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Buccal pumping mechanics of *Xenopus laevis* tadpoles: effects of biotic and abiotic factors

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SUMMARY

Biotic factors such as body size and shape have long been known to influence kinematics in vertebrates. Movement in aquatic organisms can also be strongly affected by abiotic factors such as the viscosity of the medium. We examined the effects of both biotic factors and abiotic factors on buccal pumping kinematics in *Xenopus* tadpoles using high-speed imaging of an ontogenetic series of tadpoles combined with experimental manipulation of the medium over a 10-fold range of viscosity. We found influences of both biotic and abiotic factors on tadpole movements; absolute velocities and excursions of the jaws and hyoid were greater in higher viscosity fluid but durations of movements were unaffected. Smaller tadpoles have relatively wider heads and more robust hyoid muscles used in buccal expansion and compression. Lever arm ratios were found to be constant at all sizes; therefore, smaller tadpoles have relatively higher resolved muscle forces and, like tadpoles in more viscous medium, displayed higher absolute velocities of jaw and hyoid movements. Nonetheless, small tadpoles drew in water at lower Reynolds numbers (Re) than predicted by kinematics, due to negative allometry of the buccal pump. Finally, tadpoles transitioned from a flow regime dominated by viscous forces ($Re=2$) to an intermediate regime ($Re=106$).

Key words: amphibian, feeding, scaling, viscosity.

INTRODUCTION

A variety of biotic and abiotic factors interact to determine the kinematics of animal movements, including factors such as body size, muscle physiology, lever mechanics, inertia of body parts, temperature and fluid mechanics. Body size has long been known to exert strong effects on patterns of movement, with smaller animals completing similar movements in less time than larger animals. This scaling pattern has been found in many terrestrial locomotor movements (Biewener, 1983; Hill, 1950; Jayne and Riley, 2007; Rand and Rand, 1966; Toro et al., 2003; Wilson et al., 2000; Wilson and Franklin, 2000) as well as in numerous aquatic feeding movements (Adriaens and Verraes, 1997; Deban and O'Reilly, 2005; Hernandez, 2000; Herrel et al., 2005; Holzman et al., 2008; Reilly, 1995; Richard and Wainwright, 1995; Robinson and Motta, 2002; Van Wassenbergh et al., 2005; Van Wassenbergh et al., 2007; Vincent et al., 2007; Wainwright and Shaw, 1999).

In the aquatic environment viscosity serves as an important abiotic factor, determining how fluid flows around an organism as well as the organism's performance and behavior in the fluid (McHenry and Lauder, 2005). The effects of changes in viscosity as a result of changing temperature have been studied in invertebrate locomotion (McHenry et al., 2003; Van Duren and Videler, 2003) and reproductive physiology (Brokaw, 1966); these studies showed that lower temperatures raised viscosity, which in turn reduced velocity of movement. Other studies have consistently found that organisms operating in more viscous regimes (i.e. at lower Reynolds number, Re) move with similar kinematics, but more slowly (Hunt von Herbing and Keating, 2003; Wilson and Franklin, 2000), or modify their kinematics (Hernandez, 2000; Koehl and Strickler, 1981; McHenry and Lauder, 2005).

We examined the combined effects of biotic factors (body size and shape) and abiotic factors (fluid viscosity) on buccal pumping

kinematics in tadpoles of *Xenopus laevis*, the African clawed frog. *Xenopus laevis* was chosen because its tadpoles are obligate suspension feeders which grow over a large body size range and which buccal pump continually (Seale et al., 1982). Buccal pumping in these tadpoles plays a role in both feeding and respiration, making it an ideal system for this study. The goals of this research were to investigate the effects of both biotic and abiotic factors on the kinematics of suspension feeding in *Xenopus* tadpoles to test the hypotheses that the smallest tadpoles will operate in flow regimes dominated by viscosity, and that positive allometry of the buccal pumping musculature will allow the tadpoles to maintain isometric scaling of pumping kinematics despite changes in flow regime. Four approaches were used: (1) we examined the scaling patterns of the feeding morphology; (2) we examined the scaling patterns of buccal pumping kinematics during feeding in water – deviations of the kinematics of buccal pumping from either the Hill (Hill, 1950) or Richard and Wainwright (Richard and Wainwright, 1995) models may be attributed to allometric scaling of the feeding morphology; (3) we examined the effects of viscosity on buccal pumping, including the effects of changes in shape and size – increasing viscosity effectively decreases the size of the organism in terms of Re ; and (4) we calculated the Re in an ontogenetic series of buccal pumping tadpoles. Re is calculated for each individual at the point where water enters the buccal cavity through the mouth to determine whether they operate under a viscous regime ($Re < 75$) or under an intermediate and potentially inertial regime ($75 < Re < 270$) (Fuiman and Webb, 1988; McHenry and Lauder, 2005).

MATERIALS AND METHODS

Tadpoles

Tadpoles of the African clawed frog, *Xenopus laevis* (Daudin 1802), were used for morphological examination and behavioral

experiments to investigate changes in buccal pumping kinematics as a result of changes in body size during ontogeny, of proportions of the buccal pump apparatus during ontogeny, and of changes in the viscosity of the aquatic medium. Lacking keratinized mouthparts, larval *Xenopus* are unable to scrape algae and detritus from the substrate and instead feed by entrapping particles suspended in the water column. Opening of mouth and depression of the hyoid results in water entering the buccal cavity, and subsequent closing of the mouth and elevation of the hyoid pushes water through the branchial apparatus, which entraps suspended particles.

