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Comparative feeding kinematics of tropical hylid tadpoles

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SUMMARY

Anuran larvae, which are otherwise simple in shape, typically have complex keratinized mouthparts (i.e., labial teeth and jaw sheaths) that allow them to graze upon surfaces. The diversity in these structures among species presumably reflects specializations that allow for maximal feeding efficiency on different types of food. However, we lack a general understanding of how these oral structures function during feeding. We used high-speed digital imaging (500 Hz) to observe tadpoles of six species from the anuran family Hylidae grazing on a standardized food-covered substrate. Tadpoles of these species vary in the number of labial tooth rows, belong to two different feeding guilds (benthic and nektonic), and inhabit ponds and streams. We confirmed that the labial teeth in these species serve two functions; anchoring the mouth to the substrate and raking material off of the substrate. In general, tadpoles with a larger maximum gape and those with fewer labial tooth rows opened and closed their mouths faster than tadpoles with smaller gape and more tooth rows. Nektonic feeding tadpoles released each of their tooth rows proportionally earlier in the gape cycle compared to benthic feeding tadpoles. Lastly, we found some support for the idea that deformation of the jaw sheaths during a feeding cycle is predictable based on tadpole feeding guild. Collectively, our data show that anatomic (e.g., number of labial teeth) and ecological features (e.g., feeding guild) of tadpoles significantly influence how tadpoles open and close their mouths during feeding.

Key words: Anura, biomechanics, geometric morphometrics, high-speed digital imaging, Hylidae, tadpole

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INTRODUCTION

One defining characteristic of all anuran larvae is that they have a transient lifestyle—there are no paedomorphic tadpoles and they must go through metamorphosis in order to survive and reproduce (Wassersug, 1974). Since tadpoles of most species live in temporary aquatic bodies of water, they need to be able to metamorphose before the pond dries. However, tadpoles of each species must reach a threshold minimum body size before metamorphosis is possible (Wilbur and Collins, 1973). Selection should thus favor traits that maximize larval growth rates within existing phylogenetic and/or ecological constraints.

A suite of traits that influence tadpole growth rates are those related to the anatomical hardware they have for food acquisition. The anterior and posterior keratinized jaw sheaths (also called a "beak") form the borders of the oral opening (Fig. 1). An oral disc surrounds the external edge of the jaw sheaths and is made up of soft tissue with a free, fringed margin. Between the jaw sheaths and the margin of the oral disc, lying anterior and posterior to the jaw sheaths, are multiple transverse rows of keratinized labial teeth (also called "denticles"). The number of labial tooth rows varies greatly among species, ranging from 0 to 38 rows (Altig and McDiarmid, 1999). This morphological diversity is presumed to reflect specializations that maximize feeding efficiency on various types of food, on different shaped surfaces, and adaptations to abiotic components of the aquatic environment (e.g., water current) in which the tadpoles live (Altig, 2006).

With the exception of obligatory, pelagic suspension-feeding tadpoles and some macrophagous predatory tadpoles which lack keratinized mouthparts, these specialized oral structures are essential for grazing on substrates (Altig and McDiarmid, 1999). In brief, during the opening and closing of the jaws (hereafter "gape cycle"), the labial teeth anchor the oral disc to the substrate as the keratinized jaw sheaths close and rake material off the substrate (Venesky et al., 2011; Wassersug and Yamashita, 2001). Depending on the density of food particles on the substrate, the flexible jaw sheaths can narrow as the mouth closes to better concentrate the bite force on a smaller area (Wassersug and Yamashita, 2001). Thus, part of the gape cycle includes not just the opening and closing of the jaws, but a concurrent narrowing and widening as the jaw sheaths close and open during grazing. After the jaw sheaths are closed, the posterior labial tooth rows sequentially release and rake the surface again. These combined biting actions of the jaw

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sheaths and scraping action of the labial teeth create a suspension of food that is then sucked into the tadpole's mouth during the next gape cycle as the buccal floor is depressed (in rhythmic synchrony with the opening and closing of the mouth). Damage to, or the surgical removal of, keratinized labial teeth causes the tadpole's mouth to slip off an algal covered substrate (Venesky et al., 2010a; Venesky et al., 2010b; Venesky et al., 2010c), resulting in reduced feeding efficiency (Venesky et al., 2009; Venesky et al., 2010b).

Studies to date on tadpole feeding kinematics, however, have only focused on temperate pond-dwelling tadpoles, the majority of which have 2 anterior and 3 posterior labial tooth rows (hereafter formulated as anterior tooth rows/posterior tooth rows [e.g., "2/3"]; Altig and McDiarmid, 1999). Although herpetologists have appreciated the great diversity in tadpole oral structures for well over a hundred years (e.g., Boulenger, 1891) and have used this morphological diversity to diagnose tadpoles of different species, our knowledge of how the morphological diversity relates to tadpole feeding ecology is very limited. A lack of understanding of how this morphological diversity relates to feeding kinematics has prevented us from acquiring a full understanding of tadpole feeding. As such there are a number of pertinent and rather basic questions about the ecology of tadpoles that remain unanswered. For example, how does variation in oral morphology of tadpoles (e.g., the number of labial teeth) relate to variation in feeding kinematics? Can these anatomical differences be used to predict the functional morphology and autecology of the larvae of different species? Does the oral morphology, in turn, influence resource partitioning and therefore community structure/organization in tadpole assemblages?

We used high-speed digital imaging to observe how tadpoles from the anuran family Hylidae graze on a standardized food-covered substrate. We chose to study hylid tadpoles because they represent one of the most species-rich and morphologically diverse of the anuran families. In terms of feeding guilds, the tadpoles we studied here are benthic [*Bokermannohyla alvarengai* (2/5), *B. saxicola* (3/9), *Hypsiboas albopunctatus* (2/3), and *Scinax machadoi* (2/3)] or nektonic [*Agalychnis lemur* (2/3) and *S. fuscovarius* (2/3)] larvae. Tadpoles of these species also differ in the type of water bodies in which they occur. The only true pond dwelling tadpole that we filmed was *S. fuscovarius*, which lives in both temporary and permanent ponds (Rossa-Feres and Nomura, 2006). Tadpoles of *Hypsiboas albopunctatus* are found in permanent slow shallow streams and swamps and less frequently in permanent ponds (Rossa-Feres and Nomura,

2006), whereas tadpoles of *B. saxicola* and *S. machadoi* inhabit permanent streams (Eterovick and Brandao, 2001) and tadpoles of *B. alvarengai* occur in temporary streams (Sazima and Bokermann, 1977). Tadpoles of *A. lemur* are also stream dwelling, but often occur in either the currents or side pools of small streams (Jungfer and Weygoldt, 1994).

