



The scaling of tongue projection in the veiled chameleon, *Chamaeleo calytratus*



Anthony Herrel^{a,b,*}, Chrystal L. Redding^c, J. Jay Meyers^c, Kiisa C. Nishikawa^c

^a Département d'Ecologie et de Gestion de la Biodiversité, UMR 7179 C.N.R.S/M.N.H.N., 57 rue Cuvier, Case postale 55, 75231 Paris Cedex 5, France

^b Evolutionary Morphology of Vertebrates, Ghent University, K.L. Ledeganckstraat 35, 9000 Gent, Belgium

^c Department of Biological Sciences, Northern Arizona University, PO Box 5640, Flagstaff, AZ 86011, USA

ARTICLE INFO

Article history:

Received 21 August 2013

Received in revised form

13 December 2013

Accepted 25 January 2014

Available online 19 March 2014

Keywords:

Feeding kinematics

Feeding apparatus

Prey capture

Lizard

ABSTRACT

Within a year of hatching, chameleons can grow by up to two orders of magnitude in body mass. Rapid growth of the feeding mechanism means that bones, muscles, and movements change as chameleons grow while needing to maintain function. A previous morphological study showed that the musculoskeletal components of the feeding apparatus grow with negative allometry relative to snout–vent length (SVL) in chameleons. Here, we investigate the scaling of prey capture kinematics and muscle physiological cross-sectional area in the veiled chameleon, *Chamaeleo calytratus*. The chameleons used in this study varied in size from approximately 3 to 18 cm SVL (1–200 g). Feeding sequences of 12 chameleons of different sizes were filmed and the timing of movements and the displacements and velocities of the jaws, tongue, and the hyolingual apparatus were quantified. Our results show that most muscle cross-sectional areas as well as tongue and hyoid mass scaled with isometry relative to mandible length, yet with negative allometry relative to SVL. Durations of movement also scaled with negative allometry relative to SVL and mandible length. Distances and angles generally scaled as predicted under geometric similarity (slopes of 1 and 0, respectively), while velocities generally scaled with slopes greater than 0 relative to SVL and mandible length. These data indicate that the velocity of jaw and tongue movements is generally greater in adults compared to juveniles. The discrepancy between the scaling of cross-sectional areas versus movements suggests changes in the energy storage and release mechanisms implicated in tongue projection.

© 2014 Elsevier GmbH. All rights reserved.

1. Introduction

An understanding of scaling is paramount to our understanding of how growth may constrain animal function (McMahon, 1973; Schmidt-Nielsen, 1984). Many animals change in shape as they increase in body size from birth to adulthood, as is the case in many mammals and birds (Carrier, 1983, 1995; Carrier and Leon, 1990; Trillmich et al., 2003; Genbrugge et al., 2011; Herrel et al., 2012). However, the simplest model of growth is geometric similarity in which morphological elements grow in direct proportion to each other without changes in shape (Hill, 1950). This type of growth is commonly observed in ectothermic organisms including fishes (Richard and Wainwright, 1995; Cook, 1996), amphibians (Birch, 1999), and reptiles (Toro et al., 2003; Pfaller et al., 2011;

Anderson et al., 2012). If animals increase in size without changing shape then this has functional consequences. Indeed, based on this premise, Hill (1950) predicted that in geometrically similar animals displacements would be proportional to length, velocities would be independent of changes in overall size, and accelerations would decrease linearly with body length. Given the importance of understanding how growth affects function, many studies have examined the scaling of the musculo-skeletal system in animals (e.g., Katz and Gosline, 1993; Wainwright and Richard, 1995; Ferry-Graham, 1998; Hernandez, 2000; Meyers et al., 2002; Powell et al., 2002; Herrel et al., 2011).

In addition to being of interest from a purely mechanical perspective, understanding the changes in function with changes in size during growth is important from an evolutionary perspective. Indeed, selection is often strongest on early life-history stages as these are at a disadvantage given their small size relative to competitors or predators (Carrier, 1996; Herrel and Gibb, 2006). Indeed, although each size class has its own predators, juveniles are typically eaten by all. Consequently it has been observed that juveniles compensate for their small absolute size by showing

* Corresponding author at: Département d'Ecologie et de Gestion de la Biodiversité, UMR 7179 C.N.R.S/M.N.H.N., 57 rue Cuvier, Case postale 55, 75231 Paris Cedex 5, France. Tel.: +33 140798120; fax: +33 140793773.

E-mail address: anthony.herrel@mnhn.fr (A. Herrel).

disproportionate levels of performance (see review in Herrel and Gibb, 2006). In many ballistic systems the power for rapid movements is often derived from elastic energy storage (De Groot and Van Leeuwen, 2004; Anderson and Deban, 2010; Deban and Lappin, 2011; Deban and Richardson, 2011). In those cases, the performance of the system is principally related to non-muscular components as elastic tissues can be loaded slowly and thus do not impose constraints upon contraction velocity or muscle power generation. As such, these types of systems should allow juveniles to perform at levels similar to, or outperform, adults as is suggested by studies of jumping (Toro et al., 2003; Carrier, 1995).

Here, we investigate the scaling of feeding kinematics in chameleons. Chameleon feeding is unique, not only because they have a unique mechanism of ballistic tongue projection (Zoond, 1933; Gans, 1967; Bell, 1989, 1990; Wainwright et al., 1991; Wainwright and Bennett, 1992a,b,c), but also because of the rapid growth observed during their first year of life. Many species are short-lived and need to grow to adulthood within the first year of life (e.g., Karsten et al., 2008). The energetic constraints on rapid growth are strong and thus juveniles need to be able to capture prey effectively. As such, constraints on prey capture performance are likely strong, and juveniles may need to perform at levels similar to those of adults (e.g., project the tongue as fast as adults do in absolute terms) to maximize energy intake and growth. A recent study on the scaling of the anatomy of the ballistic tongue apparatus in chameleons indicated that juveniles have a proportionately larger feeding apparatus than adults (Anderson et al., 2012). Therefore, we predict that juvenile chameleons will show levels of performance similar to those observed in adults with absolute velocities being similar and tongue projection distances being greater relative to body size, as those two elements likely determine prey capture success. However, whether this is also the case when exploring the scaling of tongue projection relative to head size remains unclear. As such, we here examine the scaling of tongue projection both relative to overall body size and mandible length. Finally, we also analyze the scaling of the force generation capacity of the principal hyolingual muscles to better understand the observed kinematic patterns. Our predictions follow Hill (1950) in predicting that (i) angles and velocities should be independent of body size, (ii) linear

dimensions, distances, and durations should scale with a slope of 1, and (iii) surface areas should scale with a slope of 2.

2. Materials and methods

Chamaeleo calypttratus were purchased from commercial animal vendors and were housed in sealed wooden cages with wire screen on two sides. Each cage contained a live plant (*Ficus* sp.) and additional branches for climbing. Water was provided by misting the animals daily and a bowl with a drip system was also provided. All cages had an incandescent light as well as a heat lamp set on a 11:13 h light:dark cycle. Chameleons were kept on a diet of crickets dusted with calcium three times a week. During summer months, chameleons were offered a variety of locally collected insects. Our growth series for the kinematic analysis was derived from chameleons bred in the lab for the purpose of this study. All experiments were approved by the Animal Care and Use Committee at Northern Arizona University.

2.1. Morphological analysis

To quantify the ontogenetic changes of the feeding system in *C. calypttratus*, we measured the tongue and hyoid muscles involved in tongue projection in preserved specimens from the private collection of the first author. Tongue and hyolingual muscles were removed from 17 specimens ranging in size from 21 to 193 mm snout–vent length (SVL). The terminology of the muscles follows Gnanamuthu (1930) and Herrel et al. (2001).