Morphology

Sixteen formalin-preserved tadpoles (4.3–18.3 mm snout–vent length, SVL) were utilized for morphological measurements and dissection. Head width at the eyes was measured using digital calipers (MyCal-Lite 700-113, Mitutoyo Corp., Kanagawa, Japan). Buccal volume was measured by first sealing the spiracles with silicone aquarium sealant, and then filling the buccal cavity with water using a micropipette. Under a dissecting microscope (M5-101796 dissecting microscope, Wild Corp., Heerbrugg, Switzerland), points of insertion and origin of the interhyoideus (IH) and orbitochoideus (OH) muscles on the palatoquadrate and ceratohyal were identified.

The paired OH muscle is the primary muscle responsible for depressing the floor of the buccal cavity (Cannatella, 1999; Larson and Reilly, 2003). It originates on the muscular process of the palatoquadrate and inserts on the lateral edge of the ceratohyals. Upon contraction, the OH rotates the lateral end of the ceratohyal dorsally. Each ceratohyal forms a lever with its fulcrum with the palatoquadrate located near its lateral end (Gradwell, 1971). Thus, rotation of the ceratohyals depresses the floor of the buccal cavity, drawing water in. The water is then pushed out the back of the buccal cavity and out the spiracles through the action of the IH muscle, which spans the width of the head, along the ventral surface of the ceratohyals, and inserts at the lateral edge of the ceratohyal. Contraction of the IH rotates the lateral ends of the ceratohyals ventrally and the medial portions dorsally, thus elevating the floor of the buccal cavity. This compression of the buccal cavity forces water out through the branchial apparatus, which entraps particles.

Using fine forceps, the IH muscle was peeled from the ventral portion of the buccal cavity and the OH was removed from both sides of the head. Muscles were stored in 10% formalin until analysis. OH length was measured and averaged; IH length was not independently measured, as the muscle extends the width of the head. Wet mass of the muscles was measured (CP225D digital scale, Sartorius AG, Goettingen, Germany); each OH muscle was measured separately, and averaged for one value per individual. These values were confirmed by calculating the volume of the muscle using the width of the head (IH length) and the cross-sectional area (CSA), which was found by embedding the muscles in paraffin and sectioning each at its center of mass with a razor blade. The IH muscle is strap-like and was sectioned at half the length of the muscle whereas the OH is conical and was sectioned at 2/3 of the total length of the muscle from the origin. The circumference of the muscle cross sections were digitized using a Wild M5-101796 dissecting microscope (Wild Corp., Heerbrugg, Switzerland) with camera lucida attachment, and analyzed using ImageJ software (v 1.41, NIH, Washington, D.C., USA) to obtain the area of the cross section.

To examine the scaling of the skeletal elements, tadpoles were cleared and stained for cartilage with Alcian blue using published protocols (Hanken and Wassersug, 1981; Deban, 1997). Specimens were digitally imaged in ventral and lateral views using a Leica MZ75

dissecting microscope (Leica Microsystems GmbH, Wetzlar, Germany) and Leica DFC290 camera (Leica Camera AG, Solms, Germany). Width of the ceratohyals, length of the branchial basket (Fig. 1), and the out-lever length for the IH and OH muscles were measured from the ventral images. The out-lever extends from articulation of the ceratohyal with the palatoquadrate to the midline where the ceratohyal abuts the median copula (Fig. 2). The in-lever of the OH was taken as the length of the portion of the ceratohyal that extends laterally beyond this articulation to the point of insertion of the OH. Lateral images were used to measure the dorsoventral thickness of the ceratohyal, which was taken as the in-lever length of the IH. Lever arm ratio of hyoid depression is calculated as the ratio of the in-lever to the out-lever (Wassersug and Hoff, 1979).

The relationship of muscle CSA and muscle mass to body length were assessed independently of each other and of the lever arm ratios. The slopes of the least-squares regressions of log-transformed variables *versus* log SVL were compared with the expected values for each variable according to published geometric similarity models (Hill, 1950; Richard and Wainwright, 1995). When regressed against body length, wet mass should scale with a slope of three, CSA with a slope of two, and lever arm ratio with a slope of zero (i.e. it is constant at all body lengths). The ratio of the IH to OH muscle cross-sectional areas should also scale with a slope of zero.

To estimate the maximum force applied to the buccal cavity during expansion and compression, the force generated by each muscle was calculated by multiplying the CSA of the muscle by the specific tension of amphibian muscle, 22 N cm^{-2} (Regnier and Herrera, 1993). This muscle force was multiplied by the lever ratio to yield the resolved force of hyoid depression and hyoid elevation at the median copula.

Buccal pumping kinematics

Seventy-five tadpoles (4–16 mm SVL) buccal pumping in water were imaged at $125 \text{ frames s}^{-1}$ (Photron PCI-1024 FastCam, Photron USA Inc., San Diego, CA, USA) in lateral view with fiber optic illumination. Only images with the entire tadpole body in the frame and presenting a lateral profile were used for digitizing. Two-dimensional x, y coordinates of four landmarks on each frame of the image sequence were recorded (Fig. 3): (A) Upper jaw tip, (B) lower jaw tip, (C) top of the head directly dorsal to the eye, and (D) hyoid apparatus directly ventral to the eye. These coordinate data were used to generate 10 kinematic variables, including two distances, four durations and four velocities: (1) gape distance (distance between points A and B), (2) hyoid depression distance

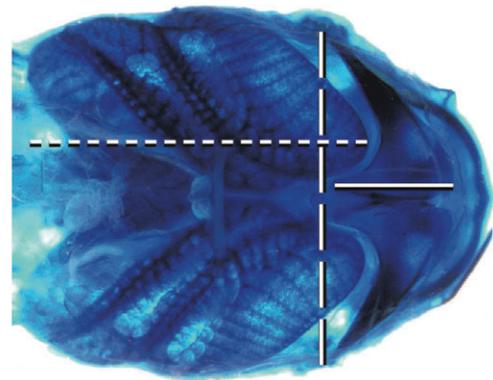


Fig. 1. Ventral view of a cleared and stained *Xenopus laevis* tadpole, illustrating morphological measurements, ceratohyal width (solid line), head width (long-dashed line) and branchial basket length (short-dashed line).

