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Author(s)	Veronica T. T. de Sousa, Fausto Nomura, Denise de C. Rossa-Feres, Gilda V. Andrade, Tiago L. Pezzuti, Richard J. Wassersug, Matthew D. Venesky
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Differential Effects of Temperature on the Feeding Kinematics of the Tadpoles of Two Sympatric Anuran Species

VERÔNICA T.T. DE SOUSA[1]*,
FAUSTO NOMURA[2], DENISE DE C. ROSSA-FERES[3], GILDA V. ANDRADE[4],
TIAGO L. PEZZUTI[5],
RICHARD J. WASSERSUG[6,7],
AND MATTHEW D. VENESKY[8]

[1]PPG Ecologia e Evolução, Universidade Federal de Goiás, Goiânia, Goiás, Brazil
[2]Departamento de Ecologia, Universidade Federal de Goiás, Goiânia, Goiás, Brazil
[3]Departamento de Zoologia e Botânica, Universidade Estadual Paulista, São José do Rio Preto, São Paulo, Brazil
[4]Departamento de Biologia, Campus do Bacanga, Universidade Federal do Maranhão, São Luis, Maranhão, Brazil
[5]Departamento de Zoologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil
[6]Sir Charles Tupper Medical Building, Department of Medical Neuroscience, Dalhousie University, Halifax, Nova Scotia, Canada
[7]Department of Urologic Sciences, Gordon & Leslie Diamond Care Centre, University of British Columbia, Vancouver, British Columbia, Canada
[8]Department of Biology, Allegheny College, Meadville, Pennsylvania

ABSTRACT   Temperature impacts ectotherm performance by influencing many biochemical and physiological processes. When well adapted to their environment, ectotherms should perform most efficiently at the temperatures they most commonly encounter. In the present study, we tested how differences in temperature affects the feeding kinematics of tadpoles of two anuran species: the benthic tadpole of *Rhinella schneideri* and the nektonic tadpole of *Trachycephalus typhonius*. Benthic and nektonic tadpoles have segregated distributions within ponds and thus tend to face different environmental conditions, such as temperature. Muscle contractile dynamics, and thus whole organism performance, is primarily temperature dependent for ectotherms. We hypothesized that changes in mean temperatures would have differential effects on the feeding kinematics of these two species. We conducted a laboratory experiment in which we used high-speed videography to record tadpoles foraging at cold and warm temperatures. In general, tadpoles filmed at warm temperatures opened their jaws faster, attained maximum gape earlier, and exhibited shorter gape cycles than tadpoles in cold temperatures, irrespective of species. We also found species x temperature interactions regarding the closing phase velocity, and the percentage of time it takes tadpoles to achieve maximum gape and to start closing their jaws. These interactions could indicate that these two co-occurring species differ in their sensitivity to differences in water temperature and have temperature-dependent feeding strategies that maximize feeding performance in their preferred environment. J. Exp. Zool. 9999A: 1–10, 2015. © 2015 Wiley Periodicals, Inc.

INTRODUCTION

Animals, especially ectotherms, are highly sensitive to the temperature of the surrounding environment. Thermal perform-ance curves predict that ectotherms will achieve maximum performance at moderate to relatively high body temperatures, whereas extreme temperatures will result in poor performance (Huey and Stevenson, '79). Ectotherms avoid environmental temperature extremes through behavioral and/or physiological thermoregulation, but their ability to do so is limited (e.g., Wilson and Franklin, '99; Wu et al., 2007; Niehaus et al., 2011). Ectotherms that inhabit environments that are prone to rapid and large changes in temperature may thus suffer performance tradeoffs as a consequence of a decreased ability to thermoreg-ulate. In small lentic bodies of water, water temperature changes as a result of the input or output of relatively small amounts of heat (Willmer et al., 2005). Consequently, ectotherms that live in lentic environments may experience substantial daily environ-mental temperature variations (Willmer et al., 2005) that should affect their performance. For anuran tadpoles, which generally develop in aquatic environments, water temperature is one of the most important factors that affect their growth, developmental rate, body size at metamorphosis, and ultimately survival (e.g., Marian and Pandian, '85; Berven, '90; Álvarez and Nicieza, 2002).

Feeding is one of the most conspicuous behaviors of tadpoles and, along with other morphological and ecological features, is used for sorting tadpoles into ecomorphological guilds (Altig and Jonhston, 1989). Two common guilds include benthic and nektonic tadpoles, both of which can feed by scraping food from submerged surfaces. They differ, however, in their preferred location in ponds: benthic tadpoles occur at or near the pond bottom, whereas nektonic tadpoles are found within the water column. Benthic and nektonic tadpoles can be found throughout the pond, but the depth where they can be found will vary according to the species preferences (e.g. Prado et al., 2009). As deep waters are comparatively colder than shallow waters (Newman, '89), tadpoles occurring in different microhabitats will experience different temperatures. Tadpoles occupying shallow waters will experience drastic temperature fluctuations compared to tadpoles in deeper waters. Indeed, tadpoles' feeding rate and many fitness correlates (e.g., growth rates and size at metamorphosis) are strongly affected by variation in temperature (Marian and Pandian, '85; Warkentin, '92; Álvarez and Nicieza, 2002; Liess et al., 2013).

Because of these ecological and biological differences, one might predict that tadpoles that experience different average temperatures should evolve to exhibit temperature-dependent feeding strategies that maximize food consumption in those environments. We conducted a laboratory study aimed at exploring how differences in temperature affect feeding behavior of larvae of two anuran species: the benthic tadpoles of the toad *Rhinella schneideri* (Werner, 1894) and the nektonic tadpoles of the treefrog *Trachycephalus typhonius* (Linnaeus, 1758). These species occur in the same geographic areas and it is common to find their tadpoles co-occurring in the same ponds (e.g. Rossa-Feres et al., 2004; Duarte et al., 2012). In South America, tadpoles of both species are found in ponds in which temperature varies from 19.8 to 38.7°C (Duarte et al., 2012). Though species co-occur in the same ponds, tadpoles of *R. schneideri* have higher heat tolerance compared to *T. typhonius* (CT_{max} of 42.5°C and 41.9°C, respectively) (Duarte et al., 2012).