Intact muscle lengths (prior to removal) were measured whenever possible. Muscles that had been removed from the specimen were measured only if the muscle origin and insertion were intact following removal. Given that we were unable to dissect muscles in all individuals without damaging them, sample sizes differ for each muscle (Table 1). Muscles were blotted dry to remove excess alcohol and then weighed. For each muscle, right and left sides were weighed together and their weight was divided by 2 to obtain an average muscle weight. Small muscles were weighed on a Mettler-Toledo AT21 comparator scale (Mettler-Toledo, Greifensee, Switzerland), accurate to 0.001 mg.

Table 1

Scaling relationships of the \log_{10} -transformed physiological cross-sectional area (PCSA in cm^2) of the hyolingual musculature of *Chamaeleo calypttratus* relative to \log_{10} -transformed snout–vent length and mandible length as well as results of a *t*-test testing for differences between the observed and predicted slope (P_{gs}).

	R	P-value	Intercept	Slope	P_{gs}
Mandible length					
Tongue + accelerator mass (g, $N=17$)	0.95	<0.001	−4.67	3.01 ± 0.27	0.88
Hyoid mass (g, $N=17$)	0.97	<0.001	−5.51	3.07 ± 0.21	0.20
m. intermandibularis anterior (cm^2 , $N=9$)	0.81	0.008	−4.60	1.79 ± 0.49	0.26
m. intermandibularis posterior (cm^2 , $N=8$)	0.91	0.002	−4.53	1.76 ± 0.34	0.10
m. mandibulohyoideus II (cm^2 , $N=13$)	0.96	<0.001	−5.56	2.01 ± 0.19	0.86
m. mandibulohyoideus I (cm^2 , $N=14$)	0.89	<0.001	−4.86	1.89 ± 0.28	0.18
m. sternohyoideus superficialis (cm^2 , $N=15$)	0.88	<0.001	−3.95	1.36 ± 0.20	<0.001
m. sternohyoideus profundus (cm^2 , $N=14$)	0.76	0.001	−4.27	1.38 ± 0.34	<0.001
m. accelerator (cm^2 , $N=4$)	0.99	0.01	−3.97	2.09 ± 0.24	0.56
Snout–vent length					
Tongue + accelerator mass (g, $N=17$)	0.96	<0.001	−5.95	2.70 ± 0.20	<0.001
Hyoid mass (g, $N=17$)	0.98	<0.001	−6.70	2.69 ± 0.16	<0.001
m. intermandibularis anterior (cm^2 , $N=9$)	0.79	0.01	−5.14	1.50 ± 0.44	0.01
m. intermandibularis posterior (cm^2 , $N=8$)	0.92	0.001	−5.18	1.54 ± 0.26	0.002
m. mandibulohyoideus II (cm^2 , $N=13$)	0.94	<0.001	−6.26	1.74 ± 0.20	<0.001
m. mandibulohyoideus I (cm^2 , $N=14$)	0.90	<0.001	−5.60	1.68 ± 0.24	<0.001
m. sternohyoideus superficialis (cm^2 , $N=15$)	0.87	<0.001	−4.46	1.19 ± 0.19	<0.001
m. sternohyoideus profundus (cm^2 , $N=14$)	0.72	0.003	−5.08	1.34 ± 0.37	<0.001
m. accelerator (cm^2 , $N=4$)	0.99	0.007	−4.72	1.83 ± 0.15	0.14

Bold type indicates variables that show a slope not significantly different from predictions of geometric similarity (2 for cross-sectional areas and 3 for mass). Note that whereas the m. intermandibularis, the m. mandibulohyoideus and the m. accelerator all play a role in tongue protrusion and projection, the m. sternohyoideus is principally a hyoid retractor. Yet, these muscles play an important role in the triggering of tongue projection by accelerating the hyoid forward at the end of tongue protraction through the posterior rotation of the ceratobranchial relative to the entoglossal process.

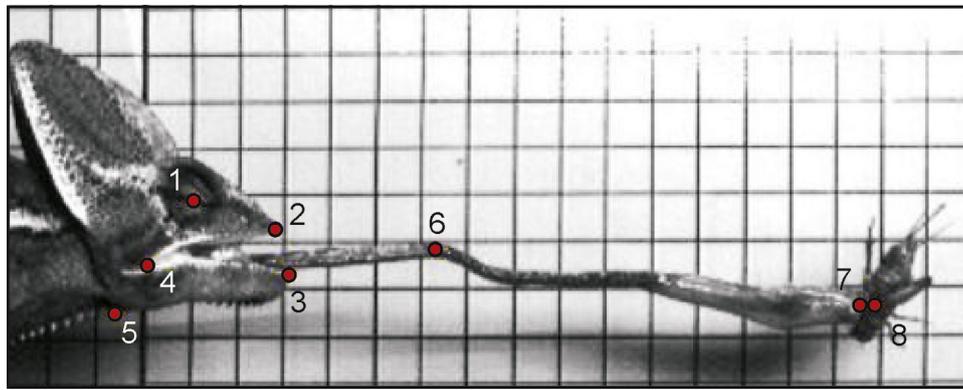


Fig. 1. Landmarks digitized as illustrated for a feeding sequence of an adult male *Chamaeleo calyptratus*. 1, eye; 2, upper jaw tip; 3, lower jaw tip; 4, jaw vertex; 5, posterior mandible; 6, hyoid tip; 7, tongue tip; 8, prey item.

Large muscles were weighed on a Fisher Scientific A-160 scale (Thermo Fisher Scientific, Waltham, MA, USA), accurate to 0.1 mg. Although dehydration due to the preservation in ethanol may have affected the mass of the muscles, the relative water loss will be the same in all muscles and thus scaling patterns should remain unaffected.

The length of the musculus (m.) intermandibularis anterior was measured from its insertion on the mid-line raphe to its origin on

the inner side of the mandible. The m. intermandibularis posterior was measured from the posterior side of the lower jaw to its insertion on the mid-line raphe. The m. mandibulohyoideus II was measured from the jaw symphysis to the posterior ventral end of the ceratohyal. The m. mandibulohyoideus I muscle was removed, flattened, and measured from origin to insertion. The m. sternohyoideus superficialis was measured from the ventral side of the sternum to the distal posterior ventral side of the hyoid. The m.