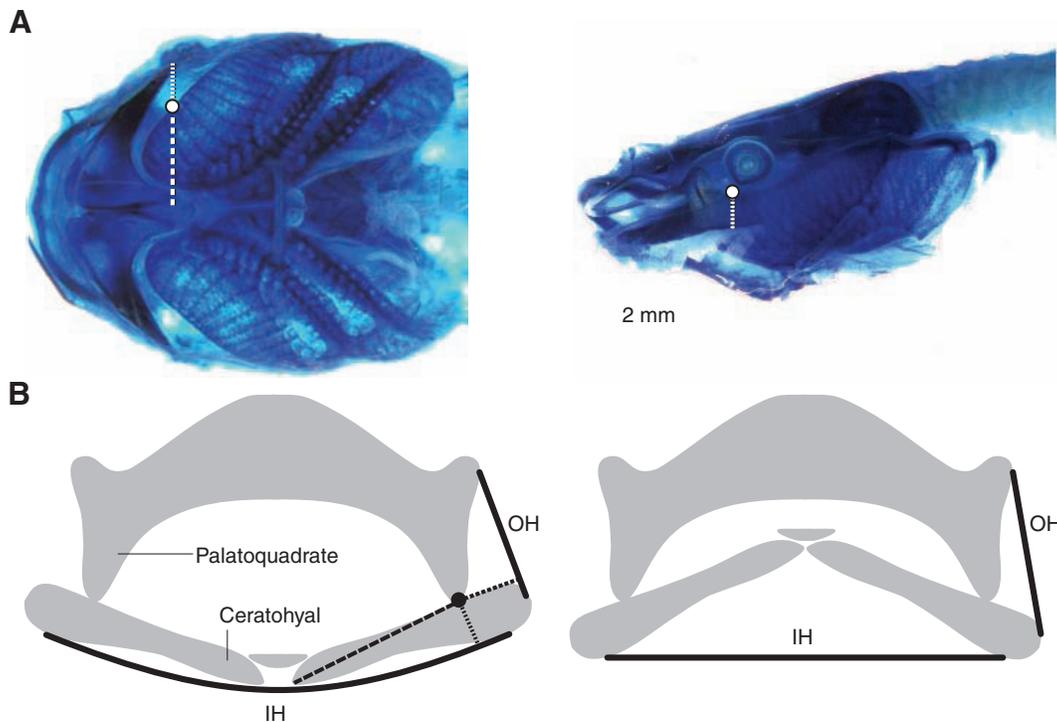


Fig. 2. (A) Ventral and lateral views of a cleared and stained *Xenopus* tadpole, illustrating the ceratohyal and the lever arm lengths for the orbitohyoideus (OH) muscle (left) and interhyoideus (IH) muscle (right). The out-lever for both muscles is shown as a dashed line and the in-levers as dotted lines. (B) Schematic diagrams of the buccal pumping mechanism in rostral view, illustrating the actions of OH and IH muscles (lines) expanding (left) and compressing (right) the buccal cavity. The shorter dashed line indicates the in-lever for the acting muscle, the longer dashed line indicates the out-lever, and the point represents the fulcrum upon which the ceratohyal rotates. B is redrawn from Gradwell (Gradwell, 1968).

(distance between points C and D), durations of (3) mouth opening, (4) mouth closing, (5) hyoid depression and (6) hyoid elevation, and velocities of (7) mouth opening, (8) mouth closing, (9) hyoid depression and (10) hyoid elevation. Ten buccal pumping cycles were recorded from each individual and individual means were generated for each variable.

To examine the effects on kinematics of food in the water, a subset of 15 tadpoles were first imaged without food particles suspended in water, then imaged again once food had been introduced, measuring the same variables mentioned previously. Food consisted of ground up commercial algae flakes (Nutrafin Spirulina Algae Flake Food, Hagen Inc., Montreal, Canada). For tadpoles that were imaged with food in the water, frames were used only if particles were observed moving into the mouth.

Reynolds number calculations

Re of water flowing into the mouth was calculated using the equation $Re = U \cdot l / \nu$, where U is the velocity of the object moving through the water, l is the characteristic length of the object, and ν is the viscosity of the fluid (Vogel, 1994).

Re was calculated for water entering the buccal cavity through the mouth, using head width as the characteristic length. Fluid velocity was calculated as the mean velocity of three food particles measured over the time they started moving until they passed the jaw joint. Only particles starting their trajectory directly in front of the mouth were used for these calculations.

Fluid velocity measured from the image sequences was then compared with fluid velocity predicted from the morphology and kinematics of the tadpoles, to determine how well a simple model based on the principle of continuity could predict fluid velocity over

a range of body sizes given changes in both kinematics and morphology of the buccal pump. We used a piston model in which the buccal floor area (square of the head width) was multiplied by the hyoid depression velocity to predict fluid velocity coming into the mouth.