Our aim was to compare feeding kinematics for the six species as the larvae graze on a common substrate. We controlled for phylogenetic relationships among our study taxa and tested whether maximum gape, the total number of tooth rows of each species, and their feeding guild were good predictors of the speed at which tadpoles open and close their mouths. We hypothesized that tadpoles with a larger maximum gape and those with more labial teeth would have a longer gape cycle because it would require more time for the greater number of teeth to pass along the substrate. Findings from our previous research suggest that tadpoles in different feeding guilds vary in their jaw kinematics during feeding (Venesky et al. 2011). Thus, we hypothesized that tadpoles of species with similar feeding guilds (e.g., nektonic feeders) would have similar feeding kinematics. Lastly, we used geometric morphometrics to explore the change in shape of the jaw sheaths at different positions during the gape cycle. If benthic feeding tadpoles regularly change the shape of their jaw sheath to scrape food from a variety of surfaces that differ in surface regularity, texture, and hardness (e.g., rocks and leaves), we hypothesized that benthic feeding tadpoles would exhibit greater jaw sheath deformation during feeding than nektonic tadpoles.

MATERIAL AND METHODS

Tadpoles

With the exception of *A. lemur*, all of the tadpoles used in our experiment were field-collected from the states of São Paulo and Minas Gerais, Brazil from January 25–February 5, 2011. Tadpoles ranged in size from 25.53–72.48 mm (see Table S1). Immediately after collection, tadpoles were transported to the laboratory at São Paulo State University. Prior to filming, tadpoles were maintained at a density of 2–4 tadpoles/L in plastic containers filled with approximately 10L of pond water that was continually aerated. Tadpoles were maintained at 22C on a natural photoperiod and were fed daily a powdered commercial (Sera Micron®) algal-based food containing *Spirulina* and sea algae meal. Tadpoles of *A. lemur* were captive-born tadpoles

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from a captive colony at The Atlanta Botanical Gardens, USA and were filmed in 2010 at The University of Memphis under similar laboratory conditions as described above.

Feeding trials

In order to produce a standardized planar substrate on which the tadpoles could graze, we suspended Sera Micron® in water and brushed the mixture on one side of each of 25 glass microscope slides and allowed them to air dry. Each slide contained a uniform layer of dried algae (0.7g ± 0.1g; calculated by subtracting the mass of an empty slide from the mass of a slide with food brushed on it).

Before the start of each feeding trial, we mounted the clean side of the food-covered slide against the inside wall of a glass container (8.5 x 8.5 x 8.5 cm) where the tadpoles were digitally imaged. The container was filled with approximately 175mL of water, which was continually aerated during each trial. We prefocused the camera (Fastec TroubleShooter LE 250; Fastec Imaging, California USA) on the food-covered surface of the microscope slide and adjusted the vertical field of view as necessary during filming. Since kinematics of tadpole feeding is influenced by the resistance they encounter (Wassersug and Yamashita, 2001), we used different food-covered slides for each trial to ensure that tadpoles had access to a similar density of food.

We filmed the tadpoles at 500 Hz in individual trials while they grazed on the food-covered surface, recording a single "feeding bout" (i.e., the point where the mouth of the tadpole first touched the microscope slide until the tadpole fully released from the slide and swam away) for each tadpole. Each feeding bout consisted of a continuous rapid series "gape cycles" (4.5 ± 0.2; mean ± standard error), during which the tadpoles scraped food from the microscope slide. As per Venesky et al., (2011), we define a "gape cycle" as: (1) starting with the jaw sheaths fully closed and the anterior and posterior tooth rows in closest proximity; (2) proceeding to the point where the mouth is fully open and the labial tooth rows reached maximum gape; and (3) ending with full closure of the jaw sheaths and anterior and posterior tooth rows again in closest proximity.

Feeding kinematics

We quantified six kinematic variables that were common to all six species when they actively graze upon a substrate. *Gape cycle*—the duration of time from when the jaws begin to open until

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they are fully closed. *Time to maximum gape*—the duration of time from when the mouth starts to open to when maximum gape is achieved. *Percentage of time to maximum gape*—the duration of time, as a percentage of the total gape cycle, when maximum gape is achieved. *Release time of P-1*—the point in time, as a percentage of the total gape cycle, when posterior tooth row 1 (P-1) begins to move. *Release time of P-2*— the point in time, as a percentage of the total gape cycle, when posterior tooth row 2 (P-2) begins to move. *Release time of P-3*— the point in time, as a percentage of the total gape cycle, when posterior tooth row 3 (P-3) begins to move. Since species differed in the number of posterior tooth rows they have, we only included the shared data on P1-3 in our statistical model; however, we made qualitative observations of the kinematics of the supernumerary tooth rows present in tadpoles of *Bokermannohyla* spp.

We analyzed our digital images frame by frame with Midas OS (Xcitex Inc., 2012). All time measurements were recorded in milliseconds.

Statistical Analyses of the Kinematic Data

Closely related species share a recent evolutionary history and thus might not have truly independent responses. To evaluate the potential influence of phylogeny on our results, we tested whether a statistical model that controlled for phylogeny was more parsimonious than a model that did not control for phylogeny by comparing their AIC_c values (Burnam and Anderson, 2002). First we created a composite phylogeny of the study species (Fig. S1) using previously published data. Genus level topology and branch lengths for this phylogeny were based on the phylogeny of Wiens et al. (2006), because it was well resolved, its relevant genus level topology is consistent with subsequent phylogenetic hypotheses (e.g., Wiens et al., 2010), and its branch lengths were time calibrated. We assigned divergence times between our *Scinax* species based on the Wiens et al. (2006) phylogeny using the divergence time of *S. catharinae* from *S. fuscovarius* as a surrogate for the divergence time of *S. machadoi* from *S. fuscovarius*, because *S. machadoi* is placed within the *S. catharinae* group (Faivovich, 2002) yet was not itself included in the phylogeny. Finally, as divergence times within the genus *Bokermannohyla* were not available, we divided this terminal branch equally for divergence between *B. alvarengai* and *B. saxicola*. For each response variable, the AIC_c value from the phylogenetically corrected model was always more than 28 points lower than the model not controlling for phylogeny (Table S2).

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Thus, we subsequently used phylogenetically corrected statistical models when analyzing our kinematic data.

