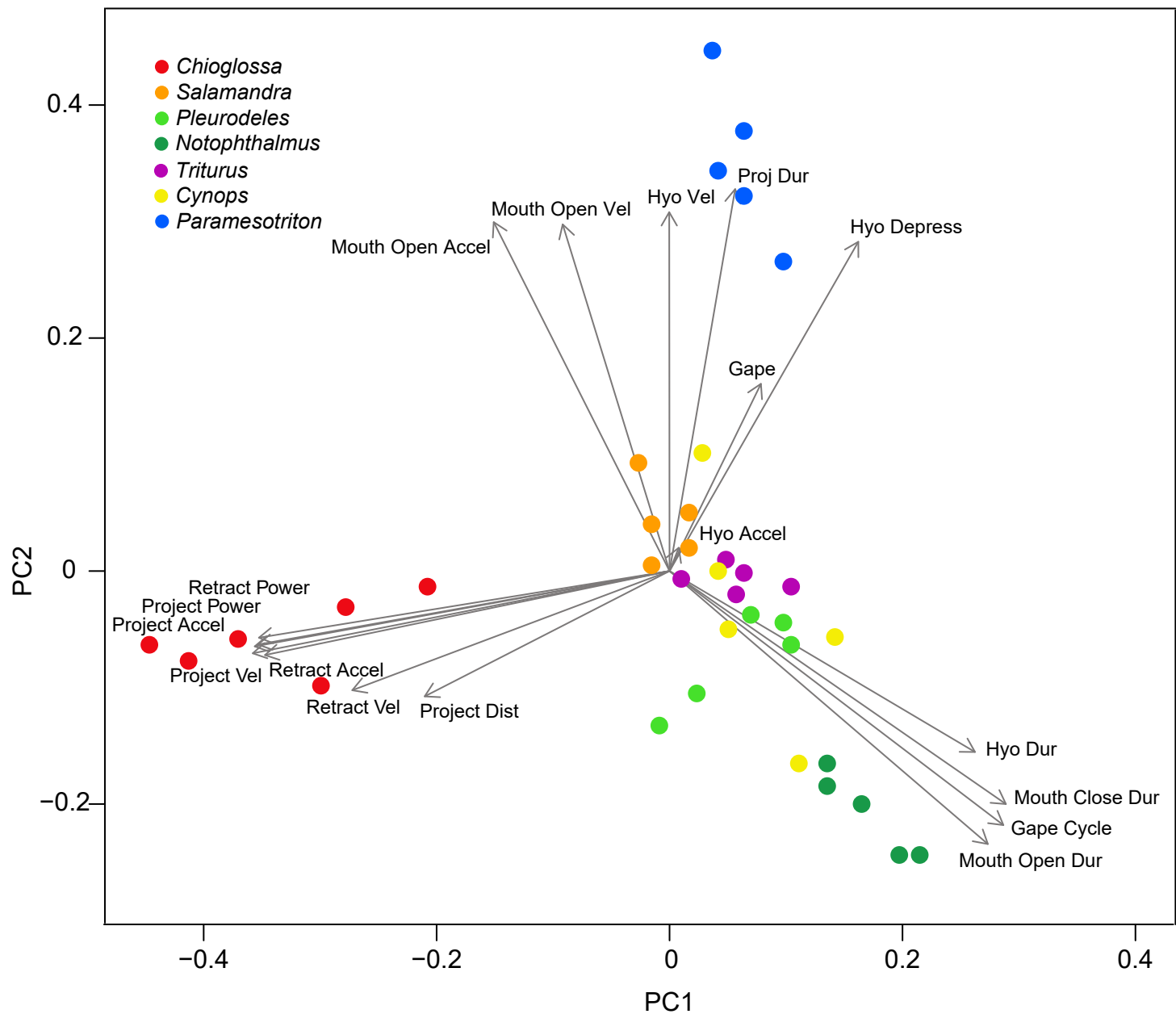


Figure S2. Principle components analysis of kinematic and performance data. Indications as in figure S1 (*Chioglossa*, N=6; *Salamandra*, *Pleurodeles*, *Notophthalmus*, *Triturus*, *Cynops*, and *Paramesotriton*, N=5). The fully terrestrial salamandrid, *Chioglossa*, has the greatest tongue-projection performance, and is distinct from the other salamandrids in principle components space. Compared to most semi-aquatic and aquatic newts, *Salamandra* shows specializations for terrestrial feeding, however, it is not as extreme as *Chioglossa*. The fully aquatic newt, *Paramesotriton*, also forms a distinct cluster with the greatest mouth opening velocity and acceleration, and hyobranchial depression velocity, suggesting specializations for aquatic feeding. The first principle component describes 40.5% of the variance in the data set, while the second describes 26%.

Table S1. Post hoc analyses summarizing adjusted p-values of morphological measurements between species.

[Click here to Download Table S1](#)

Table S2. Post-hoc analyses summarizing adjusted p-values of terrestrial feeding kinematics between species.

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