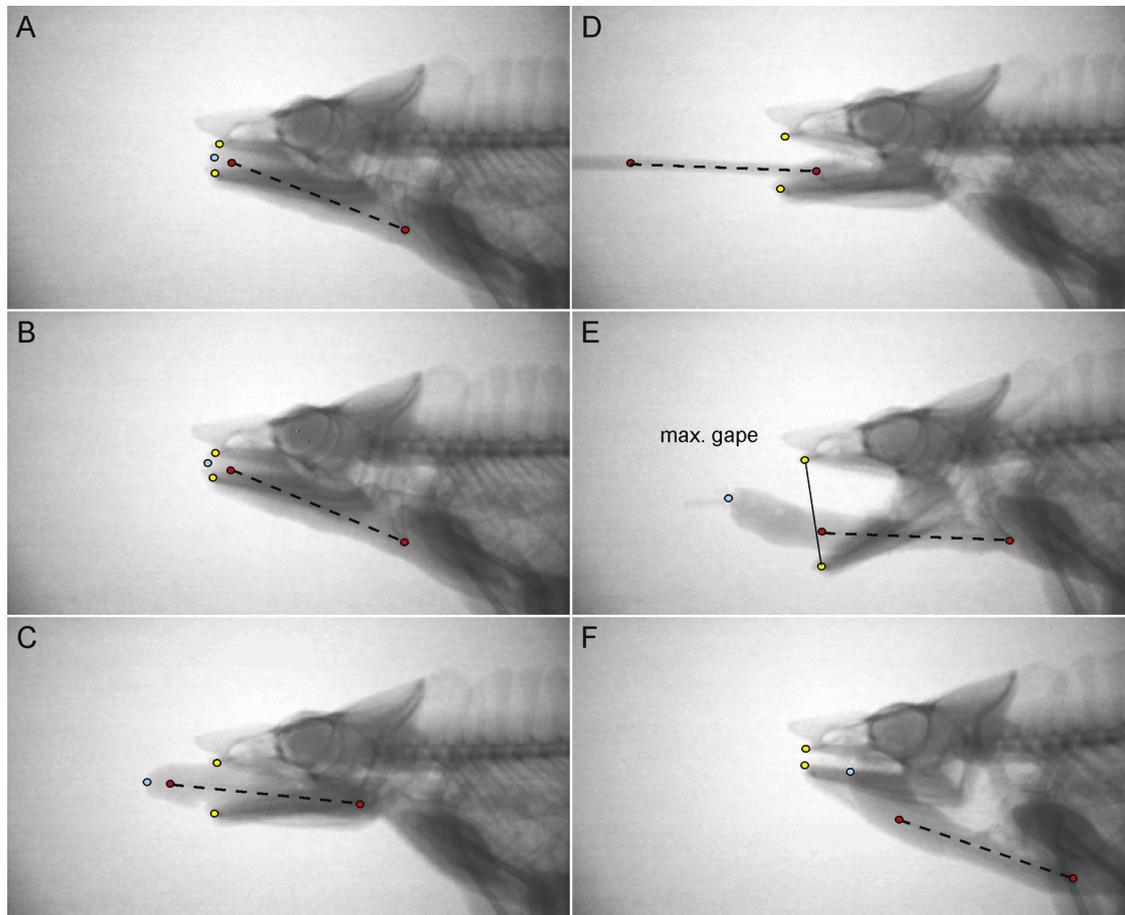


Fig. 2. Selected frames from an X-ray video recording of prey capture in a *Chamaeleo melleri* to illustrate the different kinematic phases. The yellow dots represent the jaw markers, the blue dot the tip of the tongue and the two red dots with the dashed line between them illustrate the position of the entoglossal process. From (A) to (B) a slight protrusion of the tongue and hyoid within the mouth cavity can be observed. This phase is defined as *protraction*. From (B) to (C) the tongue and hyoid tips move beyond (i.e., anterior to) the jaws. This phase is defined as *protrusion*. From (C) to (D) the tongue is projected off the hyoid and these events define the *projection* phase. From (D) to (E) the jaws open maximally to allow the retraction of the tongue with *maximal gape* being illustrated in (E) and coinciding with the retraction of the hyoid to within the jaw margins. From (D) to (F) the tongue is retracted and this phase is called *retraction*. For the timing of the different kinematic events we refer to Figs. 3 and 4.

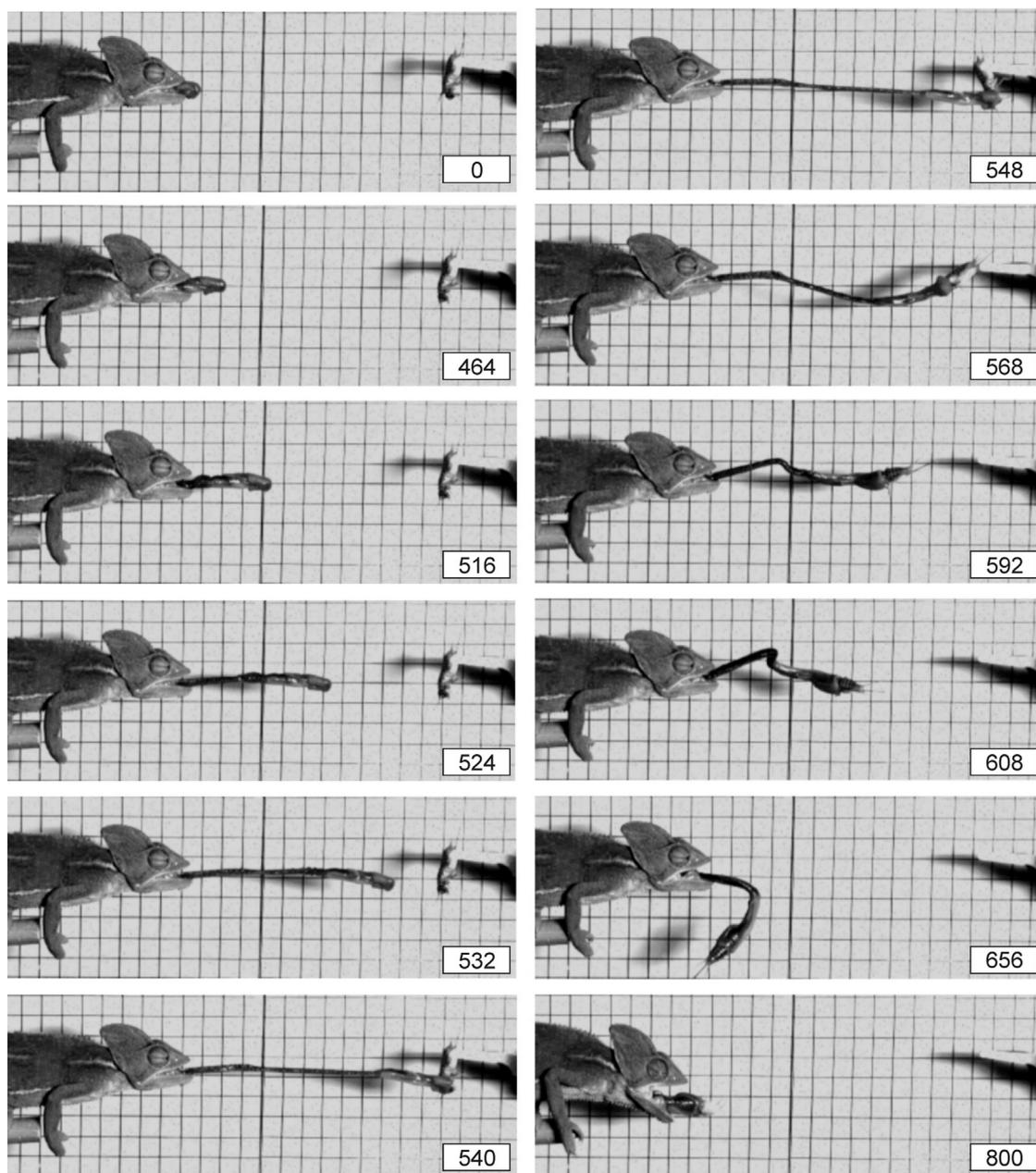


Fig. 3. Representative feeding sequence of an adult female *Chamaeleo calytratus*. In this sequence, tongue projection to prey contact occurs in about 40 ms. The time in ms is given in the bottom right of each frame. The background grid is composed of 5 mm squares.

sternohyoideus profundus was removed and the length of the muscle was measured. The physiological cross-sectional area of the m. hyoglossus was not included due to the difficulty of estimating its length in preserved specimens where the muscle is tightly coiled and retained by connective tissue. For the m. accelerator we measured the actual fiber length obtained after nitric acid digestion (see Herrel et al., 2001) in four individuals (Table 1).

Using mass and muscle length data, the physiological cross-sectional area (PCSA) was calculated by multiplying mass (g) by muscle density (1.06 g/cm^3 ; Mendez and Keys, 1960) and dividing it by muscle length or fiber length (cm). Note that we used whole muscle length for most muscles as the long strap-like and parallel-fibered muscles of the hyobranchial system are composed of fibers in series. As such, the use of fiber lengths would lead to an overestimate of the actual cross-sectional area.