In order to maintain statistical power in a phylogenetically corrected model with only six taxa, we used the Contrast program within PHYLIP version 3.69 (Felsenstein, 2004), using the composite phylogeny described previously (Fig. S1), with the W menu option in the program invoked. This menu option calculates contrasts based on both within- and among-species covariation by including all individuals in the model rather than using an average value for each species (Felsenstein, 2008). We tested for an effect of the total number of tooth rows, maximum gape, and feeding guild (i.e., benthic or nektonic) on each of the six kinematic response variables by calculating 95% confidence limits around the regression coefficients based on the mathematical relationship between the standard error of the regression coefficient and the covariance, correlation coefficient, and regression coefficient (Bailey, 1995; Anderson et al., 2012). An effect was determined to be significant if the 95% confidence limits failed to encompass zero. The categorical variable feeding guild was coded as a binary variable so that we could use it in our analysis. We log transformed all of our data prior to analysis.

We were unable to statistically compare species-differences in feeding using phylogenetically corrected analyses because there was no variation in the predictor variable (species). Thus, we discuss qualitative differences in feeding among species.

Deformation in jaw sheath shape

In addition to measuring variables associated with the duration of time it takes for the labial teeth to reach specific points in the gape cycle, we used geometric morphometrics to describe the change in shape of the tadpole oral aperture resulting from deformation of the jaw sheaths during feeding. We obtained digital images of the tadpole's mouth from our high-speed video files. We focused on three different stages of the gape cycle: *opening*—the point in the gape cycle where the anterior and posterior jaw sheaths are opening but are still in contact with each other (Fig. S2A); *maximum gape*—the point in the gape cycle where the anterior and posterior jaw sheaths are furthest apart from each other (Fig. S2B); and *closing*—the point in the gape cycle where the anterior and posterior jaw sheaths are closing and regain contact with each other (Fig. S2C). For each section of the gape cycle, we placed five digital landmarks on the anterior and posterior jaw

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sheaths (Fig. S2). The landmarks in each jaw sheath represent the exterior margins, the center, and a point equidistant between the exterior and center. Criteria for selecting our landmarks were based on our ability to easily identify the same portion of the anatomical structure, their visibility throughout the entire gape cycle, and their ability to represent the change in shape of the structure during feeding.

Landmark data of 255 frames from 20 individuals (N = 3 for *B. alvarengai*, *B. saxicola*, *S. machadoi*, and *S. fuscovarius*; N = 6 for *A. lemur*; and N = 2 for *H. albopunctatus*) representing the shape variation in the oral aperture were transformed by a Generalized Least Squares Procrustes Superimposition (Rohlf and Slice, 1990). This process aligns the landmarks to a Cartesian plane while eliminating effects of translation, rotation, and size. Differences in landmark coordinates that remained after the Procrustes superimposition were due only to variation in shape. We then calculated partial warp scores using a Thin-Plate Spline transformation (Zelditch et al., 2004), producing 2p – 4 (p = number of landmarks) Euclidean shape variables, without the affine component. Generalized Least Square Procrustes Superimposition and the Thin-Plate Spline analyses were performed in PAST version 2.16 (Hammer et al., 2001)

Statistical Analyses of Jaw Shape

We use the partial warps to perform a principal components analysis (PCA) to compare all the relative transformations related to compression and shear in the general modification of the oral aperture during the opening phase, the maximum opening, and the closing phase of each gape cycle. We also compared the differences in the oral aperture related to the variation affecting local subsets of landmarks by generating the local partial warps excluding the uniform component (Zelditch et al., 2004). We compared these relative modifications in shape variations using the partial warps without the uniform component by a two-way non-parametric multivariate analysis of variance (NPMANOVA) with species (six levels) and gape cycle phase (three levels) as factors.

We used the program TpsUtility 1.4 to position the digital landmarks on all of the images; the geometric data were obtained using with TpsDig 2.12 (both software packages developed by F. J. Rohlf and available at http://life.bio.sunysb.edu/morph/). All other statistical procedures were performed in Past 2.16 (Hammer et al., 2001).

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RESULTS

Gape cycle

As predicted, tadpoles with a larger maximum gape and those with more labial tooth rows open and close their mouths slower than tadpoles that have smaller mouths or fewer tooth rows (Table 1; Fig. 2A, C).

Nektonic feeding tadpoles had shorter gape cycles than benthic feeding tadpoles (mean ± SE: 77.11 ± 8.27 and 93.95 ± 7.87, respectively); however, after controlling for phylogeny, feeding guild was not a significant predictor of the gape cycle (Table 1). Similarly, the duration of time to achieve maximum gape did not differ between nektonic and benthic feeding tadpoles (Table 1). However, when considering the duration of time it takes a tadpole to achieve maximum gape as a function of the duration of the gape cycle, benthic feeding tadpoles achieved maximum gape significantly earlier than nektonic feeding tadpoles (Table 1).

Lastly, it appears that the species differed qualitatively in the duration of their gape cycle (Fig. S3A, Table S3). Tadpoles of *B. saxicola* and *B. alvarengai*, which have the greater number of labial tooth rows of the species we examined, appear to have longer gape cycles relative to every other species (Fig. S3A). On the opposite end of the spectrum, tadpoles of *S. fuscovarius* and *S. machadoi* had the fastest gape cycle relative to the other species, but they do not appear to differ from each other (Fig. S3A). These results also suggest that species with the same labial tooth row formulae differ in the amount of time in which they open and close their mouths: e.g., tadpoles of *H. albopunctatus* have a longer gape cycle than *S. fuscovarius* and *S. machadoi*. In addition, our data indicate that tadpoles of *A. lemur* reach maximum gape proportionally later in the gape cycle relative to the other species we examined (Fig. S3B, Table S3).

Labial teeth

In general, neither maximum gape nor the total number of labial tooth rows were significant predictors of the when the labial tooth rows start to move when the jaws close (Table 1). Feeding guild, however, was a significant predictor of when the labial tooth rows are released from the substrate (Table 1). Nektonic feeding tadpoles released each of their labial tooth rows earlier in the gape cycle compared to benthic feeding tadpoles (Table 1; Fig. 3).

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Our data also qualitatively suggest that tadpoles of different species release their labial teeth at different times within the gape cycle (Fig. S4) and tadpoles with fewer labial tooth rows released their innermost tooth row (P1) sooner in the gape cycle than tadpoles with more labial tooth rows (Fig. S4). For example, tadpoles of *S. fuscovarius* and *B. alvarengai* appear to release their teeth early in the gape cycle whereas tadpoles of *H. albopunctatus* and *S. machadoi* appear to release their teeth late in the gape cycle (Fig. S4). Of the species that differed in terms of when their labial teeth release, one particularly interesting result was a difference between tadpoles of *S. fuscovarius* and *S. machadoi*. Not only do tadpoles of *S. fuscovarius* release their teeth earlier than *S. machadoi* in the gape cycle, but tadpoles of *S. fuscovarius* release their teeth concurrently whereas *S. machadoi* released rows P2 and P3 sequentially and later than P1(Fig. S4).