Viscosity manipulations

To examine the effects of increasing viscosity on buccal pumping frequencies and kinematics, a methylcellulose solution (MP Biomedicals, #155496, Thermo Fisher Scientific, Waltham, MA, USA) was chosen for its non-toxic properties, as well as its digestibility (Hunt von Herbing and Keating, 2003). In addition to

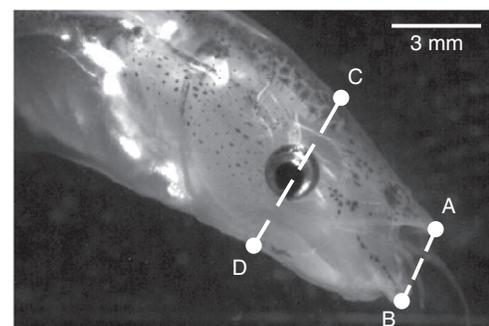


Fig. 3. Representative movie image of a *Xenopus laevis* tadpole buccal pumping, showing maximum gape and hyoid depression with landmarks and distances used for kinematic analysis. A, upper jaw tip; B, lower jaw tip; C, top of the head directly dorsal to the eye; D, hyoid apparatus directly ventral to the eye.

water, three separate solutions were chosen based on calculations of *Re* experienced by small *Xenopus* tadpoles. Dynamic viscosities were measured at 1.0 (water), 3.5, 6.9 and 10.3 centistokes (cSt) using a Cole-Parmer Viscometer (Cannon-Ubbelohde viscometers, Cole-Parmer Instrument Company, Vernon Hills, IL, USA).

Sixty-five tadpoles (4.7–15.7 mm SVL) were placed individually in the experimental fluid, chosen at random, and mean pumping frequency over one hour was measured. Tadpoles were then imaged as above to record 10 buccal pumping cycles. Each tadpole was exposed to only one experimental fluid. Kinematic variables for the 10 pumps were averaged, yielding one value per variable for each tadpole.

Statistical analyses

Linear regressions were performed on all log-transformed morphological and kinematic variables *versus* log SVL, and the slopes of the regression lines and their 95% confidence intervals and compared with slopes expected from two geometric similarity models (Hill, 1950; Richard and Wainwright, 1995). Slopes are reported as scaling exponents, which are identical to exponents obtained from power regressions on non-log-transformed data. Regressions were computed in Microsoft Excel (Office 2008 edition, Microsoft Corp., Redmond, WA, USA) on an Apple iMac computer (Apple Inc., Cupertino, CA, USA).

The effects of body size and viscosity on buccal pumping kinematics were examined using an analysis of covariance (ANCOVA) in which viscosity was the main effect and log SVL was the covariate. An interaction term was included in the model, but was removed when it was found to be non-significant for all variables, to increase statistical power. All kinematic variables were log transformed prior to analysis. Analyses were conducted using JMP 5.1 software (SAS Institute Inc., Cary, NC, USA) on an Apple iMac computer.

RESULTS

Morphology

IH muscle length (i.e. ceratohyal length or head width) and buccal volume scaled hypoallometrically with respect to SVL, with exponents of 0.63±0.08 (all variables are indicated as scaling exponent ± 95% confidence interval, unless otherwise noted) and 1.22±0.13, respectively (Table 1, Fig. 4). By contrast, ceratohyal

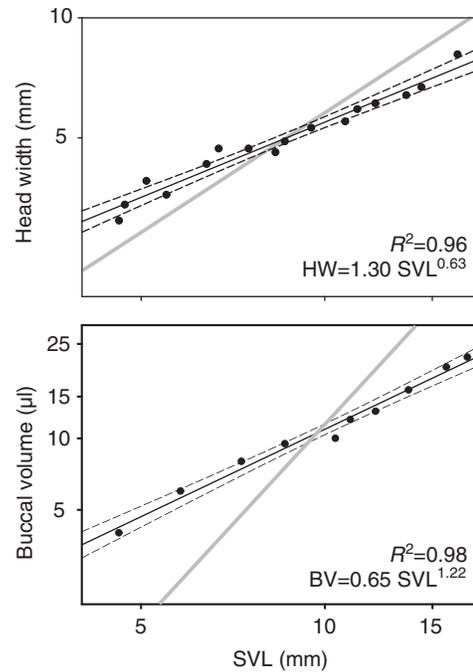


Fig. 4. Scaling of head width (HW) and buccal volume (BV) to snout-vent length (SVL) in *Xenopus* tadpoles, illustrating negative allometry. 95% confidence intervals of slopes do not include gray lines representing isometric slopes (head width, 1; buccal volume, 3).

width, branchial basket length and OH length all scaled isometrically, with exponents of 0.81±0.21, 0.91±0.15 and 1.00±0.18, respectively.

IH mass exhibited isometric growth with an exponent of 2.77±0.53 that was not significantly different from 3.0, while OH mass showed negatively allometric growth with an exponent of 1.61±0.43 (Table 1; Fig. 5). The cross-sectional areas of both the OH (exponent=0.44±0.17) and IH (exponent=0.78±0.22) exhibited negatively allometric growth (Table 1; Fig. 6), with the OH exhibiting significantly stronger negative allometry than the IH ($F=4.55, P<0.05$). The ratio of the IH to the OH exhibited positive allometry (scaling exponent=0.35±0.08; Fig. 7).

Table 1. Scaling exponents with 95% confidence intervals (CI) of the morphological and biomechanical variables *versus* snout-vent length, and expected exponents based on geometric similarity (GSM)

Variable	Exponent	Lower CI	Upper CI	R ²	F	P	GSM
Head width	0.63	0.55	0.70	0.96	338.97	<0.001	1*
CH width	0.81	0.60	1.03	0.85	69.96	<0.001	1
Branch basket length	0.91	0.76	1.06	0.93	165.51	<0.001	1
OH length	1.00	0.82	1.18	0.95	164.55	<0.001	1
Buccal volume	1.22	1.09	1.35	0.98	479.71	<0.001	3*
OH mass	1.61	1.18	2.03	0.85	67.55	<0.001	3*
IH mass	2.77	2.21	3.28	0.92	125.96	<0.001	3
OH CSA	0.44	0.26	0.61	0.69	29.13	<0.001	2*
IH CSA	0.78	0.56	1.00	0.82	58.32	<0.001	2*
IH/OH CSA ratio	0.35	0.27	0.42	0.88	99.46	<0.001	0*
OH lever arm ratio	0.24	-0.48	0.95	0.04	0.54	0.47	0
IH lever arm ratio	0.25	-0.29	0.79	0.12	1.09	0.33	0
OH resolved force	0.68	-0.02	1.38	0.27	4.48	0.06	2*
IH resolved force	1.06	0.28	1.85	0.55	9.73	0.014	2*
IH/OH resolved force ratio	0.09	-0.87	1.06	0.01	0.05	0.832	0

Asterisks indicate variables that show allometry, i.e. variables with expected GSM exponents that are not encompassed by the 95% confidence intervals of the observed exponent. CH, ceratohyal; OH, orbitohyoideus; IH, interhyoideus.