2.2. Kinematic analyses

After emerging from the egg, *C. calytratus* weigh $\leq 1.0 \text{ g}$ and have an SVL of approximately 3 cm. Within three days of hatching, these juveniles perform prey capture. Feeding behavior of 12 *C. calytratus* ranging in size from 3.3 to 18 cm SVL was recorded using a digital high-speed camera (Redlake MotionScope; DEL Imaging Systems, Cheshire, CT, USA) set at 250 frames per second. Although this recording speed is relatively low, it allowed us to capture the feeding events accurately, with the fastest event (tongue projection) being composed of at least five frames in the single fastest prey capture across all trials. Animals were placed on a wooden dowel in front of a 1 cm background grid. Prey size was varied with chameleon size; chameleons ranging from 3 to 6 cm SVL were given 6 mm crickets, chameleons ranging from 6 to 12 cm SVL were given 12 mm crickets, and chameleons ranging from 12 to 18 cm SVL were

given 24 mm crickets. Crickets were held with flexible insect forceps approximately one SVL (± 1 cm) in front of the chameleon. Only successful prey capture sequences in which chameleons remained perpendicular to the camera were used. To determine that animals were positioned in the plane perpendicular to the camera we measured head length and only recordings where this variable did not change by more than 1% were used. At least three sequences were recorded for each individual and the maximal performance was retained for analysis. Note that maximum performance for different traits (e.g., distance or velocity) did not always come from the same sequence. Thus, whereas peak velocity may have been observed in the first recording, peak distance may have occurred in the third feeding event recorded.

Video files were converted into image sequences using QuickTime (Apple Inc., Cupertino, CA, USA) and were then analyzed using Didge v. 2.02 (A. Cullum, Creighton University, Omaha, NE, USA). On each frame, the X and Y coordinates of eight points were digitized: (1) the eye, (2) upper jaw, (3) lower jaw, (4) jaw vertex (note that the jaw vertex does not represent an osteological landmark but the fleshy corner of the mouth where upper and lower jaws meet), (5) posterior mandible, (6) hyoid tip, (7) tongue tip, and (8) prey item (Fig. 1). Because the tip of the entoglossal process is difficult to see before tongue projection, its position was estimated by the tongue tip until the tongue was projected. Three feeding sequences were digitized for each individual. Raw displacement profiles were smoothed using a zero phase shift, fourth-order low pass Butterworth digital filter at 10 Hz (Winter, 1990). Velocities were calculated from the filtered displacement data by numerical differentiation.

The different tongue and hyoid movements were defined as follows (Figs. 2–4): (i) protraction is defined as forward movement of the tongue and hyoid inside the mouth; (ii) protrusion is defined as slow forward movement of the tongue and hyoid beyond the mandible tip; and (iii) projection is the ballistic movement of the tongue as it is propelled off the hyoid (Meyers and Nishikawa, 2000). The following timing events, angles, and distances were calculated for each prey capture event: (1) the duration of mouth opening, calculated from the onset of mouth opening to maximal gape; (2) the duration of hyoid protraction, calculated from the onset of hyoid protraction to peak hyoid protraction; (3) the duration of hyoid protrusion from the onset to peak hyoid protrusion; (4) the duration of tongue projection; (5) the duration of tongue retraction calculated as the time between prey contact and the end of the retraction of the tongue between the jaws; (6) the duration of tongue recovery as the time from prey contact to complete mouth closing; (7) the duration of the feeding sequence from the onset of mouth opening to the end of mouth closing; (8) the duration of mouth closing from peak gape to complete mouth closure; (9) maximum gape angle calculated as the peak angle formed by the upper jaw tip, the jaw vertex, and the lower jaw tip; (10) maximal gape distance as the greatest distance between the upper jaw tip and the lower jaw tip; (11) maximum tongue reach as the greatest distance between the lower jaw tip and the tongue tip; (12) distance to prey as the distance between the lower jaw tip and the prey item at the onset of tongue projection; (13) the time to maximal hyoid protraction; (14) the time to prey contact; (15) the time to maximal tongue projection; (16) the time to maximal gape; (17) the maximal mouth opening velocity; (18) the maximal mouth closing velocity; (19) the maximal tongue projection velocity; (20) the maximal tongue retraction velocity; (21) the maximal hyoid protraction velocity; (22) the maximal hyoid retraction velocity.

2.3. Statistical analyses

Slopes, intercepts, *P*-values, and confidence intervals were calculated using ordinary least squares regression in SPSS v. 20 (IBM

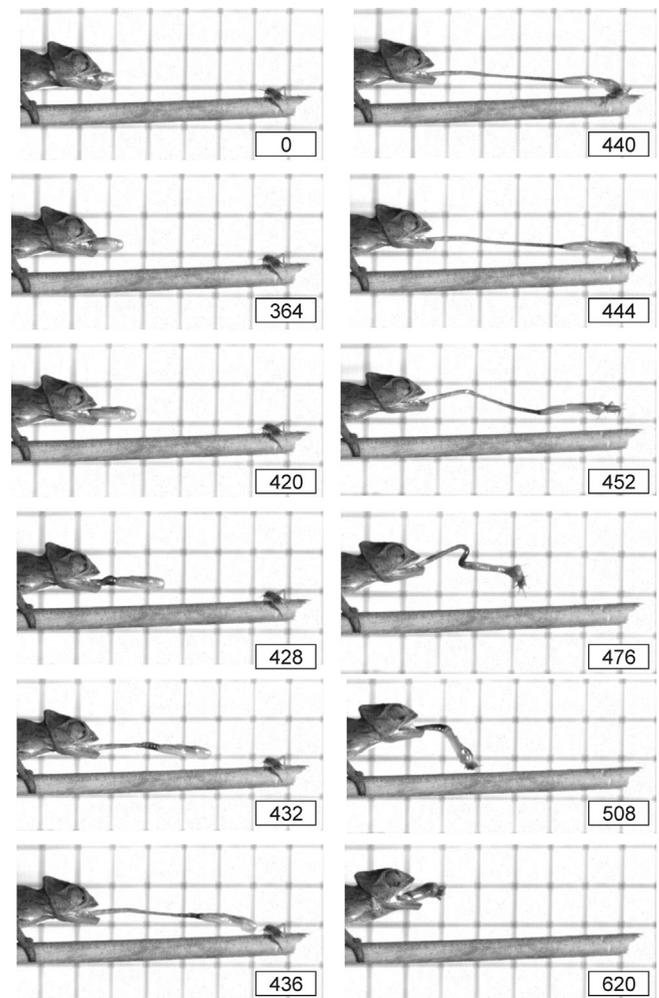


Fig. 4. Representative feeding sequence of a juvenile male *Chamaeleo calyptratus*. The time from tongue projection to prey contact is 24 ms. The time in ms is given in the bottom right of each frame. The background grid is composed of 5 mm squares.

Corp., Armonk, NY, USA). All morphological and kinematic variables were \log_{10} -transformed and regressed against \log_{10} of mandible length and SVL. The null hypothesis (isometric growth versus changes in snout–vent length or mandible length) was tested using a two-sided *t*-test (Sokal and Rohlf, 1995). Predictions for cross-sectional areas were that the area should scale with a slope of 2; for durations, the timing of events, and distances the predicted slope is 1; for angles and velocities the predicted slope equals 0 (Hill, 1950). Scaling patterns that correspond to predictions of geometric similarity were considered as showing isometry; deviations from the slopes predicted under geometric similarity are referred to as being allometric.

3. Results

3.1. Tongue and hyoid mass

Whereas tongue and hyoid mass scaled isometrically with mandible length, they scaled with negative allometry relative to SVL (Table 1).

3.2. Cross-sectional areas

With a few exceptions, PCSAs of the muscles were found to scale with isometry relative to mandible length (Table 1; Fig. 5).