Deformation in jaw sheath shape

The first three axis of each PCA ordination explained approximately 70% of the total variation of jaw sheath shape (opening = 68.29%; maximum gape = 74.44%; closing = 69.23%).

We found significant main effects of species and gape cycle phase, and their interaction, on the shape of the jaw sheaths (Table 2). During the opening phase of the gape cycle, species differed primarily in the proportional expansion of the jaw sheaths laterally (indicated as warmer colors in Fig. 4A, B), suggesting that tadpoles open their mouths wider so that their mouths can cover a larger area. One surprising finding was that tadpoles opened their mouths asymmetrically, with greater deformations occurring on the left size of the anterior jaw sheath and in the right size of the posterior jaw sheath (indicated as warmer colors in Fig. 4B).

In contrast, during maximum gape and the closing phase of the gape cycle, the anterior jaw sheath changed more than the posterior jaw sheath (indicated as warmer colors in Figs. 5,6). Much of these were positional, rather than actual shape changes of the anterior jaw sheaths. Prior to the tadpole raking the substrate and closing its' jaw sheaths, the anterior jaw sheath acts a support for the scraping force applied by the posterior jaw sheaths. Thus the positional changes of the anterior jaw sheaths maximize the contact with the substrate in order to provide a better traction.

We found some support for the hypothesis that differences in the shape change of the jaw sheaths during feeding are predictable based on tadpole feeding guild. For example, the shape of the jaw sheaths of the nektonic feeding *Scinax fuscovarius* and benthic feeding *S. machadoi* are

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similar during the opening phase of the gape cycle (i.e., considerable overlap in the purple and green lines of Fig. 4); however, the shape of the jaw sheaths differ significantly between these species at maximum gape (Fig. 5). This differences is most pronounced during the closing phase of the gape cycle as the jaw sheaths of the benthic feeding *S. machadoi* get wider whereas the jaw sheaths of the nektonic *S. fuscovarius* do not change shape as much (Fig. 6). We also found some support for the notion that phylogenetic and behaviorally similar tadpoles have common feeding kinematics (e.g., benthic feeding tadpoles of *Bokermannohyla alvarengai* and *B. saxicola* exhibited a high degree of similarity during the three phases of gape cycle; Figs. 4-6).

Lastly, our results support the hypothesis that the shape change of the jaw sheaths during the gape cycle differs among species. The strongest evidence comes from the observed differences in the shape change of the jaw sheaths during feeding of tadpoles of *A. lemur* and *S. fuscovarius*. Tadpoles of both of these species are nektonic and have the same tooth row configuration; however, they open and close their jaws fundamentally differently (i.e., the separation of the pink and purple lines in Figs. 4,5).

DISCUSSION

Ecologists seek to understand the relationship between the phenotype of an organism and the habitat in which it lives. Amphibian biologists have long noted correlations between the oral morphology of tadpoles and their habitats (e.g., Noble, 1931) and the study of tadpole ecomorphological diversity continues to be an active field of research (Van Buskirk, 2009; Vera Candioti and Altig, 2010). However, despite nearly a century of research on tadpole ecomorphology, we have [at best] a very limited understanding of how variation in tadpole oral morphology relates explicitly to feeding. Our kinematic data show that anuran species with anatomically similar tadpoles fundamentally differ in how their larvae open and close their mouths during feeding and that these differences are predictable based on anatomic traits and ecomorphological guild.

Gape Cycle

Our digital imagery data reveal clear differences in the duration of time in which tadpoles open and close their jaws, indicating differences in velocity. This is best observed when we controlled for phylogenetic differences among species and only considered maximum gape and labial tooth

The Journal of Experimental Biology – ACCEPTED AUTHOR MANUSCRIPT

353 row number as continuous predictors. Results from this analysis show that tadpoles with a larger
354 maximum gape and those with more labial tooth rows open and close their mouths at a slower
355 speed compared to tadpoles with fewer tooth rows (Fig. 2A, B). This corroborates the findings of
356 previous work on tadpole feeding (e.g., Venesky et al., 2011). Tadpoles with more labial tooth
357 rows likely obtain more food per gape cycle than tadpoles with fewer teeth (when feeding on a
358 common substrate) because the extra tooth rows can pass over a food source multiple times
359 during each bite. Thus, it is our hypothesis that tadpoles with fewer tooth rows accelerate the rate
360 at which they open and close their mouths so that they can optimize their food intake. Future
361 studies that test whether tadpoles adaptively change the speed at which they feed on surfaces
362 with biofilms of different densities (e.g., Wassersug and Yamashita, 2001) and studies that
363 quantify food intake are needed to discriminate between this, and other, hypotheses. The
364 characterization of morphological differences at smaller scales (i.e., the curvature and number of
365 individual labial teeth and/or morphological asymmetries; Fig. 1) might also affect how the teeth
366 engage and disengage from a food source (Vera Candioti and Altig, 2010) and could be
367 considered in future studies.

368 We did not find any support for the hypothesis that gape cycle speed is predictable based
369 on the ecological guilds to which tadpoles belong. However, after controlling for differences in
370 the total duration of the gape cycle, benthic tadpoles reached maximum gape approximately 10%
371 earlier than nektonic tadpoles, highlighting the point that benthic feeding tadpoles spend
372 proportionally more time closing their jaws than nektonic feeding tadpoles. Ecological correlates
373 with morphology have been well recognized for tadpole oral features, but only in terms of the
374 static structures and not their active (kinematic) function. For example, lentic tadpoles generally
375 have more teeth than lotic tadpoles (Altig and Johnson, 1989) and tadpoles that eat large prey
376 have wide mouths (Vera Candioti, 2005). More recently, Van Buskirk (2009) examined tadpoles
377 of 82 hylid and myobatrachid species and found that stream- and pond-dwelling tadpoles
378 differed in the shape of their jaw sheaths and suggested that these differences might reflect
379 adaptations to different diets. Our finding that benthic feeding tadpoles spend proportionally
380 more time closing their jaws than nektonic feeding tadpoles further supports the hypothesis that
381 tadpole functional morphology correlates with the feeding guild of the larvae. One possible
382 explanation for why nektonic tadpoles feed faster than benthic tadpoles is that gape cycle speed
383 in benthic feeding tadpoles feed is traded off with other morphological features, such as flattened

body or ventral positioning of the mouth (Altig and Johnson, 1989; Altig and McDiarmid, 1999). That is, a reduced gape cycle might represent an anatomical constraint of mouth position on the tadpole body.