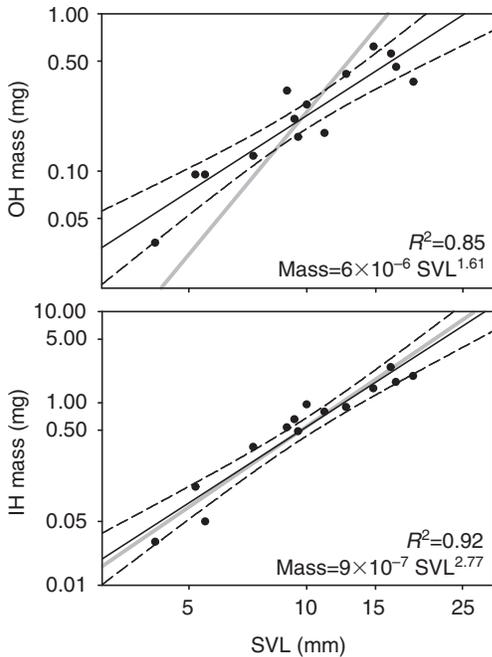


Fig. 5. Scaling wet mass of interhyoideus (IH) and orbitohyoideus (OH) muscles, illustrating negative allometry of the OH and isometry of the IH. Gray lines indicate isometric slope of 3. Dashed lines are 95% confidence intervals of slopes.

No significant relationship was found between the lever arm ratio of the OH (exponent=0.24±0.71) or of the IH (0.25±0.55) and body length (Fig. 6). Resolved force generated by both muscles increased

with size (OH exponent=0.68±0.70; IH exponent=1.06±0.79). The IH/OH CSA ratio increased significantly with increasing SVL (exponent=0.35±0.08) while IH/OH resolved force showed no relationship with SVL (exponent=0.09±0.96) (Table 1; Fig. 7).

Buccal pumping kinematics

In *Xenopus* tadpoles, a buccal pumping event consisted of the tadpole opening its mouth, and depressing the hyoid to draw water into the buccal cavity, then closing the mouth and elevating the hyoid to expel water through the branchial apparatus and out the paired spiracles.

Results of the ANOVAs revealed no differences in kinematic variables between feeding and non-feeding trials ($F=0.07-2.82$, $P=0.10-0.79$). Tadpole pumping frequency, however, was greater in feeding trials [feeding=58.68±8.57 pumps min⁻¹ (mean ± s.e.m.), non-feeding=30.95±5.61, $F=13.286$, $P=0.003$]. Together these results indicate that the presence of food particles encouraged tadpoles to pump more often but did not change the kinematics of buccal movement.

Kinematics of buccal pumping in water did not exhibit scaling patterns consistent with either scaling model. Only maximum hyoid distance (scaling exponent=0.60±0.40) and duration of mouth closing (0.74±0.51) scaled isometrically, consistent with Hill's (Hill, 1950) and Richard and Wainwright's (Richard and Wainwright, 1995) models of geometric similarity, respectively. The only negatively allometric variable was maximum gape distance (0.52±0.45). Duration of mouth opening (-0.05±0.72), duration of hyoid depression (0.40±0.60) and velocity of hyoid elevation (-0.31±0.45) showed no effect of increasing SVL. Kinematic variables decreasing with increasing SVL included velocity of mouth opening (-0.72±0.23), velocity of mouth closing (-0.51±0.41) and