We first hypothesized that temperature would influence tadpole feeding kinematics. Irrespective of species, we predicted that tadpoles acclimated in warmer temperatures would have faster kinematics compared to tadpoles raised in colder temper-atures, because muscle contractile dynamics is primarily temper-ature dependent (James, 2013). Next we hypothesized that temperature would have differential effects on the feeding kinematics of these two species. Tadpoles of *R. schneideri* are lungless, negatively buoyant, and consequently benthic (Ultsch et al., '99). Because these benthic tadpoles occur primarily on the bottom of ponds in shallow depths (< 25 cm, Prado et al., 2009), and cannot regulate their position in the water column because of their negative buoyancy, they should experience relatively large diurnal shifts in environmental temperatures. In contrast, tadpoles of *T. typhonius* have functional lungs long before

*Correspondence to: Verônica T. T. de Sousa, Universidade Federal de Goiás, PPG Ecologia e Evolução, Av. Esperança, s/n, Setor Itatiaia, CEP 74001-970, Goiânia, Goiás, Brasil.
E-mail: veronicathiemi@outlook.com

metamorphosis and can achieve neutral buoyancy (Zweifel, '64). They are nektonic and can swim throughout the water column. Thus, they should be better buffered from extreme shifts in temperature because they can choose a microhabitat that is closest to their optimal temperature.

Because the opening of the jaws depends mostly on muscle contractile dynamics (Gradwell, '72; Cannatella, '99), whereas the closing of the jaws is affected by resistance on a substrate (Wassersug and Yamashita, 2001), we predicted that tadpoles of *R. schneideri* would have relatively stable feeding kinematics as they opened their mouths for feeding in both cold and warm temperatures (i.e., they would feed effectively at both temperatures). In contrast, we predicted that tadpoles of *T. typhonius* would exhibit faster kinematics and attain maximum gape earlier during the gape cycle when they fed at warm temperatures but not in colder water.

MATERIAL AND METHODS

Study Area

Tadpoles of *R. schneideri* and *T. typhonius* were obtained in ponds located in Nova Itapirema (21°04'44.83"S, 49°32'22.00"W), northwestern of São Paulo State, Brazil. The climate of this region is Aw Köppen-Geiger (Alvares et al., 2013), characterized by hot and wet summers (October–March) and dry winters (April–September). The annual rainfall varies from 1,200 to 1,650 mm (Carvalho and Assad, 2005) and the onset of the rainy season varies each year (Rossa-Feres and Jim, 2001). In the beginning of the rainy season (during October), the temperature in ponds may be as low as 21°C, and it may reach 37°C in the middle of the season (during January) (Rossa-Feres, unpublished data). The original vegetation cover, of Mesophytic Semideciduous Forest (Atlantic Forest Domain) with patches of Cerrado, was intensively deforested for agro-pastoral activities, and the remaining fragments of original vegetation are few and small (SMA/IF, 2005).

Study Species

R. schneideri and *T. typhonius* are widespread through South America (Frost, 2014). In São Paulo State, Brazil, *R. schneideri* and *T. typhonius* are sympatric. Both species breed explosively at the beginning of the rainy season and tadpoles can co-occur in the same temporary ponds, where, as noted above, they tend to use different microhabitats (Rossa-Feres et al., 2004), i.e., *R. schneideri* on the bottom and *T. typhonius* in the water column. Tadpoles of these species differ in labial tooth row formula, with *T. typhonius* having more tooth rows (3/5 in the present study, but up to 4/6 in individuals from other populations) than *R. schneideri* tadpoles (2/3) (Rossa-Feres and Nomura, 2006). Despite the distinct external oral morphology, tadpoles of *R. schneideri* and *T. typhonius* both appear to mainly ingest planktonic algae, but also graze on substrate (Rossa-Feres et al., 2004).

Collection and Maintenance of Tadpoles

Fieldwork was carried out from November to December 2012. Tadpoles of *R. schneideri* and *T. typhonius* were collected from a temporary pond on farmland and transported to a laboratory at the Universidade Estadual Paulista (IBILCE/UNESP, campus of São José do Rio Preto, São Paulo State, Brazil). Tadpoles were collected within a period of 5 (*T. typhonius*) to 8 days (*R. schneideri*) before the beginning of the trials. Each species was maintained in a different polyethylene aquaria (37 × 30 × 10 cm) filled with dechlorinated tap water, which was continuously aerated. They were fed ad libitum once a day with a mixture of flocculated (Alcon BASIC®, Alcon, Camboriú, Santa Catarina, Brazil) and powdered (Sera Micron®, SERA, Heinsberg, Germany) commercial fish food until the beginning of the experiments (which is a standard diet used to maintain healthy tadpoles of these two species in the laboratory). Tadpoles were collected under a permit from the Brazilian Institute for Environment and Natural Renewable Resources (IBAMA) and Chico Mendes Institute for Biodiversity Conservation (ICMBio), Brazil (SISBIO no. 18163-1 to D.C.R.F.) and maintained with approval from the Ethics Committee on the use of Animals (CEUA-IBILCE/UNESP) in accordance with the National Council for Control of Animal Experimentation (CONCEA).

Feeding Trials

To evaluate the effect of temperature on feeding kinematics, 10 tadpoles of *R. schneideri* and 10 tadpoles of *T. typhonius* were randomly selected and allocated to one of two treatment groups: cold (22°C) or warm temperatures (30.5–32°C). The temperatures in which tadpoles were tested fell within the range of the water temperature of the ponds in which they naturally occurred. Tadpoles of different groups were kept in two separate rooms and acclimated at their respective temperatures for 5 days before the start of the feeding trials. Ideally, we would have placed all of the tadpoles in the same room and controlled each container individually. However, we had technical difficulties regulating the appropriate temperature in the small chamber that we used during our feeding trials because tadpoles did not always feed immediately when placed in the filming chamber. Thus, we elected to film in two separate rooms so that we would minimize temperature variation between each replicate. Water temperature was measured with a thermometer immediately before the start of each feeding trial.