Although qualitative, we also found that species that have similar labial tooth row formulae and those that are in the same ecological guild appears to differ in their feeding kinematics; yet, this is not always the case. For instance, the nektonic tadpoles of *S. fuscovarius* and *A. lemur* have a 2/3 tooth row formulae but appear to differ in the speed of their gape cycle (Fig. S3A). These differences in feeding could be attributed to a number of factors, such as diet preferences (Rossa-Feres et al., 2004). However, we suspect that these differences reflect specific adaptations to living in ponds and streams (Jungfer and Weygoldt, 1994; Rossa-Feres and Nomura, 2006). A slower gape cycle might be advantageous in lotic environments, especially if tadpoles have to feed while avoid being swept downstream where food resources might be scarce. Although we did not statistically test for this relationship, the stream-dwelling tadpoles that we examined in our study (e.g., *H. albopunctatus*, *B. alvarengai*, and *B. saxicola*) generally had relatively longer gape cycles (Fig. S3A).

Labial Teeth

The labial teeth of temperate pond-dwelling tadpoles have two functions during feeding: they first anchor the oral disc to the substrate and they rake food off the substrate as the jaws close (Venesky et al., 2011; Wassersug and Yamashita, 2001). Our video data of tropical pond- and stream-dwelling tadpoles corroborate the findings of previous research and confirm that this is indeed a common feature of tadpole feeding kinematics.

Neither maximum gape nor the number of tooth rows were good predictors of the proportion of the gape cycle when the labial teeth start to release from the substrate (with the exception that labial tooth row P1 moves proportionally earlier in gape cycle when tadpoles have fewer labial teeth; Table 1). However, after controlling for phylogenetic relationships among our taxa, we found that benthic feeding tadpoles released their three posterior tooth rows proportionally later in the gape cycle compared to nektonic tadpoles (Fig. 3). These results complement our previous finding that benthic feeding tadpoles close their jaws slower than nektonic tadpoles and suggest that the kinematic profile of benthic tadpoles might be advantageous for tadpoles that regularly scrape irregularly surfaces for food. In other words,

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closing the jaws slowly allows the labial teeth of benthic feeding tadpoles more time to remove more food from a substrate. Future studies that correlate feeding kinematics with how much food is actually removed from a substrate during feeding would help determine whether this strategy is effective.

In terms of differences among species with similar tooth row formulae, we highlight two observations. First, species with similar labial tooth row formulae appear to have different labial tooth row kinematics, suggesting that feeding kinematics is not necessarily fixed by the anatomical hardware present. This finding complements and builds upon previous research demonstrating that tadpole feeding behavior (Smith and Vanbuskirk, 1995) and kinematics (Venesky et al., 2011) are quite flexible and can change along with aspects of the tadpoles' environment, such as the density of food particles (Wassersug and Yamashita, 2001) and the viscosity of the water (Ryerson and Deban, 2010). Second, tadpoles of *S. machadoi* released their tooth rows sequentially whereas the other species released their teeth synchronously. Although tadpoles of *S. machadoi* are stream-dwelling (Eterovick and Brandao, 2001), the sequential release of their labial tooth rows is not likely associated with living in a lotic environment for two reasons: not all stream-dwelling tadpoles release their labial teeth sequentially (e.g., *B. alvarengai*) and this phenomenon is observed in pond-dwelling tadpoles (Venesky et al., 2011). It remains to be seen whether the pattern of release of the labial tooth rows changes significantly when the tadpoles graze upon substrates with more irregular topography and/or biofilms of varying firmness and thickness.

Deformation in jaw sheath shape

The deformation in a tadpole jaw sheaths (i.e., their ability to not just change position but change shape) is one of the most elegant subtleties of tadpole feeding. In general, tadpole feeding can be broken into three discrete phases: a positioning phase (opening the mouth), an attachment phase (at maximum gape) and a food removal phase (closing) (Wassersug and Yamashita, 2001). As we documented, the shape of the jaw sheaths changes during each phase of the gape cycle and this appears to be associated with whether tadpoles are positioning their mouths to grasp the substrate or closing their mouths to remove material from the substrate. For example, during the opening phase (positioning phase), the deformation in the shape of jaw sheaths is more extensive. This may facilitate increasing the surface area scraped by the jaws as they close.

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It is hypothesized that tadpoles narrow their posterior jaw sheath to concentrate their bite force over a smaller area to remove more food (Wassersug and Yamashita, 2001). An alternative hypothesis, however, is that narrowing the jaw sheaths might not be beneficial if the jaws can penetrate through the entire biofilm because they would get less food per bite. However, qualitative examinations of the algal slides used in our experiments suggest that tadpoles do not appear to fully penetrate through the entire film on the glass. As such, one might predict that benthic feeding tadpoles might narrow their jaw sheath more readily during feeding compared to nektonic feeding tadpoles because they rely for food on what they scrape off of surfaces whereas nektonic tadpoles may acquire proportionately more nutrition from material already in suspension. The results from our geometric morphometric analyses generally support this hypothesis and are clearest when comparing the jaw sheath shape change of the nektonic feeding tadpole of *S. fuscovarius* and the benthic feeding tadpole of *S. machadoi*. During the opening phase of the gape cycle, the shape of their jaw sheaths is very similar (Fig. 4) but as the tadpoles close their mouths, the jaw sheaths of *S. machadoi* narrows whereas the jaw sheaths of *S. fuscovarius* remain relatively unchanged (Fig. 5C).

If feeding behavior was the only factor influencing the shape of jaw sheaths during feeding, we would expect the jaw sheath shape change of *S. fuscovarius* would be similar to tadpoles of *Agalychnis lemur* since they share a feeding guild and have a similar tooth row formula and feeding guild; however, the shape of the jaw sheaths differ during all phases of the gape cycle. The gape in tadpoles is considered to be a phylogenetically independent trait, being affected mainly by ecological and behavioral processes, which explain a significant amount of the prey-size variation among tadpoles (Vera Candioti, 2007). In our analysis of tadpoles of six hylid species, we observed vast differences in the deformation of the jaw sheaths, even among species that share similar ecological guilds. This result suggests that (a) there is a high degree of behavioral differences of tadpoles, even among members of the same guild and (b) fundamental differences in feeding behavior among species, not differences in food type, influence how tadpoles partition of food resources (Diaz-Paniagua, 1985; Inger, 1986; Rossa-Feres et al., 2004).