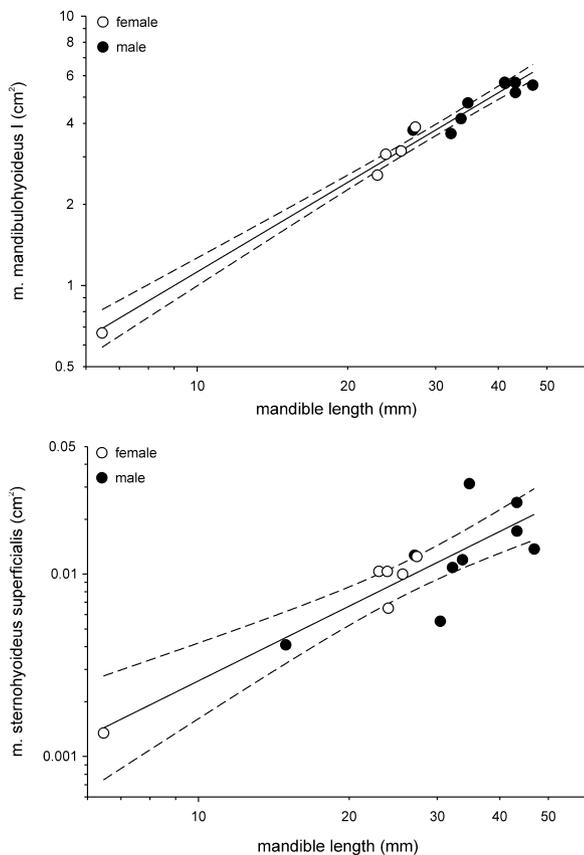


Fig. 5. Graphs illustrating the scaling of the physiological cross-sectional areas of a hyoid protractor (m. mandibulothyoideus I, top) and a hyoid retractor (m. sternohyoideus superficialis, bottom). Full lines indicate actual regression slopes with 95% confidence limits. Sexes are indicated in different colors (female = white; male = black). Note the log-scale on the axes.

Those that scaled as predicted under geometric similarity were the m. intermandibularis anterior and posterior, the m. mandibulothyoideus I and II, and the m. accelerator. The PCSA of the mm. sternohyoideus superficialis and profundus scaled with negative

Table 2
Scaling relationships for kinematic measurements of *Chamaeleo calyptrotus* ($N = 12$). Results of a linear regression of \log_{10} -transformed kinematic variables on \log_{10} mandible length as well as results of a t -test testing for differences between the observed and predicted slope (P_{gs}).

Kinematic variables	R	P	Intercept	Slope	P_{gs}
Duration mouth opening (ms)	0.16	0.61	3.06	0.12 ± 0.23	<0.001
Duration hyoid protraction (ms)	0.11	0.75	2.84	0.08 ± 0.23	<0.001
Duration of tongue protrusion (ms)	0.23	0.46	2.66	0.23 ± 0.30	<0.001
<u>Duration tongue projection (ms)</u>	<u>0.78</u>	<u>0.003</u>	<u>0.95</u>	<u>0.45 ± 0.11</u>	<u><0.001</u>
<u>Duration tongue retraction (ms)</u>	<u>0.76</u>	<u>0.004</u>	<u>1.81</u>	<u>0.32 ± 0.09</u>	<u><0.001</u>
Duration recovery (ms)	0.51	0.09	2.26	0.26 ± 0.14	<0.001
Duration feeding sequence (ms)	0.19	0.55	3.06	0.14 ± 0.23	<0.001
Duration mouth closing (ms)	0.90	<0.001	1.15	0.54 ± 0.08	<0.001
Max. gape angle (degrees)	0.15	0.64	1.66	0.04 ± 0.07	0.08
Gape distance (mm)	0.98	<0.001	-1.13	0.97 ± 0.06	0.12
Tongue reach (mm)	0.97	<0.001	-0.37	1.02 ± 0.09	0.47
Prey distance (mm)	0.98	<0.001	-0.47	1.07 ± 0.06	0.002
Time to max. hyoid protraction distance (mm)	0.17	0.61	3.23	-0.13 ± 0.25	<0.001
Time to prey contact (ms)	0.17	0.60	3.23	-0.13 ± 0.25	<0.001
Time to max. tongue protraction (ms)	0.17	0.61	3.23	-0.13 ± 0.25	<0.001
Time to max. gape (ms)	0.06	0.85	3.21	-0.04 ± 0.19	<0.001
Max. mouth opening velocity (m/s)	0.51	0.09	-0.42	0.52 ± 0.28	<0.001
Max. mouth closing velocity (m/s)	0.64	0.03	-0.11	0.72 ± 0.28	<0.001
Max. tongue projection velocity (m/s)	0.44	0.15	1.66	0.18 ± 0.12	<0.001
Max. tongue retraction velocity (m/s)	0.71	0.01	0.92	0.44 ± 0.14	<0.001
Max. hyoid protraction velocity (m/s)	0.43	0.17	0.21	0.35 ± 0.24	<0.001
Max. hyoid retraction velocity (m/s)	0.16	0.62	0.51	0.12 ± 0.24	0.13

Underlined variables are those that increase with an increase in mandible length; bold type indicates variables that show a slope not significantly different from predictions of geometric similarity (1 for durations, times to kinematic events and distances; 0 for angles and velocities). Max., maximal.

allometry and showed slopes smaller than 2 (see Table 1). Relative to SVL all muscles with the exception of the m. accelerator scaled with a slope smaller than 2 (Table 1).

3.3. Kinematic variables

Most of the duration and timing variables did not change with changes in mandible or snout-vent length (Tables 2 and 3; Figs. 6 and 7). Only the duration of tongue projection, the duration of tongue retraction and the duration of mouth closing increased with increasing mandible and body size, yet with slopes smaller than the predicted slope of 1. This indicates that larger chameleons are relatively slower than predicted during the phases of tongue projection and retraction. Gape distance, tongue reach and prey distance also increased with size (Tables 2 and 3; Fig. 6). Whereas these variables scaled with slight negative allometry relative to SVL, slopes relative to mandible length were not different from 1. The velocity of tongue and jaw movement increased with an increase in body size with a slope greater than predicted (Tables 2 and 3). However, hyoid retraction velocity did not change with changes in mandible or SVL and scaled as predicted under geometric similarity (Tables 2 and 3).

4. Discussion

Our data did not accord with our predictions as in our data set larger animals were generally faster than smaller animals. Yet, the scaling of cross-sectional areas and tongue mass did not deviate from the predictions under a geometric scaling model. Although data on the scaling of muscle cross-sectional areas are scarce, previous studies have suggested that muscles associated with the feeding system scale with positive allometry (e.g., Herrel et al., 2005) or isometry (Herrel et al., 2008). Our results on the scaling of physiological cross-sectional areas relative to mandible length suggest that PCSA increases with size with a slope that is not significantly different from 2, as predicted by geometric similarity. Only the mm. sternohyoideus superficialis and profundus scaled with slopes less than 2, thus deviating significantly from predictions of geometric similarity. However, relative to SVL all muscles with exception of the m. accelerator scaled with negative

Table 3

Scaling relationships for kinematic measurements of *Chamaeleo calytratus* (N = 12). Results of a linear regression of log₁₀-transformed kinematic variables on log₁₀ snout–vent length as well as results of a t-test testing for differences between the observed and predicted slope.

Kinematic variables	R	P	Intercept	Slope	P _{gs}
Duration mouth opening (ms)	0.19	0.56	2.97	0.13 ± 0.21	<0.001
Duration hyoid protraction (ms)	0.16	0.63	2.74	0.10 ± 0.21	<0.001
Duration of tongue protrusion (ms)	0.26	0.41	2.51	0.23 ± 0.27	<0.001
Duration tongue projection (ms)	0.80	0.002	0.74	0.41 ± 0.10	<0.001
Duration tongue retraction (ms)	0.75	0.005	1.68	0.29 ± 0.08	<0.001
Duration recovery (ms)	0.51	0.09	2.15	0.23 ± 0.12	<0.001
Duration feeding sequence (ms)	0.22	0.50	2.96	0.14 ± 0.20	<0.001
Duration mouth closing (ms)	0.90	<0.001	0.92	0.48 ± 0.08	<0.001
Max. gape angle (degrees)	0.12	0.70	1.66	0.026 ± 0.07	0.24
Gape distance (mm)	0.99	<0.001	-1.56	0.88 ± 0.05	<0.001
Tongue reach (mm)	0.98	<0.001	-0.84	0.94 ± 0.06	0.007
Prey distance (mm)	0.99	<0.001	-0.94	0.97 ± 0.05	0.07
Time to max. hyoid protraction distance (mm)	0.12	0.71	3.22	-0.09 ± 0.22	<0.001
Time to prey contact (ms)	0.12	0.71	3.22	-0.09 ± 0.23	<0.001
Time to max. tongue protraction (ms)	0.12	0.71	3.22	-0.09 ± 0.23	<0.001
Time to max. gape (ms)	0.02	0.96	3.18	-0.01 ± 0.17	<0.001
Max. mouth opening velocity (m/s)	0.46	0.13	-0.56	0.42 ± 0.26	<0.001
Max. mouth closing velocity (m/s)	0.61	0.04	-0.37	0.62 ± 0.26	<0.001
Max. tongue projection velocity (m/s)	0.42	0.18	1.60	0.16 ± 0.11	<0.001
Max. tongue retraction velocity (m/s)	0.69	0.01	0.76	0.38 ± 0.13	<0.001
Max. hyoid protraction velocity (m/s)	0.40	0.20	0.09	0.30 ± 0.22	<0.001
Max. hyoid retraction velocity (m/s)	0.11	0.72	0.51	0.08 ± 0.22	0.26