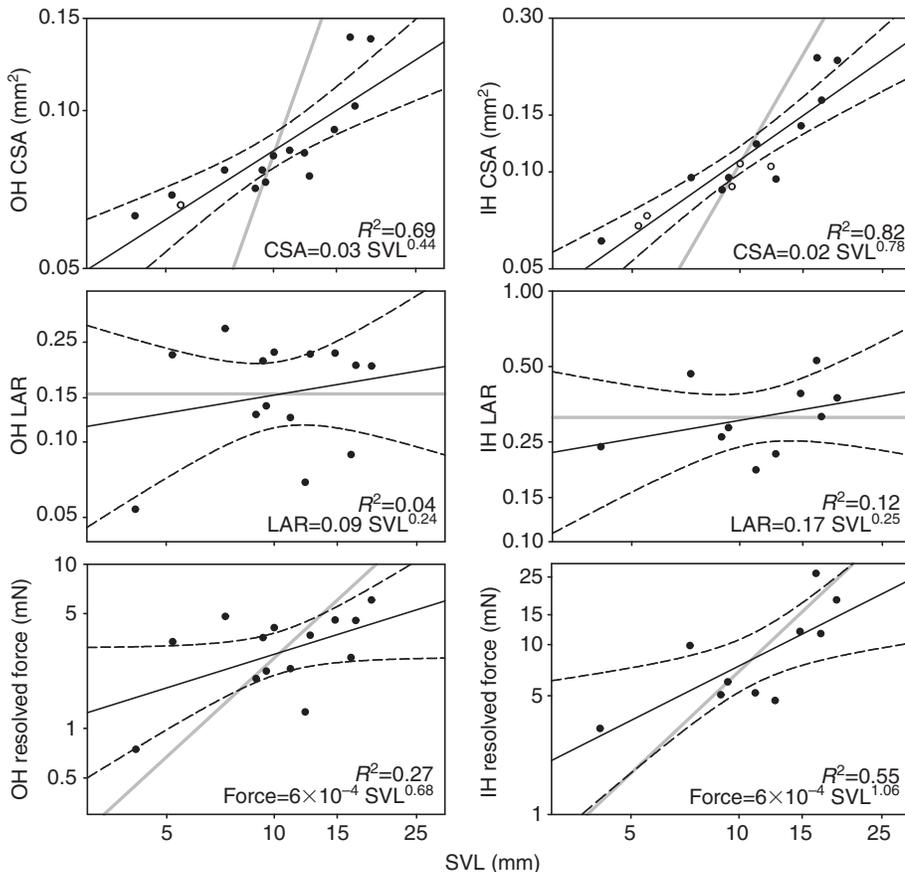


Fig. 6. Scaling of cross-sectional area (CSA), lever arm ratio (LAR) and resolved force of orbitohyoideus (OH) and interhyoideus (IH) muscles. Gray lines represent lines of isometric slope: CSA, 2; LAR, 0; Force, 2. Dashed lines are 95% confidence intervals of slopes. Open circles in upper graphs are individuals not used in calculations of LAR and resolved force.

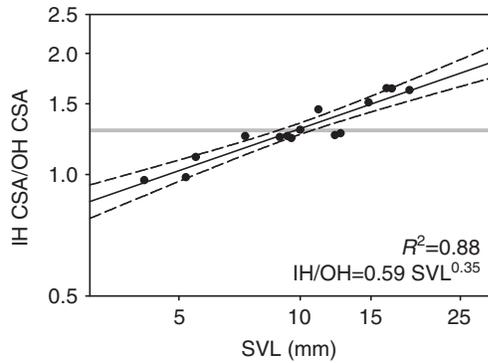


Fig. 7. Ratio of cross-sectional areas of the interhyoideus (IH) muscle to the orbitohyoideus (OH) muscle, illustrating the ontogenetic increase in the relative cross section of the IH muscle. Below is the ratio of resolved force, showing isometry. Isometry is indicated by the gray line.

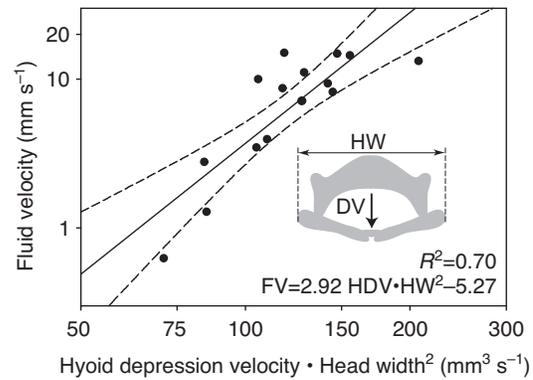


Fig. 8. Measured fluid velocity (FV) entering the mouth as a function of buccal piston velocity. The latter is modeled as head width (HW) squared multiplied by the hyoid depression velocity (HDV) measured kinematically.

velocity of hyoid depression (-0.65 ± 0.18), inconsistent with either Hill's (Hill, 1950) or Richard and Wainwright's (Richard and Wainwright, 1995) models of geometric similarity (Table 2).

Reynolds number

The minimum velocity of fluid observed entering the mouth was 0.6 mm s^{-1} and the maximum velocity was 13.2 mm s^{-1} (Fig. 8) and scaled to SVL with a slope of 1.77 ± 0.94 . Re for fluid entering the mouth ranged from 2 for the smallest tadpole (4.3 mm SVL) to 106 for the largest in this study (16.5 mm SVL). Re scaled to SVL with a slope of 2.40 ± 0.99 . If Re were to scale isometrically and velocities were constant, the expected slope would be 1, increasing in direct proportion with head width, the characteristic length.

Effects of viscosity

When viscosity and SVL were considered together in an ANCOVA, significant effects of SVL on eight of the 10 kinematic variables were revealed (Table 3). Durations increased significantly in larger tadpoles, and the velocity of mouth closing and velocity of hyoid elevation declined. Only four of the 10 kinematic variables were significantly affected by the viscosity treatments, i.e. maximum gape distance, maximum hyoid distance, velocity of mouth closing and velocity of hyoid elevation (Table 3). In all cases, higher viscosity produced greater distances and velocities. Interactions of SVL and viscosity were not significant for any kinematic variable.

DISCUSSION
Morphology

Study of the scaling of aquatic feeding powered by buccal movements has been limited to organisms that grow isometrically or nearly so (Deban and O'Reilly, 2005; Herrel et al., 2005; Reilly, 1995; Richard and Wainwright, 1995; Robinson and Motta, 2002). *Xenopus laevis* tadpoles are similar in that some elements of the morphology exhibit isometry with respect to SVL, including rostrocaudal width of the ceratohyal and length of the branchial basket. However, other important parameters – head width, buccal volume and muscle areas – exhibit significant allometry (Table 1). Our direct measurements of buccal volume confirmed indirect estimates of volume based on pumping frequencies (Seale, 1982).

The negative allometry of IH and OH muscle cross-sectional areas and resolved forces would be expected to allow higher velocities of mouth and hyoid movements of smaller tadpoles relative to larger tadpoles, and thus higher Re . However, we found that while smaller tadpoles indeed move faster (Table 2), they still draw water into the buccal cavity at lower Re than larger tadpoles. Thus, the effects of small size on Re are not entirely mitigated by the allometry that the tadpoles display.