The discovery of asymmetry in the deformation of the anterior and posterior jaw sheaths of tadpoles during feeding is intriguing in light of the fact that in anurans in general have numerous strongly lateralized behaviors (reviewed in Robins, 2005). In addition, the majority of anuran larvae, including all that we studied here, are unusual among vertebrates in

being externally morphologically asymmetrical; i.e., their single spiracle is always located on the left side of the body. The asymmetry that we observed in jaw deformation during the gape cycle may relate to the tadpoles using shearing to facilitate removing particularly resistant material from the substrate. This would be consistent with the turning biases previously been document for tadpoles (Malashichev and Wassersug, 2004; Wassersug and Yamashita, 2002). One prediction is that the oral asymmetry will be greater when tadpoles feed on more resistant substrates than the uniform biofilm we provided them in the current study.

Conclusions

The results from our research complement and build upon previous work at the intersection of anuran functional morphology and ecomorphology. Our results demonstrate that some aspects of tadpole feeding are predictable based on anatomical features (e.g., tadpoles with more labial tooth rows have longer gape cycles). However, other features differ among tadpoles that share anatomical and ecological features (e.g., the deformation of the jaw sheaths in nektonic feeding tadpoles with a 2/3 tooth row formulae). Future studies will be needed to test how differences in abiotic (e.g., temperature) or biotic (e.g., density or type of food) conditions influence feeding kinematics.

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REFERENCES

Altig, R. (2006). Discussions of the origin and evolution of the oral apparatus of anuran tadpoles. *Acta Herpeologica* **1**, 95-105.

Altig, R. and Johnson, G. F. (1989). Guilds of anuran larvae: relationships among developmental modes, morphologies, and habitats. *Herpetological Monographs* **3**, 81-109.

Altig, R. and McDiarmid, R. W. (1999). Body plan: development and morphology. In *Tadpoles: the biology of anuran larvae*, eds. R. W. McDiarmid and R. Altig), pp. 24-51. Chicago: University of Chicago Press.

Anderson, C. V., Sheridan, T. and Deban, S. M. (2012). Scaling of the ballistic tongue apparatus in chameleons. *Journal of Morphology* **273**, 1214-1226.

Bailey, N. T. J. (1995). Statistical Methods in Biology. Third Edition. Cambridge: Cambridge University Press.

Boulenger, G. A. (1891). A synopsis of the tadpoles of the European batrachians. *Proceedings of the Zoological Society of London* **59**, 593-678.

Burnam, K. P. and Anderson, D. R. (2002). Model selection and multimodel inference. New York: Springer-Verlang Press.

Diaz-Paniagua, C. (1985). Larval diets related to morphological characters of five anuran species in the Biological Reserve of Doñana (Huelva, Spain). *Amphibia-Reptilia* **6**, 307-322.

Eterovick, P. C. and Brandao, R. A. (2001). A description of the tadpoles and advertisement calls of members of the Hyla pseudopseudis group. *Journal of Herpetology* **35**, 442-450.

Faivovich, J. (2002). A cladistics analysis of *Scinax* (Anura: Hylidae). *Cladistics* **18**, 367-393.

Felsenstein, J. (2004). PHYLIP (Phylogeny Inference Package) version 3.69. Distributed by the author. Seattle: Department of Genome Sciences, University of Washington.

Felsenstein, J. (2008). Comparative methods with sampling error and within-species variation: Contrasts revisited and revised. *American Naturalist* **171**, 713-725.

The Journal of Experimental Biology – ACCEPTED AUTHOR MANUSCRIPT

Hammer, O., Harper, D. A. T. and Ryan, P. D. (2001). PAST: Paleontological Statistics software pachage for education and data analysis. *Paleontologica Electronica* **4**, 1-9.

Inger, R. F. (1986). Diet of tadpoles living in a Bornean rain forest. *Alytes* **5**, 153-164.

Jungfer, K. H. and Weygoldt, P. (1994). The reproductive biology of the leaf frog *Phyllomedusa lemur* (Boulenger, 1882) and a comparison with other members of the Phyllomedusinae (Anura: Hylidae). *Revue française d'aquariologie* **21**, 57-64.

Malashichev, Y. B. and Wassersug, R. J. (2004). Left and right in the amphibian world: which way to develop and where to turn? *Bioessays* **26**, 512-522.

Noble, G. K. (1931). In *The Biology of the Amphibia*. USA: McGraw-Hill.

R. (2012). R: a language and environment for statistical computing.

Robins, A. (2005). Lateralized visual processing in anurans. *Landes Bioscience* **1**, 162-173.

Rohlf, F. J. and Slice, D. (1990). Extensions of the procrustes method for the optimal superimposition of landmarks. *Systematic Zoology* **39**, 40-59.

Rossa-Feres, D. C., Jim, J. and Fonseca, M. G. (2004). Diets of tadpoles from a temporary pond in southeastern Brazil (Amphibia, Anura). *Revista Brasileira de Zoologia* **21**, 745-754.

Rossa-Feres, D. C. and Nomura, F. (2006). Characterization and taxonomic key for tadpoles (Amphibia: Anura) from the northwestern region of São Paulo State, Brazil. *Biota Neotropica* **6**, 1-26.

Ryerson, W. G. and Deban, S. M. (2010) Buccal pumping mechanics of *Xenopus laevis* tadpoles: effects of biotic and abiotic factors. *Journal of Experimental Biology* **213**, 2444-2452.

Sazima, I. and Bokermann, W. C. A. (1977). Anfíbios da Serra do Cipó, Minas Gerais, Brasil. 3: Observações sobre a biologia de *Hyla alvarengai* Bok. (Anura, Hylidae). *Revista Brasileira de Biologia* **37**, 413-417.

Smith, D. C. and Vanbuskirk, J. (1995). Phenotypic design, plasticity, and ecological performance in two tadpole species. *American Naturalist* **145**, 211-233.

Van Buskirk, J. (2009). Getting in shape: adaptation and phylogenetic inertia in morphology of Australian anuran larvae. *Journal of Evolutionary Biology* **22**, 1326-1337.

Venesky, M. D., Parris, M. J. and Storfer, A. (2009). Impacts of *Batrachochytrium dendrobatidis* infection on tadpole foraging performance. *EcoHealth* **6**, 565-575.