Our feeding experiment followed the protocol described in Venesky et al. (2011). Briefly, prior to each feeding trial, we made a mixture of Sera Micron® and water, brushed it on one side of a glass microscope slide, and allowed it to air dry. As tadpoles feeding kinematics is influenced by the resistance they encounter (Wassersug and Yamashita, 2001), all slides were made with the same concentration of Sera Micron®. We then mounted the clean side of one food-covered slide against the inside wall of a glass container (8.5 × 8.5 × 8.5 cm) and filled it with aged

dechlorinated tap water. We prefocused a Fastec TroubleShooter LE 250 camera (Fastec Imaging, San Diego, CA) on the food-covered surface prior to each trial and video-recorded the tadpoles in individual trials while they grazed on this surface. We used a fresh food-covered slide for each trial to ensure that tadpoles had access to a substrate of similar density and food concentration. Video images were capture at 500 frames per second and the camera's vertical field of view was adjusted as necessary during recording.

In these trials, we recorded single feeding bouts, with a "feeding bout" defined as beginning when a tadpole contacted the food covered surface and opened its mouth to the time when the tadpole completely closed its mouth and swam away. Each feeding bout consisted of a continuous series of "gape cycles", during which the tadpoles scraped food from the slide. As per Venesky et al. (2011), a "gape cycle" (1) starts with the jaw sheaths fully closed and the anterior and posterior tooth rows in closest proximity; (2) proceeds to the point where the mouth is fully open and the labial tooth rows reached maximum gape; and (3) ends with full closure of the jaw sheaths and anterior and posterior tooth rows again in closest proximity to each other.

After each feeding trial, we euthanized the tadpoles with an overdose of an anesthetic (2% lidocaine hydrochloride) and then fixed them in a 1:1 solution mixture of 70% alcohol and 15% formalin. The developmental stage of the tadpoles was determined according to Gosner ('60) and the total length of each larvae was measured. All specimens were subsequently deposited in the amphibian collection of the Department of Zoology and Botany of IBILCE/UNESP (DZSJRP-Amphibia-Tadpoles; *T. typhonius*: DZSJRP 2420.1, *R. schneideri*: DZSJRP 2430.1).

Feeding Kinematics

In order to compare the kinematics of tadpoles grazing behavior under cold and warm temperatures, we quantified six kinematic variables that were common to feeding in both settings: (i) *Maximum gape*—the length (in millimeters) from the internal border of the upper jaw sheath to the internal border of the lower jaw sheath as a percentage of the upper jaw sheath width; (ii) *Duration of the full gape cycle*—the duration of time (in milliseconds) from when the jaws begin to open until they are fully closed; (iii) *Percentage of time to maximum gape*—the duration of time, as a percentage of the total time of the gape cycle, from when the mouth starts to open to when maximum gape is achieved; (iv) *Percentage of time to lower jaw narrowing*—the duration of time, as a percentage of the total time of the gape cycle, from when the mouth starts to open to when the lower jaw starts to narrow; (v) *Opening phase velocity*—the velocity at which upper and lower jaws move from the initial position of full contact between jaws to the maximum gape position; and (vi) *Closing phase velocity*— the velocity at which upper and lower jaws move from the initial position of maximum gape to the final position of jaws fully closed.

Velocity was calculated as the displacement (in millimeters) of the jaws during opening and closing phases of a gape cycle divided by the time elapsed during the considered phase of the gape cycle. We randomly extracted data from 4–5 gape cycles from each tadpole. Thus, 48 gape cycles were analyzed for *R. schneideri* and 50 for *T. typhonius*. All measurements were obtained using ImageJ 1.47m (Rasband, 2012).

Data Analyses

To evaluate how kinematic variables were affected by temperature, we calculated the Q_{10} temperature coefficient, which is a measure of the temperature sensitivity of a physiological process (IUPS, 2003). This coefficient was calculated using the following equation: $Q_{10} = (R_2/R_1)^{10/(T_2 - T_1)}$, where R_1 and R_2 represent the reaction rates at the lowest (T_1) and highest (T_2) temperatures, respectively (Willmer et al., 2005). We calculated Q_{10} coefficients for all kinematic variables except for the duration variables for which we calculated the inverse Q_{10} (i.e., $1/Q_{10}$) because duration can be expressed as a rate by its reciprocal. A Q_{10} value can be interpreted as the factor by which a reaction rate changes as temperature increases by 10°C. If the rate of the reaction is completely temperature independent, the resulting Q_{10} will be equal to 1. If the reaction rate increases with increasing temperature, the Q_{10} will be greater than 1. If the reaction rate decreases with increasing temperature, the Q_{10} will be less than 1.

In order to verify whether Q_{10} values indicate that feeding kinematic variables are affected by the temperature, we tested if the Q_{10} values calculated for each variable differed significantly from a value of 1 using the one-sample t-test. This procedure verifies whether a given sample is likely to have been taken from a population with a given (theoretical) mean. The 95% confidence interval for the mean is calculated using the t distribution, based on a bootstrapping algorithm. Also, we verified whether Q_{10} values for each kinematic variable differed between species using t-tests for equal means. All t-tests were calculated using the function "t.test" in R software "stats" package (R Core Team, 2013).