Our examination of the OH lever arm ratio confirmed values from previous research (0.18–0.22), similar to other microphagous suspension-feeding tadpoles (Wassersug and Hoff, 1979). The isometric IH lever arm ratio we measured has not been previously reported. The lack of a relationship between lever arm ratio and body

Table 2. Least squares regression slopes (i.e. scaling exponents) with 95% confidence intervals (CI) of the kinematic variables versus snout-vent length, and expected slopes from two models of geometric scaling (i.e. Hill, 1950 and Richard and Wainwright, 1995)

Variable	Exponent	Lower CI	Upper CI	R^2	F	P	Richard and	
							Hill, 1950	Wainwright, 1995
Maximum gape distance	0.52	0.07	0.97	0.47	7.15	0.03	1*	1*
Maximum hyoid distance	0.60	0.20	1.00	0.60	11.92	0.01	1	1
Duration of mouth opening	-0.05	-0.77	0.67	0.003	0.03	0.88	1*	0
Duration of mouth closing	0.74	0.14	1.33	0.51	8.26	0.02	1	0*
Duration of hyoid depression	0.40	-0.20	1.00	0.23	2.41	0.16	1	0
Duration of hyoid elevation	0.69	0.05	1.33	0.44	6.27	0.04	1	0*
Velocity of mouth opening	-0.72	-0.95	-0.50	0.88	56.24	<0.001	0*	1*
Velocity of mouth closing	-0.51	-0.92	-0.10	0.51	8.28	0.02	0*	1*
Velocity of hyoid depression	-0.65	-0.83	-0.48	0.90	74.71	<0.001	0*	1*
Velocity of hyoid elevation	-0.31	-0.76	0.15	0.23	2.42	0.16	0	1*

Asterisks indicate variables that differ from a given model; i.e. variables with expected slopes that are not encompassed by the 95% confidence intervals of the observed slope.

Table 3. Results of the ANCOVA examining effects of snout–vent length and viscosity on buccal pumping kinematics

Variable	Viscosity			Snout–vent length		
	F	P	Slope	F	P	Slope
Duration of mouth opening	0.007	0.9350	n.s.	9.01	0.0048*	+
Duration of mouth closing	0.008	0.4122	n.s.	21.18	<0.0001*	+
Duration of hyoid depression	0.0016	0.9684	n.s.	11.43	0.0017*	+
Duration of hyoid elevation	0.0653	0.7997	n.s.	25.51	<0.0001*	+
Maximum gape distance	15.50	0.0004*	+	13.34	0.0008*	+
Maximum hyoid distance	26.10	<0.0001*	+	28.79	<0.0001*	+
Velocity of mouth opening	5.71	0.0221	n.s.	8.32	0.0065	n.s.
Velocity of mouth closing	33.94	<0.0001*	+	11.81	0.0015*	–
Velocity of hyoid depression	3.74	0.0608	n.s.	6.06	0.0186	n.s.
Velocity of hyoid elevation	55.76	<0.0001*	+	9.56	0.0038*	–

Interactions were not significant (n.s.) and were dropped from the model. Asterisks indicate significant effects at the Bonferroni-corrected α (0.005 for 10 tests).

length indicates that the lever arm ratio is not responsible for changing the magnitude of the force applied to the buccal cavity by the OH or the IH as the tadpole grows. Rather, the scaling of CSA of each muscle determines the scaling of the applied force, which shows negative allometry (Fig. 6). The greater relative force applied to the buccal cavity in smaller tadpoles may partially compensate for the higher viscous forces at small body sizes (i.e. lower Re).

Although the ratio of IH/OH cross-sectional areas of the *Xenopus* tadpoles shows positive allometry, the ratio of forces applied to the buccal cavity scales with isometry (Fig. 7). Small tadpoles are thus applying ingestion and expulsion forces in the same ratio as large tadpoles, with the IH applying 1–10 times the force to the buccal cavity as the OH. The ratio of muscle forces was previously examined to investigate a link between morphology and feeding ecology in a wide range of anuran species. Tadpoles species whose OH produce more force than their IH (as determined by cross-sectional areas) were found to be macrophagous, while tadpoles whose IH produce more force were found to be microphagous suspension feeders (Satel and Wassersug, 1981). *Xenopus* tadpoles have the highest recorded IH/OH CSA ratio (1.67), but another pipid, *Hymenochirus* tadpoles have the second lowest ratio (0.07), and a member of the closest relative of the pipids, *Rhinophrynus*, have a similar ratio to *Xenopus* (1.22). Similarly, the ceratohyal lever arm ratio (i.e. the length of the in-lever divided by the length of the out-lever) was found to be correlated with feeding mode (Wassersug and Hoff, 1979). When the ratios were compared with the feeding mode of each taxon, the two pipids investigated lie at opposite extremes of the spectrum. *Hymenochirus* has the highest lever arm ratio (0.50) similar to other macrophagous carnivores, while *Xenopus* has the lowest ratio (0.14), much like other midwater suspension feeders. A low IH/OH ratio, correlated with a high lever arm ratio, is indicative of large force generation during the mouth opening and hyoid depression phase, imperative for a suction-feeding tadpole to capture elusive prey. A higher IH/OH ratio and lower lever arm ratio are indicative of the importance of pushing fluid through the branchial apparatus, as opposed to the initial stage of drawing food into the buccal cavity. The large applied force ratio of our *Xenopus* tadpoles and a lack of a significant change across a range of body sizes (Fig. 7) indicates that even at very small body size these tadpoles have typical suspension-feeding biomechanics of the buccal pump, which they maintain through larval ontogeny.

Buccal pumping kinematics

Relative distance of mouth and hyoid movement declines with increasing body size in the *X. laevis* tadpoles, while durations of movement increase and velocities decrease. The lack of congruence

of scaling exponents of the tadpoles with geometric similarity models suggests that either suspension feeding has different performance demands or constraints than suction feeding, despite the similar mechanics, or that morphological allometry (i.e. negative allometry of buccal volume, muscle CSA and resolved muscle forces) may be responsible for the negative allometry of movement velocities.