The Journal of Experimental Biology – ACCEPTED AUTHOR MANUSCRIPT

Venesky, M. D., Wassersug, R. J., Jorgensen, M. E., Riddle, M. and Parris, M. J. (2011). Comparative feeding kinematics of temperate pond-dwelling tadpoles (Anura, Amphibia). *Zoomorphology* **130**, 31-38.

Venesky, M. D., Wassersug, R. J. and Parris, M. J. (2010a). Fungal pathogen changes the feeding kinematics of larval anurans. *Journal of Parasitology* **96**, 552-557.

Venesky, M. D., Wassersug, R. J. and Parris, M. J. (2010b). How does a change in labial tooth row number affect feeding kinematics and foraging performance of a ranid tadpole (*Lithobates sphenocephalus*)? *Biological Bulletin* **218**, 160-168.

Venesky, M. D., Wassersug, R. J. and Parris, M. J. (2010c). The impact of variation in labial tooth number on the feeding kinematics of tadpoles of southern leopard frog (*Lithobates sphenocephalus*). *Copeia* **2010**, 481-486.

Vera Candioti, M. F. (2005). Morphology and feeding in tadpoles of *Ceratophrys cranwelli* (Anura : Leptodactylidae). *Acta Zoologica* **86**, 1-11.

Vera Candioti, M. F. (2007). Anatomy of anuran tadpoles from lentic water bodies: systematic relevance and correlation with feeding habits. *Zootaxa*, 1-175.

Vera Candioti, M. F. and Altig, R. (2010). A survey of shape variation in keratinized labial teeth of anuran larvae as related to phylogeny and ecology. *Biological Journal of the Linnean Society* **101**, 609-625.

Wassersug, R. J. (1974). Evolution of anuran life-cycles. *Science* **185**, 377-378.

Wassersug, R. J. and Yamashita, M. (2001). Plasticity and constraints on feeding kinematics in anuran larvae. *Comparative Biochemistry and Physiology Part A-Molecular and Integrative Physiology* **131**, 183-195.

Wassersug, R. J. and Yamashita, M. (2002). Assessing and interpreting lateralised behaviours in anuran larvae. *Laterality* **7**, 241-260.

Wiens, J. J., Graham, C. H., Moen, D. S., Smith, S. A. and Reeder, T. W. (2006). Evolutionary and ecological causes of the latitudinal diversity gradient in Hylid frogs: Treefrog trees unearth the roots of high tropical diversity. *American Naturalist* **168**, 579-596.

Wiens, J. J., Kuczynski, C. A., Hua, X. and Moen, D. S. (2010). An expanded phylogeny of treefrogs (Hylidae) based on nuclear and mitochondrial sequence data. *Molecular Phylogenetics and Evolution* **55**, 871-882.

The Journal of Experimental Biology – ACCEPTED AUTHOR MANUSCRIPT

Wilbur, H. M. and Collins, J. P. (1973). Ecological aspects of amphibian metamorphosis. *Science* **182**, 1305-1314.

Zelditch, M. L., Swiderski, D. L., Sheets, H. D. and Fink, W. L. (2004). Geometric morphometrics for biologists. San Diego: Elsevier Academic Press.

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Figure Legends

Fig. 1. Images of the oral apparatus of an individual of each of the species that we studied, noting the differences in the configurations of the labial tooth rows. Top row (left to right): *Hypsiboas albopunctatus*, *Agalychnis lemur*, and *Scinax fuscovarius*. Bottom row (left to right): *Scinax machadoi*, *Bokermannohyla alvarengai*, and *Bokermannohyla saxicola*.

Fig. 2. The relationship between maximum gape (mm) and (A) the duration of time (in milliseconds) of the full gape cycle and (B) the percentage of the full gape cycle at which tadpoles achieved maximum gape. The relationship between the total number of labial tooth rows and (C) the duration of time (in milliseconds) of the full gape cycle and (D) the percentage of the full gape cycle at which tadpoles achieved maximum gape. Both maximum gape and the number of labial tooth rows were significant positive predictors of the duration of the gape cycle and time to maximum gape. Tadpoles with a large gape, or those with more tooth rows, open and close their jaws slower compared to tadpoles with a smaller gape or fewer tooth rows.

Fig. 3. The point in time, as a percentage of the total gape cycle, when the posterior tooth rows 1-3 (indicated as “P1”, “P2” and “P3”) begin to move. Histograms represent the grand means from the gape cycles of all individuals; error bars are 1 SE of the mean. After statistically controlling for phylogenetic differences among species, benthic feeding tadpoles released each of their labial tooth rows significantly later in the gape cycle than nektonic feeding tadpoles.

Fig. 4. PCA scatter diagrams of the affine components in the shape change of the jaw sheaths of tadpoles during the opening phase of the gape cycle. Different species are indicated by different colored lines (*Agalychnis lemur* ; *Bokermannohyla alvarengai* ; *B. saxicola* ; *Hypsiboas albopunctatus* ; *Scinax machadoi* ; and *S. fuscovarius* . These findings demonstrate that within a species, the jaw sheaths undergo different changes in shape during feeding and that species with similar ecological guilds generally have similar changes in shape of the jaw sheaths. Inserts (A and B) are thin-plate spline transformation grids for the opening phase of the tadpole gape cycle. Warmer colors indicate areas of expansion and colder

colors indicate contraction for the grid elements. Panel A shows the transformations undergone by the jaw sheaths on positive PC1 eigenvalues. Along this axis, one can see that the species with greater positive eigenvalues had narrower upper jaw sheath width and position the lower jaw sheath close to the extremities of the upper jaw sheath (wider lower jaw sheaths). Panel B shows the transformations undergone by the jaw sheaths on positive PC2 eigenvalues. Along this axis, one can see that the shape of the upper jaw sheath of species with greater positive eigenvalues forms a depression at the apex of the jaw sheath and the asymmetrical position of the lower jaw sheath relative to the upper jaw sheath.