To test for species and species x temperature interactions in feeding kinematics, we fit a linear mixed effects model for each kinematic variable by using the "lmer" function in the "lme4" package (Bates et al., 2014) built in R software (R Core Team, 2013). In each model, we nested each gape cycle within an individual tadpole. We tested for main and interactive effects of species and temperature (predictors) on the kinematic variables (response variables). Tadpoles used in the experiment were at similar developmental stages (Gosner developmental stages range, *T. typhonius*: 35–38, *R. schneideri*: 34–40), but, on average, tadpoles of *T. typhonius* were longer than *R. schneideri* tadpoles (*T. typhonius*: 36.0 ± 3.0 mm, *R. schneideri*: 23.7 ± 2.7 mm). Thus, we included tadpole size (total length) as a covariate in the models. We assessed statistical significance at $P < 0.05$ by using likelihood ratio tests using the "lrtest" function in the "lmtest" package (Zeileis and Hothorn, 2002).

RESULTS

In general, tadpoles of the two species move their mouths differently (Table 1). Tadpoles of *R. schneideri* are capable of attaining a larger maximum gape, exhibited shorter gape cycles, and opened their mouths faster than *T. typhonius* tadpoles irrespective of the temperature to which they were exposed. We also found that the kinematic variables are affected by temperature (Table 2).

For both species, we found $Q_{10} > 1$ for the duration of the gape cycle, indicating that tadpoles exhibited shorter gape cycles in higher temperatures (Table 2). Although the Q_{10} value for the two species appear qualitatively similar for this variable, we detected a significant difference when testing for species differences in duration of the gape cycle (Table 3).

Species did not differ in relation to the changes in the maximum gape achieved by their tadpoles (Table 3), i.e., tadpoles of both species attained a smaller maximum gape in warmer temperature, which resulted in a $Q_{10} < 1$ for this variable (Table 2). However, species respond differently in time to achieve maximum gape as a percentage of the entire gape cycle as well as the percentage of time when the lower jaw starts to narrow (Table 3). The fact that the Q_{10} value was smaller than 1 for *R. schneideri* but greater than 1 for *T. typhonius* (Table 2) indicates that *T. typhonius* increased the rate of their feeding kinematics in warmer temperature by opening and closing their jaw relatively sooner in the gape cycle than *R. schneideri*. In contrast, for *R. schneideri* tadpoles, Q_{10} values <1 indicate that they achieved maximum gape and also started closing their mouth proportionally later in their gape cycle when in warm temperatures.

Although Q_{10} values indicate that tadpoles do not adjust the velocity at which they open their jaws according to the temperature they experience (i.e., Q_{10} values were not different from 1; Table 2), both species tended to increase the velocity of opening phase when in warmer water (Table 1). For the velocity of closing phase, Q_{10} values were significantly different from a value of 1 (Table 2), indicating that changes in the mean temperature affected this component of feeding. Tadpoles of *T. typhonius* slightly decreased the velocity at which they close their jaws in warmer water, while *R. schneideri* increased it (Tables 1 and 2).

Results from the linear mixed effects modeling (Table 4) corroborated most of the results we obtained for the temperature coefficient (Table 2) except for the opening phase velocity and the percentage of time it took tadpoles to attain maximum gape (Table 4). For the opening phase velocity, modeling results indicate that the effect of the temperature on the velocity at which tadpoles open their jaws is significant (Table 4). For the percentage of time tadpoles take to achieve maximum gape, the effect of the temperature was only found in the species x temperature interaction term (Table 4, Fig. 1A). A significant interaction was also detected for the percentage of time it takes tadpoles to start to close their jaws (Table 4, Fig. 1B) and for the closing phase velocity (Table 4, Fig. 1B). These interactions indicate that our *R. schneideri* tadpoles achieved maximum gape and started to close their mouths proportionally later in the gape cycle, but faster than tadpoles of *T. typhonius* when in warm water (Table 1, Fig. 1). We found these effects even when controlling for size as a covariate in our statistical models (Table 4).

Table 1. Mean and standard deviation (SD) of kinematic variables collected from tadpoles of *Rhinella schneideri* and *Trachycephalus typhonius* while feeding in cold (22°C) and warm (30.5—32°C) water. Maximum gape was measured as a percentage of the upper jaw sheath width (UJW).

Kinematic variable	*Trachycephalus typhonius*		*Rhinella schneideri*	
	Mean	SD	Mean	SD
Cold temperaure				
Maximum gape (as % of UJW)	118.520	10.681	135.674	14.389
Duration of gape cycle (ms)	61.267	5.977	53.250	6.067
% Time to maximum gape	49.356	4.347	42.370	5.387
% Time to lower jaw starts to narrow	66.311	4.246	61.637	5.181
Opening phase velocity (mm/s)	67.520	13.075	88.892	16.218
Closing phase velocity (mm/s)	65.523	11.397	64.506	7.588
Warm temperature				
Maximum gape (as % of UJW)	113.713	7.877	123.523	15.784
Duration of gape cycle (ms)	53.200	8.859	42.083	4.393
% Time to maximum gape	44.976	4.656	47.743	7.484
% Time to lower jaw starts to narrow	59.114	6.447	67.580	4.091
Opening phase velocity (mm/s)	76.631	18.733	100.644	26.095
Closing phase velocity (mm/s)	61.930	11.837	90.827	21.391

Table 2. Temperature sensitivity of kinematic variables measured for tadpoles of *Rhinella schneideri* and *Trachycephalus typhonius* anuran species during the feeding experiment. Sensitivity was evaluated through the Q_{10} or $1/Q_{10}$ temperature coefficients. For each kinematic variable, one-sample t-test was applied to verify whether Q_{10} (or $1/Q_{10}$) differed from 1, which would indicate that the kinematic variable was affected by the temperature.