Underlined variables are those that increase with an increase in snout–vent length; bold type indicates variables that show a slope not significantly different from predictions of geometric similarity (1 for durations, times to kinematic events and distances; 0 for angles and velocities). Max., maximal.

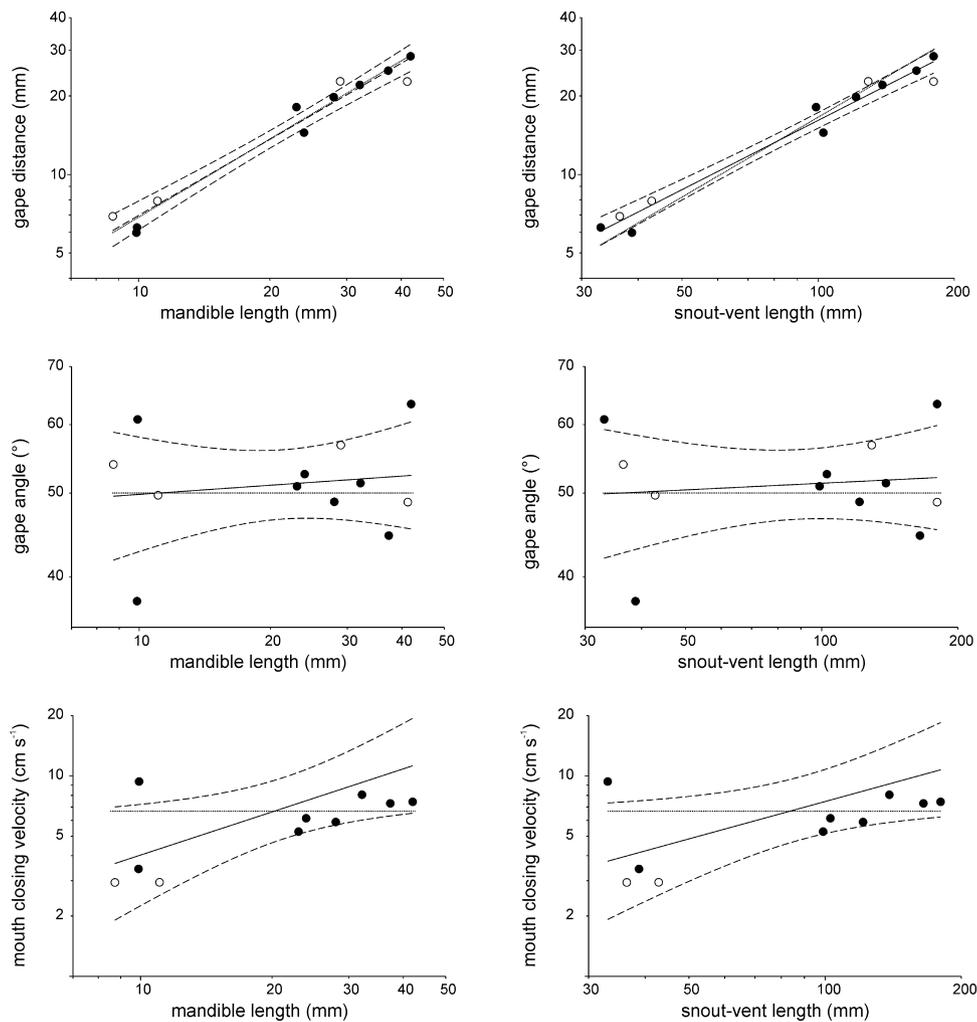


Fig. 6. Selected graphs illustrating the scaling of gape distance (top), gape angle (middle), and mouth closing velocity (bottom). On the left the scaling relationships relative to mandible length are illustrated; on the right the scaling relationships relative to snout–vent length are illustrated. Dotted lines illustrate predicted slopes under geometric similarity; full lines indicate actual regression slopes with 95% confidence limits. Sexes are indicated in different colors (female = white; male = black). Note the log-scale on the axes.

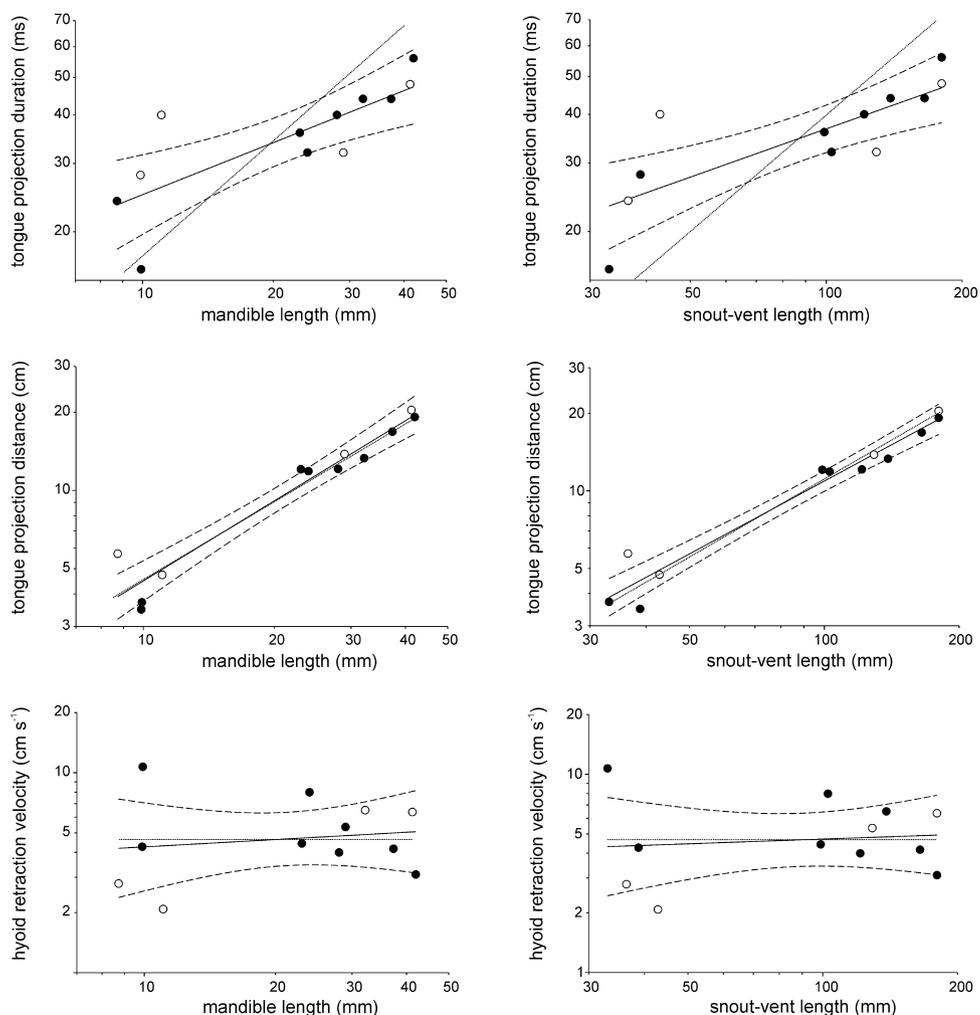


Fig. 7. Selected graphs illustrating the scaling of tongue projection duration (top), tongue projection distance (middle), and hyoid retraction velocity (bottom). On the left the scaling relationships relative to mandible length are illustrated; on the right the scaling relationships relative to snout-vent length are illustrated. Dotted lines illustrate predicted slopes under geometric similarity; full lines indicate actual regression slopes with 95% confidence limits. Sexes are indicated in different colors (female = white; male = black). Note the log-scale on the axes.