Two models that have been applied to the scaling of movement are Hill's (Hill, 1950) geometric similarity model and Richard and Wainwright's (Richard and Wainwright, 1995) geometric similarity model, which differ in their predicted patterns of movement. Both models assume that morphology grows isometrically, with linear dimensions growing in proportion with one another. The Hill model is based on the principles of muscle physiology, and the isometric slopes are derived from the variables' dimensions and Newton's $F=ma$. The Hill model predicts that durations of movement remain proportional to linear dimensions and velocities of movement remain constant despite changes in size. These predictions have been met in the jaw and hyobranchial movements during suction feeding in the aquatic salamander, *Cryptobranchus alleganiensis*, which grows with near isometry (Deban and O'Reilly, 2005). Richard and Wainwright's model differs in that it predicts that durations remain constant and that velocities of movement increase in proportion to linear dimensions (Richard and Wainwright, 1995). This model is based on the assumption that muscle length grows in direct proportion to other linear dimensions and that muscle contraction velocity is proportional to muscle length because longer muscles have more sarcomeres in series. Kinematic predictions of this model have been borne out in the suction feeding in the largemouth bass, *Micropterus salmoides*, which also grows with isometry (Richard and Wainwright, 1995). This latter scaling pattern was also found in another suction feeder, the nurse shark *Ginglymostoma cirratum*, which grows with isometry (Robinson and Motta, 2002).

In the *Xenopus* tadpoles examined here, velocities of mouth opening and closing and hyoid depression scale with exponents that are significantly more negative than the predicted exponents of the Hill and Richard and Wainwright models (Hill, 1950; Richard and Wainwright, 1995), indicating that small tadpoles are moving faster than predicted by either model (Table 2). Small tadpoles are thus using their proportionally larger resolved forces (exponents <2; Table 1) to force water through the buccal and branchial chambers faster than would be predicted by their body size were they growing with geometric similarity.

Reynolds number and effects of viscosity

Tadpoles in this study were calculated to draw water into the mouth with Re ranging from 2 to 106, increasing with increasing SVL.

The range of values point to a shift from low *Re* regime – where viscous forces dominate – into an intermediate *Re* regime at approximately 10mm SVL. A low *Re* regime differs by over an order of magnitude among authors, from as low as 10 (Weihs, 1980) to over 300 (McHenry and Lauder, 2005). We use a scale commonly used in studies of fish locomotion (Fuiman and Webb, 1988; Müller et al., 2008; Videler et al., 2002), in which a low *Re* regime extends to 75 and an intermediate regime falls between 75 and 270.

Larvae of the zebrafish *Danio rerio* were shown to produce flow into the mouth during feeding at *Re* as low as 5 (Hernandez, 2000) while similarly sized suction-feeding tadpoles *Hymenochirus boettgeri* generated *Re* up to 300 (Deban and Olson, 2002). *Hymenochirus* is a carnivorous pipid tadpole that is much smaller than *X. laevis* at first feeding (<1 mm versus 4 mm) (Deban and Olson, 2002). Compared with suspension feeding, suction feeding, as seen in *Danio* and *Hymenochirus*, is distinguished by rapid water intake and relatively much slower water expulsion.

The observed increases in fluid velocity and *Re* with increasing SVL are the result of interacting morphological and kinematic allometries. Both buccal volume and the velocity of hyoid depression show negative allometry (Tables 1 and 2). Modeling the floor of the buccal cavity as a piston moving with the velocity of hyoid depression proved to be a good predictor of fluid velocity ($R^2=0.7$) entering the mouth (Fig. 8). As body length increases, buccal floor area increases at a faster rate than the rate at which the velocity decreases, producing an increase in fluid velocity and *Re* of water entering the mouth. The smallest tadpoles generated lower *Re* than one would expect given only the relative faster movements of the mouth and hyoid during feeding, if one did not consider morphological allometry. *Re* is low despite a proportionally greater head width and buccal floor area. The absolutely smaller ‘buccal piston’ resulted in a lower fluid velocity, despite the increased velocities of movement. Lower fluid velocity and larger morphology result in a lower *Re* than expected from kinematics and assumptions of isometry.

Viscosity treatments did not have an effect on the pumping frequency of the tadpoles. Evidence for *X. laevis* altering pumping frequency is prolific in the literature (Seale, 1982; Seale et al., 1982; Seale and Wassersug, 1979; Wassersug and Hoff, 1979). Our results from the viscosity manipulations indicate that while tadpoles are able to sense the amount of food in the water and regulate pumping frequency accordingly (Seale, 1982), changes in viscosity did not provoke a similar increase in pumping frequency. Increasing the viscosity does not provide a stimulus similar to food in the water, and therefore should not have altered pumping frequency.

Pumping kinematics, however, were influenced by viscosity. In more viscous fluid the tadpoles opened the mouth and lowered the hyoid farther, and closed the mouth and elevated the hyoid with greater velocity, resulting in no significant change in the durations of movements. These results indicate that the tadpoles were compensating for increased resistance to flow, mirroring the decreases in velocity of mouth closing and hyoid elevation with increases in SVL. Smaller tadpoles that are operating at lower *Re* and tadpoles in more viscous fluid are moving more quickly.

Conclusions

This study examined the role of changes in body size, shape and abiotic properties of the medium in buccal pumping mechanics in *X. laevis* tadpoles through analyses of morphology, kinematics and environmental manipulation. Tadpoles of a range of sizes show a constant ratio of resolved force of the hyoid elevator muscle (m. interhyoideus) to the depressor muscle (m. orbitohyoideus),

suggesting that body size has no effect on the relative compressive and expansive phase forces required during buccal pumping. This result might indicate that morphological allometry of the pump and branchial apparatus compensates for changes in *Re* to keep this force ratio constant.

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