Kinematic variables	Q_{10}	95%IC	t	P
Trachycephalus typhonius				
Maximum gape	0.932	0.895–0.967	−4.023	<0.001
Duration of gape cycle	1.177[a]	1.092–1.261	4.379	<0.001
% Time to maximum gape	1.120[a]	1.031–1.209	2.817	0.011
% Time to lower jaw starts to narrow	1.169[a]	1.099–1.238	5.105	<0.001
Opening phase velocity	1.119	0.912–1.325	1.206	0.242
Closing phase velocity	0.884	0.825–0.942	−4.167	<0.001
Rhinella schneideri				
Maximum gape	0.904	0.823–0.987	−2.407	0.025
Duration of gape cycle	1.318[a]	1.228–1.410	7.265	<0.001
% Time to maximum gape	0.899[a]	0.804–0.994	−2.211	0.038
% Time to lower jaw starts to narrow	0.914[a]	0.868–0.959	−3.961	<0.001
Opening phase velocity	1.188	0.984–1.390	1.914	0.069
Closing phase velocity	1.504	1.281–1.727	4.697	<0.001

[a]The Q_{10} temperature coefficient was calculated as Q_{10} inverse (i.e., $1/Q_{10}$).

DISCUSSION

Temperature has the potential to significantly impact ectotherm performance by influencing many of their physiological processes. In the present study, we show how temperature alters tadpoles feeding kinematics. Irrespective of species, tadpoles that feed in warm water have shorter gape cycles and faster opening phase. The fact that we observed a main effect of temperature on the duration of gape cycle and opening of the jaws is not surprising because these movements are determined by the jaws muscles contractile speed, which is temperature dependent (e.g., James, 2013). According to the theoretical thermal performance curve (Huey and Stevenson, '79), physiological and biochemical processes tend to function better when organisms are exposed to moderate to relatively high temperatures. Thus, when tadpoles occupy warmer water, their muscles should perform more efficiently and they should open their jaws faster than when they occupy colder water. Tadpoles also exhibit a smaller maximum gape when feeding in warm water compared to when they feed in cold water. This may indicate that there is a tradeoff between the speed in which tadpoles feed and the surface area from which they are able to scrape food from. Whether this affects food consumption remains untested and is an important gap in our knowledge about tadpole feeding behavior (Hoff et al., '99; Borges and Rocha, 2013).

We also found that tadpoles of *R. schneideri* and *T. typhonius* generally differ in their feeding kinematics, corroborating findings of previous studies in which the feeding kinematics of divergent taxa on a common substrate was compared (e.g. Venesky et al., 2011 Venesky et al., 2013). Regardless of the water temperature in which tadpoles were foraging, *T. typhonius* consistently exhibited longer gape cycles, slower opening phases, and smaller maximum gapes than *R. schneideri*. Differences in feeding kinematics were expected because tadpoles of these species belong to different ecomorphological guilds (Altig and Johnston, '89). However, our results show the opposite patterns compared to a recent study on how tadpoles of ecomorphological guilds feed (Venesky et al., 2013). In their previous work, Venesky et al. (2013) found that benthic feeding tadpoles had longer gape

Table 3. Results of the two-sample t-test for equal means applied to verify whether tadpoles of *Rhinella schneideri* and *Trachycephalus typhonius* exhibited comparatively different temperature sensitivity for each kinematic variable measured. Sensitivity was evaluated through the Q_{10} or $1/Q_{10}$ temperature coefficients.

Kinematic variables	t	P
Maximum gape	−0.608	0.548
Duration of gape cycle	2.392	0.021
% Time to maximum gape	−3.538	<0.001
% Time to lower jaw starts to narrow	−6.442	<0.001
Opening phase velocity	0.492	0.625
Closing phase velocity	5.595	<0.001

Table 4. Results from the linear mixed effects modeling on the effects of species, temperature, and species x temperature interaction on each feeding kinematic variable (Maximum gape, Duration of the full gape cycle, Percentage of time to maximum gape, and Percentage of time to lower jaw narrowing) for tadpoles of *Rhinella schneideri* and *Trachycephalus typhonius*.

Source of variation	χ^2	P
Maximum gape		
Species	13.559	0.001
Temperature	7.946	0.019
Total length	2.506	0.113
Species x temperature	3.689	0.055
Duration of gape cycle		
Species	12.040	0.002
Temperature	13.505	0.001
Total length	0.826	0.363
Species x temperature	0.802	0.370
% Time to maximum gape		
Species	11.597	0.003
Temperature	5.497	0.064
Total length	0.309	0.578
Species x temperature	6.457	0.011
% Time to lower jaw starts to narrow		
Species	22.344	<0.001
Temperature	10.886	0.004
Total length	0.455	0.500
Species x temperature	12.348	<0.001
Opening phase velocity		
Species	18.210	<0.001
Temperature	8.267	0.016
Total length	5.100	0.024
Species x temperature	2.778	0.096
Closing phase velocity		
Species	19.191	<0.001
Temperature	12.883	0.002
Total length	2.173	0.141
Species x temperature	7.482	0.006

cycles compared to nektonic tadpoles. Thus, our data suggest that differences in tadpole feeding behavior might not be as simple and straightforward as previously thought. For example, Venesky et al. (2013) found that the total number of labial tooth rows that a tadpole had was a better predictor of the duration of the gape cycle than whether a particular species was categorized as a benthic or nektonic tadpole. When tadpoles feed, one of the functions of their labial tooth rows is to anchor the oral disc to the substrate so that the jaws can scrape food from the surface (Wassersug and Yamashita, 2001). During the closing phase of the gape cycle, the posterior tooth rows are generally released in a

serial fashion, with the inner row being the first to disengage from the substrate and the outer row, the last (Wassersug and Yamashita, 2001; Venesky et al., 2010). Because of this, the duration of the gape cycle should be positively related to the number of tooth rows (Venesky et al., 2010). Thus, the inconsistency between the results of our present study and those of Venesky et al. (2013) is likely explained by the differences in the oral morphology of the study species in each guild, or an interaction between feeding guild and the number of tooth rows for the species in either guild.