allometry. A recent interspecific study of morphological scaling in chameleons (Anderson et al., 2012) showed that muscle masses and the linear dimensions of the cranial elements generally scaled with negative allometry relative to SVL. Thus, it seems intuitive that cross-sectional areas would also increase with negative allometry as observed here. However, the muscles involved in the protraction of the hyoid and the projection of the tongue scaled isometrically relative to jaw size, yet is greater in juveniles compared to adults relative to overall body size, at least in this species. This may be important as constraints on projection velocity and distance are likely strong. By having large trophic structures and strong associated muscles directly after birth, young chameleons may in part offset the disadvantages of their small body size. The retraction of the hyolingual system after prey capture appears less constrained given the negative allometry of the hyoid retractors. This may be due to the fact that during retraction the prey has already been trapped by the powerful suction mechanism of the chameleon tongue (Herrel et al., 2000). Given that constraints on muscular performance for muscles involved in prey capture are likely strong, the negative allometry observed here for the hyoid retractors is presumably rather rare and

only made possible by the evolution of the unique prey prehension system in chameleons (Herrel et al., 2000). This may explain the fact that hyoid retraction velocity did not change with changes in body size, according to the predictions of geometric similarity.

Our kinematic analysis indicates that durations and timing-related variables are generally invariant during growth or increase with slopes much lower than the predicted slope of 1, thus deviating from predictions of geometric similarity (Hill, 1950; Pennycuik, 1992). The significant increase of mouth closing duration with jaw and mandible is, however, likely the direct result of the increase in tongue retraction time with body size as the mouth cannot be fully closed until the tongue has been fully retracted into the mouth. Tongue reach and prey distance increased with a slope of 1 relative to mandible and body length, respectively, which may explain the longer duration of tongue projection and retraction; the further the prey is from the mouth, the further the tongue is projected and the longer the duration of projection and retraction.

Most movement velocities had slopes significantly different from 0, indicating that animals get faster as they get bigger. This is somewhat surprising given the isometric scaling of the cross-sectional areas of the hyolingual musculature and tongue mass. Other studies that have examined the scaling of prey capture behavior have found mixed results. Whereas the duration and timing

variables of prey capture remained invariant with variation in body size in *Sceloporus* lizards, the scaling of velocities generally showed a linear increase with an increase in body length (Meyers et al., 2002). Thus, the data for *Sceloporus* are generally in accordance with the empirical scaling model proposed by Richard and Wainwright (1995). Our data for the scaling of velocity in the feeding system of chameleons differ from data for prey capture in snakes (Herrel et al., 2011) in which strike velocity was also independent of changes in body size throughout ontogeny. As in chameleons, where the velocity of the tongue and its reach are likely paramount to capture success, arboreal vipers rely on rapid accelerations of the head toward the prey. One notable difference between the two systems is, however, that whereas strike distance was independent of body size in vipers (Herrel et al., 2011), it increased with jaw and body size in chameleons. This may not be surprising, however, as strike distance (i.e., tongue reach in chameleons) is entirely dependent on tongue length in chameleons, whereas this is a function of behavior more than anything else in snakes (i.e., the length of the body recruited during the strike). The similar negative allometry of the size of the organ accelerated toward the prey relative to body length observed in both systems (Herrel et al., 2011; Anderson et al., 2012) suggests that this may be a general phenomenon in animals relying on rapid accelerations in order to capture prey.

The isometric growth of the tongue relative to the jaw coupled to the maintenance of the force generation capacity of the principal muscles throughout ontogeny leads to a puzzling observation suggesting a decrease in performance with size. Indeed, whereas the force generation capacity increases to the second power relative to jaw length, the mass of the organ increases to the third power. This should lead to a decrease in tongue acceleration with mandible length and lead to the independence of tongue velocity of changes in mandible length. Yet, tongue velocity scaled with a slope different from 0 suggesting that the elastic mechanisms providing the power for tongue projection may change throughout ontogeny. However, this remains to be investigated further through detailed histological studies of the connective tissue sheets surrounding the entoglossal process (De Groot and Van Leeuwen, 2004).

Our results also indicate that gape distance increased linearly with body length, yet gape angle was invariant across the size range examined here suggesting that animals open their mouth proportional to their overall size. Thus, prey size is expected to increase with overall body size as well. This contrasts with the observation that young individuals have a proportionately larger tongue apparatus relative to their body size, allowing them, at least in theory, to capture disproportionately large prey (Anderson et al., 2012). Unfortunately, ecological data on the diet and prey sizes of chameleons are scarce (but see, e.g., Pleguezuelos et al., 1999). A recent review of the literature on chameleon diets suggests that in contrast to their ability to capture large prey (Herrel et al., 2000), the majority of the diet in chameleons consists of small prey (Measey et al., 2013). Therefore, isometric changes of prey size and gape distance during growth seem reasonable. The disproportionately large tongue apparatus in juveniles may thus rather be the result of selection on the ability to capture 'any prey', with constraints on projection distance and velocity, rather than the reflection of a constraint on the capture of large prey. In summary, our results suggest that selection on prey capture performance may drive much of the scaling of the ballistic tongue projection in chameleons.

Acknowledgement

This research was supported by National Science Foundation grant IOS-0623791 to K.C.N.