As described by Wassersug and Yamashita (2001) and corroborated by other studies (e.g. Venesky et al., 2011; Venesky et al., 2013), tadpoles start to open their mouths as they approach a food source. The labial teeth then anchor a tadpole's oral disc to the substrate while the keratinized jaw sheaths close and rake material off the substrate. As the jaws sheaths start to close, the lower jaw may narrow allowing the bite force to be focused on a smaller area of the lower jaw. Simultaneously with the closing of the jaws, the posterior labial tooth rows release sequentially (or concurrently) from the substrate, passing over the surface as they move toward the oral opening. These combined actions generate a suspension of material that is drawn into the tadpole's mouth in the next gape cycle as the buccal floor is depressed during opening phase.

During feeding, six hyoid and mandibular muscles are activated, with different phases of a gape cycle being controlled by different muscles (Larson and Reilly, 2003). These include: the hyoangularis and the orbitohyoideus muscles, which are active during the opening phase; suspensorioangularis, which regulates the width of the jaws; and anterior interhyoideus, intermandibularis and levator mandibulae longus superficialis, which assist during the closing phase of the jaws. The fact that the tadpoles can adjust both the opening and closing kinematics of their jaws with respect to temperature and that these changes are not in the same direction (i.e., temperature may have a positive effect on some kinematic variables but negative effects on others) suggests that the effect of temperature on muscles that control the opening and closing of the jaws is not similar. Exactly how temperature influences the function on individual hyoid and mandibular muscles of tadpoles remains to be assessed.

One of our most intriguing results was that temperature does not necessarily shift the feeding kinematics of both species in the same direction. In cold water, the benthic feeding tadpoles of *R. schneideri* (and also the species with fewer tooth rows) achieved the maximum gape and started to close their mouths proportionally earlier in the gape cycle compared to the nektonic feeding tadpoles of *T. typhonius* (the species with more tooth rows). In warm water, this pattern is inverted. In relation to the closing phase velocity, tadpoles close their jaws in a similar rate in cold temperatures; but in warm water, tadpoles of *R. schneideri* increases the velocity at which they close their jaws, while *T. trachycephalus* slightly decreases it. The interaction between temperature and species is logical, if one considers the fact that
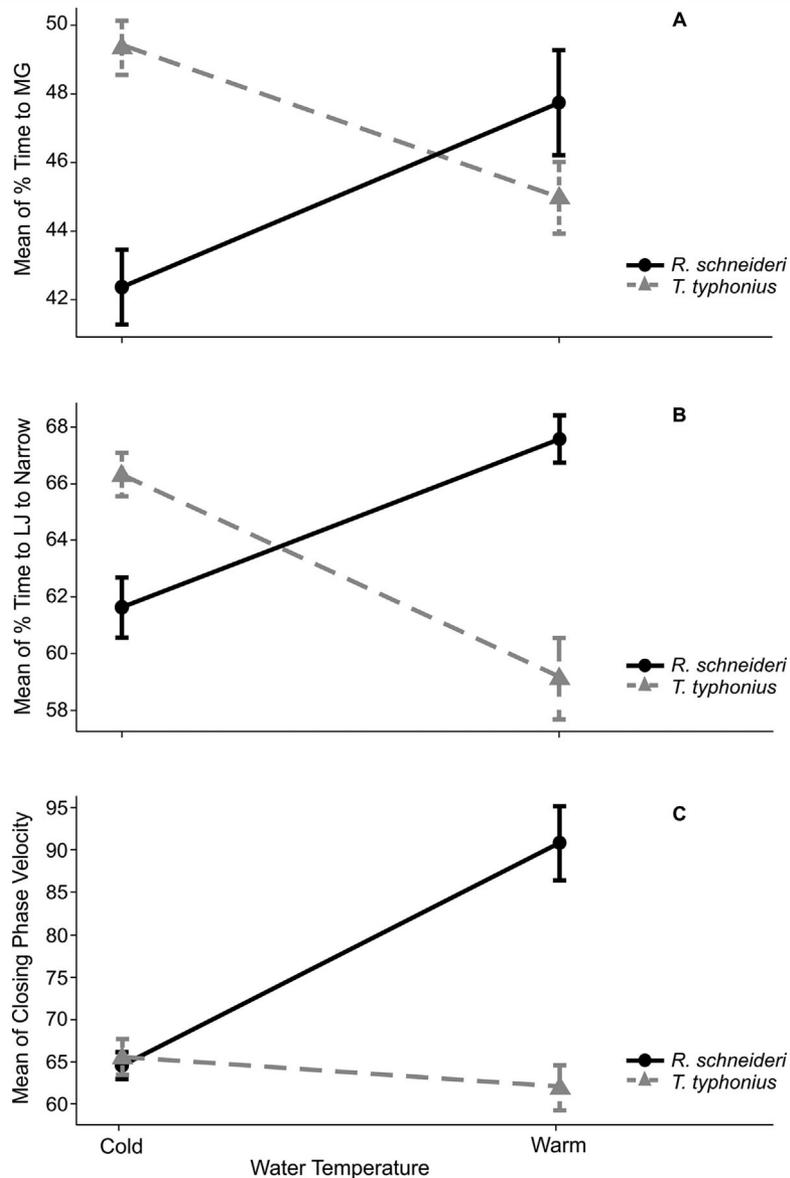
Figure 1. The species x temperature interaction effect on (**A**) the percentage of time tadpoles take to achieve maximum gape (MG), (**B**) the percentage of time tadpoles take to narrow the lower jaw (LJ) as mouth start closing, and (**C**) the velocity at which tadpoles close their mouth. Solid triangles and circles represent mean values and bars indicate standard error.

tadpoles of *R. schneideri* should be well adapted to larger thermal variation given their ecology and behavior. As tadpoles of *R. schneideri* occur in the bottom of ponds but in shallow waters (Prado et al., 2009), they experience both colder and warmer temperatures. This can be seen in the results found for their feeding at the colder temperature. It is known that tadpoles with larger maximum gapes open and close their jaws slower than the ones that exhibit smaller maximum gapes (Venesky et al., 2013),

but that is not how *R. schneideri* behaves. In colder water, tadpoles of *R. schneideri* achieve larger maximum gapes than both tadpoles of the same species subjected to the warmer temperatures, and tadpoles of *T. typhonius* in colder temperature. Yet, tadpoles of *R. schneideri* achieved a larger maximum gape sooner in cold water than in warm water, indicating that their jaw movements are not restrained by the cold temperature and, possibly that they function better in colder temperatures.

Although this article focused primarily on the functional morphology of tadpole feeding, the interaction between species and temperature on tadpole feeding kinematics could have broader ecological implications. Our data show that temperature changes can affect feeding kinematics of sympatric tadpoles in different ways. It is thus plausible that climate-related changes in pond temperature could indirectly drive changes in the structure of the community within ponds upon which tadpoles feed. Given that tadpoles can act as "ecosystem engineers" by modifying habitat structure and biomass availability in waterbodies (e.g. Flecker et al., '99; Ranvestel et al., 2004; Wood and Richardson, 2010), such changes in feeding behavior for different species could lead to cascading effects on the full food webs (e.g., Wilbur, '97). These effects in pond communities could become more pronounced because of the projected increase in extreme temperature events (Easterling et al., 2000).

Tadpoles, however, can exhibit behaviors that do not necessarily correlate with explicit morphological adaptations, such as the diurnal–benthic and nocturnal–neustonic habits of *R. schneideri* tadpoles (Rossa-Feres et al., 2004) and likely exhibit plasticity in their feeding behaviors (de Sousa et al., 2014). Thus, we can not predict how changes in tadpole feeding kinematics associated with temperature change in a pond will affect the aquatic community overall. Future studies that more thoroughly explore how phylogeny, ecomorphology, behavioral plasticity, and muscle physiology interact to affect feeding kinematics are needed to fully understand tadpole feeding at the individual and community level.

ACKNOWLEDGMENTS

LITERATURE CITED

Altig R, Johnston GF. 1989. Guilds of anuran larvae: relationships among developmental modes, morphologies, and habitats. Herpetol Monogr 3:81–109.

Alvares CA, Stape JL, Sentelhas PC, de Moraes Gonçalves JL, Sparovek G. Köppen's climate classification map for Brazil. Meteorol Z 22:711–728.

Álvarez D, Nicieza G. 2002. Effects of temperature and food quality on anuran larval growth and metamorphosis. Funct Ecol 16:640–648.

Berven KA. 1990. Factors affecting population fluctuations in larval and adult stages of the wood frog (*Rana sylvatica*). Ecology 71:1599–1608.

Bates D, Maechler M, Bolker B, Walker S. 2014. lme4: Linear mixed-effects models using Eigen and S4. R package version 1.1-7. Available online at: http://CRAN.R-project.org/package=lme4 [Accessed on July, 28th 2014].

Borges Júnior VNT, Rocha CFD. 2013. Tropical tadpole assemblages: which factors affect their structure and distribution? Oecol Aust 17:27–38.

Cannatella D. 1999. Architecture: cranial and axial musculoskeleton. In: McDiarmid RW, Altig R, editors. Tadpoles: the biology of anuran larvae. Chicago, IL: The University of Chicago Press. p 52–91.

Carvalho JRP, Assad ED. 2005. Análise espacial da precipitação pluviométrica no Estado de São Paulo: comparação de métodos de interpolação. Eng Agr - Jaboticabal 25:377–384.

de Sousa VTT, Nomura F, Venesky MD, et al. 2014. Flexible feeding kinematics of a tropical carnivorous anuran tadpole. J Zool 293:204–210.

Duarte H, Tejedo M, Katzenberger M, et al. 2012. Can amphibians take the heat? Vulnerability to climate warming in subtropical and temperate larval amphibian communities. Global Change Biol 18:412–421.

Easterling DR, Meehl GA, Parmesan C, et al. 2000. Climate extremes: observations, modeling, and impacts. Science 289:2068–2074.

Flecker AS, Feifarek BP, Taylor BW. 1999. Ecosystem engineering by a tropical tadpole: density-dependent effects on habitat structure and larval growth rates. Copeia 1999:495–500.

Frost DR. 2014. Amphibian species of the world: an online reference. Version 6.0. Available online at: http://research.amnh.org/herpetology/amphibia/index.html [Accessed on June 23rd, 2014].

Gosner KL. 1960. A simplified table for staging anuran embryos and larvae with note on identification. Herpetologica 16:183–190.

Gradwell N. 1972. Gill irrigation in *Rana catesbeiana*. Part II. On the musculoskeletal mechanism. Can J Zool 50:501–521.

Hernández LP. 2000. Intraspecific scaling of feeding mechanics in an ontogenetic series of zebrafish, *Danio rerio*. J Exp Biol 203:3033–3043.

Herrel A, Van Wassenbergh S, Wouters S, Adriaens D, Aerts P. 2005. A functional morphological approach to the scaling of the feeding system in the African catfish, *Clarias gariepinus*. J Exp Biol 208:2091–2102.

Hill AV. 1950. The dimensions of animals and their muscular dynamics. Sci Prog 38:209–230.

Hoff KS, Blaustein AR, McDiarmid RW, Altig R. 1999. Behaviour - Interactions and their consequences. In: McDiarmid RW, Altig R, editors. Tadpoles: the biology of anuran larvae. Chicag, IL: The University of Chicago Press. p 215–239.

Huey RB, Stevenson RD. 1979. Integrating thermal physiology and ecology of ectotherms: a discussion of approaches. Am Zool 19:357–366.

James RS. 2013. A review of the thermal sensitivity of the mechanics of vertebrate skeletal muscle. J Comp Physiol B 183:723–733.

Kupferberg S. 1997. Facilitation of peryphiton production by tadpole grazing: functional differences between species. Freshwater Biol 37:427–439.