References

- Anderson, C.V., Deban, S.M., 2010. Ballistic tongue projection in chameleons maintains high performance at low temperature. *Proc. Natl. Acad. Sci. U. S. A.* 107, 5495–5499.
- Anderson, C.V., Sheridan, T., Deban, S.M., 2012. Scaling of the ballistic tongue apparatus in chameleons. *J. Morphol.* 273, 1214–1226.
- Bell, D.A., 1989. Functional anatomy of the chameleon tongue. *Zool. Jb. Anat.* 119, 313–336.
- Bell, D.A., 1990. Kinematics of prey capture in the chameleon. *Zool. Jb. Physiol.* 94, 247–260.
- Birch, J.M., 1999. Skull allometry in the marine toad, *Bufo marinus*. *J. Morphol.* 241, 1–13.
- Carrier, D.R., 1983. Postnatal ontogeny of the musculo-skeletal system in the black-tailed jack rabbit (*Lepus californicus*). *J. Zool.* 201, 27–55.
- Carrier, D.R., 1995. Ontogeny of jumping performance in the black-tailed jack rabbit (*Lepus californicus*). *Zoology* 94, 309–313.
- Carrier, D.R., 1996. Ontogenetic limits on locomotor performance. *Physiol. Zool.* 69, 467–488.
- Carrier, D.R., Leon, L.R., 1990. Skeletal growth and function in the California gull (*Larus californicus*). *J. Zool.* 222, 375–389.
- Cook, A., 1996. Ontogeny of feeding morphology and kinematics in juvenile fishes: a case study of the cottid fish *Clinocottus analis*. *J. Exp. Biol.* 199, 1961–1971.
- Deban, S.M., Lappin, A.K., 2011. Thermal effects on the dynamics and motor control of ballistic prey capture in toads: maintaining high performance at low temperature. *J. Exp. Biol.* 214, 1333–1346.
- Deban, S.M., Richardson, J.C., 2011. Cold-blooded snipers: thermal independence of ballistic tongue projection in the salamander *Hydromantes platycephalus*. *J. Exp. Zool.* 315, 618–630.
- De Groot, J.H., Van Leeuwen, J.L., 2004. Evidence for an elastic projection mechanism in the chameleon tongue. *Proc. R. Soc. Lond. B* 271, 761–770.
- Ferry-Graham, L.A., 1998. Feeding kinematics of hatchling swellsharks, *Cephaloscyllium ventriosum* (Scyliorhinidae): the importance of predator size. *Mar. Biol.* 131, 703–718.
- Gans, C., 1967. The chameleon. *Nat. Hist.* 76, 52–59.
- Genbrugge, A., Heyde, A.-S., Adriaens, D., Boone, M., Van Hoorebeke, L., Dirckx, J., Aerts, P., Podos, J., Herrel, A., 2011. Ontogeny of the cranial skeleton in a Darwin's finch (*Geospiza fortis*). *J. Anat.* 219, 115–131.
- Gnanamuthu, C.P., 1930. The anatomy and mechanism of the tongue of *Chamaeleon carcaratus*. *Proc. Zool. Soc. Lond.* 31, 467–485.
- Hernandez, L.P., 2000. Intraspecific scaling of feeding mechanisms in an ontogenetic series of zebrafish, *Danio rerio*. *J. Exp. Biol.* 203, 3033–3043.
- Herrel, A., Gibb, A.C., 2006. Ontogeny of performance in vertebrates. *Physiol. Biochem. Zool.* 79, 1–6.
- Herrel, A., Meyers, J.J., Nishikawa, K.C., Aerts, P., 2000. The mechanics of prey prehension in chameleons. *J. Exp. Biol.* 203, 3255–3263.
- Herrel, A., Meyers, J.J., Nishikawa, K.C., De Vree, F., 2001. Morphology and histochemistry of the hyolingual apparatus in chameleons. *J. Morphol.* 249, 154–170.
- Herrel, A., Van Wassenbergh, S., Wouters, S., Aerts, P., Adriaens, D., 2005. A functional morphological approach to the scaling of the feeding system in the African catfish, *Clarias gariepinus*. *J. Exp. Biol.* 208, 2091–2102.
- Herrel, A., De Smet, A., Aguirre, L.F., Aerts, P., 2008. Morphological and mechanical determinants of bite force in bats: do muscles matter? *J. Exp. Biol.* 211, 86–91.
- Herrel, A., Huyghe, K., Okovic, P., Lisic, D., Tadic, Z., 2011. Fast and furious: effects of body size on strike performance in an arboreal viper *Trimeresurus (Cryptelytrops) albolabris*. *J. Exp. Zool. A* 315, 22–29.
- Herrel, A., Fabre, A.-C., Hugot, J.-P., Keovichit, K., Adriaens, D., Van Hoorebeke, L., Cornette, R., 2012. Ontogeny of the cranial system in *Laonastes aenigmamus*. *J. Anat.* 221, 128–137.
- Hill, A.V., 1950. The dimensions of animals and their muscular dynamics. *Sci. Prog.* 150, 209–230.
- Karsten, K.B., Andriamandimbarisoa, L.N., Fox, S.F., Raxworthy, C.J., 2008. A unique life history among tetrapods: an annual chameleon living mostly as an egg. *Proc. Natl. Acad. Sci. U. S. A.* 105, 8980–8984.
- Katz, S.L., Gosline, J.M., 1993. Ontogenetic scaling of jumping in the African desert locust (*Schistocerca gregaria*). *J. Exp. Biol.* 177, 81–111.
- McMahon, T., 1973. Size and shape in biology. *Science* 179, 1201–1204.
- Measey, J.G., Raselimanana, A., Herrel, A., 2013. Ecology and life-history of chameleons. In: Tolley, K.A., Herrel, A. (Eds.), *The Biology of Chameleons*. University of California Press, Berkeley, pp. 85–114.
- Mendez, J., Keys, A., 1960. Density and composition of mammalian muscle. *Metabolism* 9, 184–188.
- Meyers, J.J., Nishikawa, K.C., 2000. Comparative study of tongue protrusion in three iguanian lizards, *Sceloporus undulatus*, *Pseudotrapelus sinaitus* and *Chamaeleo jacksonii*. *J. Exp. Biol.* 203, 2833–2849.
- Meyers, J.J., Herrel, A., Birch, J., 2002. Scaling of morphology, bite force, and feeding kinematics in an iguanian and a scleroglossan lizard. In: Aerts, P., D'Aout, K., Herrel, A., Van Damme, R. (Eds.), *Topics in Functional and Ecological Vertebrate Morphology*. Shaker Publishing, Maastricht, pp. 47–62.
- Pennycuik, C.J., 1992. *Newton Rules Biology: A Physical Approach to Biological Problems*. Oxford University Press, Oxford.
- Pfaller, J.B., Gignac, P.M., Erickson, G.M., 2011. Ontogenetic changes in jaw-muscle architecture facilitate durophagy in the turtle *Sternotherus minor*. *J. Exp. Biol.* 214, 1655–1667.

- Pleguezuelos, J.M., Poveda, J.C., Monterrubio, R., Ontiveros, D., 1999. Feeding habits of the common chameleon, *Chamaeleo chamaeleon* (Linnaeus, 1758) in the south-eastern Iberian Peninsula. *Isr. J. Zool.* 45, 267–276.
- Powell, G.L., Russell, A.P., Ryan, M.J., 2002. Ontogenetic scaling of the cranial horn array in *Phrynosoma orbiculare* (Squamata: Phrynosomatidea). *J. Herpetol.* 36, 578–589.
- Richard, B.A., Wainwright, P.C., 1995. Scaling the feeding mechanism of large-mouth bass (*Micropterus salmoides*): kinematics of prey capture. *J. Exp. Biol.* 198, 419–433.
- Schmidt-Nielsen, K., 1984. *Scaling: Why is Animal Size so Important?* Cambridge University Press, Cambridge.
- Sokal, R.R., Rohlf, F.J., 1995. *Biometry: The Principles and Practice of Statistics in Biological Research*. W.H. Freeman and Co., New York.
- Toro, E., Herrel, A., Vanhooydonck, B., Irschick, D.J., 2003. A biomechanical analysis of intra- and interspecific scaling of jumping biomechanics and morphology in Caribbean *Anolis* lizards. *J. Exp. Biol.* 206, 2641–2652.
- Trillmich, F., Bieneck, M., Geissler, E., Bischof, H.-J., 2003. Ontogeny of running performance in the wild guinea pig (*Cavia aperea*). *Mamm. Biol.* 68, 214–223.
- Wainwright, P.C., Bennett, A.F., 1992a. Kinematics of prey processing in *Chamaeleo jacksonii*: conservation of function with morphological specialization. *J. Zool.* 226, 47–64.
- Wainwright, P.C., Bennett, A.F., 1992b. The mechanism of tongue projection in chameleons. I. Electromyographic tests of functional hypotheses. *J. Exp. Biol.* 168, 1–21.
- Wainwright, P.C., Bennett, A.F., 1992c. The mechanism of tongue projection in chameleons. II. Role of shape change in a muscular hydrostat. *J. Exp. Biol.* 168, 23–40.
- Wainwright, P.C., Richard, B.A., 1995. Scaling the feeding mechanism of the large-mouth bass (*Micropterus salmoides*): motor pattern. *J. Exp. Biol.* 198, 1161–1171.
- Wainwright, P.C., Kraklau, D.M., Bennett, A.F., 1991. Kinematics of tongue projection in *Chamaeleo oustleti*. *J. Exp. Biol.* 159, 109–133.
- Winter, D.A., 1990. *Biomechanics and Motor Control of Human Movement*. Wiley, New York.
- Zoond, A., 1933. The mechanism of projection of the chameleon's tongue. *J. Exp. Biol.* 10, 174–185.