Larson PM, Reilly SM. 2003. Functional morphology of feeding and gill irrigation in the anuran tadpole: electromyography and muscle function in larval *Rana catesbeiana*. J Morphol 255:202–214.

Liess A, Rowe O, Guo J, Thomsson G, Lind MI. 2013. Hot tadpoles from cold environments need more nutrients—life history and stoichiometry reflects latitudinal adaptation. J Anim Ecol 82:1316–1325.

Marian MP, Pandian TJ. 1985. Effect growth of temperature on development, and bioenergetics of the bullfrog tadpole *Rana tigrina*. J Therm Biol 10:157–161.

Newman RA. 1989. Developmental plasticity of *Scaphiopus couchii* tadpoles in an unpredictable environment. Ecology 70:1775–1787.

Niehaus AC, Angilletta MJ, Sears MW, Franklin CE, Wilson RS. 2011. Predicting the physiological performance of ectotherms in fluctuating thermal environments. J Exp Biol 215:694–701.

Prado VHM, Fonseca MG, Almeida FVR, Necchi Jr O, Rossa-Feres DC. 2009. Niche occupancy and the relative role of micro-habitat and diet in resource partitioning among pond dwelling tadpoles. South Am J Herpetol 4:275–285.

R Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available online at: http://www.R-project.org/ [Accessed on July 27, 2014].

Ranvestel AW, Lips KR, Pringle CM, Whiles MR, Bixby RJ. 2004. Neotropical tadpoles influence stream benthos: evidence for the ecological consequences of decline in amphibian populations. Freshwater Biol 49:274–285.

Rasband WS. 2012. ImageJ. U. S. National Institutes of Health, Bethesda, Maryland, USA, Available online at: http://imagej.nih.gov/ij/ [Accessed on June 02, 2013].

Reilly SM. 1995. The ontogeny of aquatic feeding behavior in *Salamandra salamandra*: stereotypy and isometry in feeding kinematics. J Exp Biol 198:701–708.

Richard BA, Wainwright PC. 1995. Scaling the feeding mechanism of largemouth bass (*Micropterus salmoides*): kinematics of prey capture. J Exp Biol 198:419–433.

Rossa-Feres DC, Jim J. 2001. Similaridade no sítio de vocalização em uma comunidade de anfíbios anuros na região noroeste do Estado de São Paulo, Brasil. Rev Bras Zool 18:439–454.

Rossa-Feres DC, Jim J, Fonseca MG. 2004. Diets of tadpoles from a temporary pond in southeastern Brazil (Amphibia, Anura). Rev Bras Zool 21:745–754.

Rossa-Feres DC, Nomura F. 2006. Characterization and taxonomic key for tadpoles (Amphibia: Anura) from the northwestern region of São Paulo State, Brazil. Biota Neotrop 6: Available online at: http:// www.biotaneotropica.org.br/v6n1/pt/abstract?identification-key+bn00706012006.

Ryerson WG, Deban SM. 2010. Buccal pumping mechanics of *Xenopus laevis* tadpoles: effects of biotic and abiotic factors. J Exp Biol 213:2444–2452.

2005. Inventário florestal da vegetação natural do Estado de São Paulo. São Paulo: Imprensa Oficial. 200 p.

Ultsch GR, Bradford DF, Freda J. 1999. Physiology: coping with the environment. In: McDiarmid RW, Altig R, editors. Tadpoles: the biology of anuran larvae. Chicago, IL: The University of Chicago Press. p 189–214.

Van Wassenbergh S, Herrel A, James RS, Aerts P. 2007. Scaling of the contractile properties of catfish feeding muscles. J Exp Biol 210:1183–1193.

Venesky MD, Rossa-Feres DC, Nomura F, et al. 2013. Comparative feeding kinematics of tropical hylid tadpoles. J Exp Biol 216:1928–1937.

Venesky MD, Wassersug RJ, Jorgensen ME, Riddle M, Parris MJ. 2011. Comparative feeding kinematics of temperate pond-dwelling tadpoles (Anura, Amphibia). Zoomorphology 130:31–38.

Venesky MD, Wassersug RJ, Parris MJ. 2010. How does a change in labial tooth row number affect feeding kinematics and foraging performance of a ranid tadpole (*Lithobates sphenocephalus*) ? Biol Bull 218:160–168.

Warkentin KM. 1992. Effects of temperature and illumination on feeding rates of Green Frog tadpoles (*Rana clamitans*). Copeia 1992:725–730.

Wassersug RJ, Yamashita M. 2001. Plasticity and constraints on feeding kinematics in anuran larvae. Comp Biochem Physiol A Mol Integr Physiol 131:183–195.

Wilbur HM. 1997. Experimental ecology of food webs: complex systems in temporary ponds. Ecology 78:2279–2302.

Willmer P, Stone G, Johnston I. 2005. Environmental physiology of animals. Oxford: Blackwell Publishing. p 768.

Wilson RS, Franklin CE. 1999. Thermal acclimation of locomotor performance in tadpoles of the frog *Limnodynastes peronii*. J Comp Physiol B 169:445–451.

Wood SLR, Richardson JS. 2010. Evidence for ecosystem engineering in a lentic habitat by tadpoles of the western toad. Aquat Sci 72:499–508.

Wu H-J, Yen C-F, Kam Y-C. 2007. Metabolic compensation and behavioral thermoregulation of subtropical rhacophorid (*Polypedates megacephalus*) tadpoles in container habitats. Comp Biochem Physiol B Biochem Mol Biol 146:101–106.

Zeileis A, Hothorn T. 2002. Diagnostic checking in regression relationships. R News 2: 7–10. Available online at: http://CRAN.R-project.org/doc/Rnews/ [Accessed on July 28th, 2014].

Zweifel RG. 1964. Life history of *Phrynohyas venulosa* (Salientia: Hylidae) in Panamá. Copeia 1964:201–208.