Fig. 5. PCA scatter diagrams of the affine components in the shape change of the jaw sheaths of tadpoles during maximum gape of the gape cycle. Different species are indicated by different colored lines (*Agalychnis lemur* ; *Bokermannohyla alvarengai* ; *B. saxicola* ; *Hypsiboas albopunctatus* ; *Scinax machadoi* ; and *S. fuscovarius* . These findings demonstrate that within a species, the jaw sheaths undergo different changes in shape during feeding and that species with similar ecological guilds generally have similar changes in shape of the jaw sheaths. Inserts (A and B) are thin-plate spline transformation grids for the opening phase of the tadpole gape cycle. Warmer colors indicate areas of expansion and colder colors indicate contraction for the grid elements. Panel A shows the transformations undergone by the jaw sheaths on positive PC1 eigenvalues. Along this axis, one can see that the species with greater positive eigenvalues had greater upper jaw sheath deformation and are capable of a greater maximum gape (increased expansion between the upper and lower jaw sheath. However, species with a greater maximum gape had reduced deformation (i.e., less tendency to deform) in their lower jaw sheath. Panel B shows the transformations undergone by the jaw sheaths on positive PC2 eigenvalues. Along this axis, we can notice that the species with greater positive eigenvalues also increase the maximum gape by the lateral expansion of the upper jaw sheath and that the major contribution to the maximum gape transformation is related to the deformation in the shape of the upper jaw sheath.

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Fig. 6. PCA scatter diagrams of the affine components in the shape change of the jaw sheaths of tadpoles during the closing phase of the gape cycle. Different species are indicated by different colored lines (*Agalychnis lemur* ; *Bokermannohyla alvarengai* ; *B. saxicola* ; *Hypsiboas albopunctatus* ; *Scinax machadoi* ; and *S. fuscovarius* . These findings demonstrate that within a species, the jaw sheaths undergo different changes in shape during feeding and that species with similar ecological guilds generally have similar changes in shape of the jaw sheaths. Inserts (A and B) are thin-plate spline transformation grids for the opening phase of the tadpole gape cycle. Warmer colors indicate areas of expansion and colder colors indicate contraction for the grid elements. Panel A shows the transformations undergone by the jaw sheaths on positive PC1 eigenvalues. Along this axis, one can see that the species with greater positive eigenvalues had a greater upper jaw sheath width but had a laterally compressed lower jaw sheath, probably due to the muscular force applied to the lower jaw when resistance is encountered as the jaws scrape the substrate. Panel B shows the transformations undergone by the jaw sheaths on positive PC2 eigenvalues. Along this axis, one can see a lateral expansion of the upper jaw sheath, probably due to the resistance to the force applied by the lower jaw sheath during substrate contact.

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680

681 **Tables**

682

683 Table 1. Summary of phylogenetically corrected regression analysis testing for significant effect
684 of maximum gape distance and total number of tooth rows on kinematic variables. Significant
685 effects are indicated when the 95% confidence limits around the regression coefficient fails to
686 encompass zero. Bold indicates expected slope (zero) falls outside the 95% confidence interval
687 around the observed slope, indicating significant difference.

688

Maximum gape

Function	*Observed Slope ± 95% Confidence Interval*
Gape Cycle	**0.107 ± 0.040**
Time to Max Gape	**0.136 ± 0.034**
% Time Max Gape	**0.029 ± 0.027**
P1 starts to move	-0.014 ± 0.023
P2 starts to move	-0.016 ± 0.028
P3 starts to move	-0.019 ± 0.035

Number of Tooth Rows

Function	*Observed Slope ± 95% Confidence Interval*
Gape Cycle	**0.563 ± 0.200**
Time to Max Gape	**0.498 ± 0.276**
% Time Max Gape	-0.066 ± 0.148
P1 starts to move	**-0.137 ± 0.107**
P2 starts to move	-0.111 ± 0.139
P3 starts to move	0.126 ± 0.176

Feeding Guild

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Function	*Observed Slope ± 95% Confidence Interval*
Gape Cycle	-0.368 ± 0.635
Time to Max Gape	0.228 ± 0.718
% Time Max Gape	**0.691 ± 0.152**
P1 starts to move	**-0.253 ± 0.226**
P2 starts to move	**-0.447 ± 0.231**
P3 starts to move	**-0.563 ± 0.290**

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690

691 Table 2. Two-way non parametric multivariate analysis of variance (MANOVA) on the effects
692 of species and of phases of gape cycle in relative transformation of partial warps scores of cover
693 jaw sheaths of tadpoles during feeding activity.

694

695

Source	d.f.	M.S.	*F*	*P*
Species	5	0.185	13.402	< 0.001
Phase	2	1.226	88.839	< 0.001
Species x Phase	10	0.036	2.583	< 0.001
Residual	238	0.014		
Total	255			

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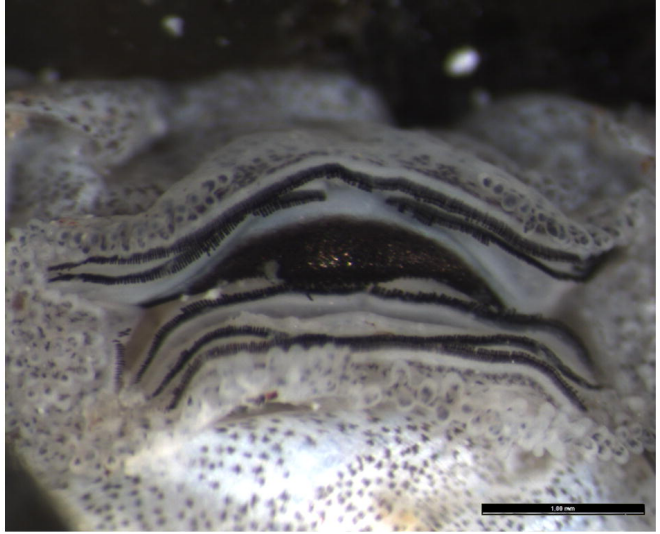
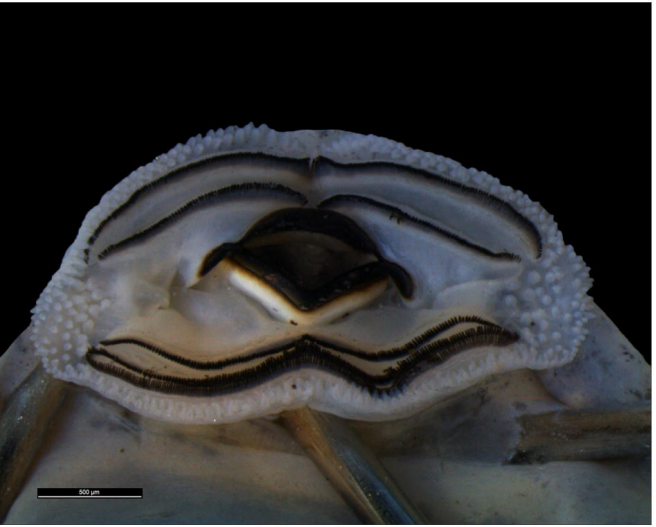
500 µm


1.00 mm

500 µm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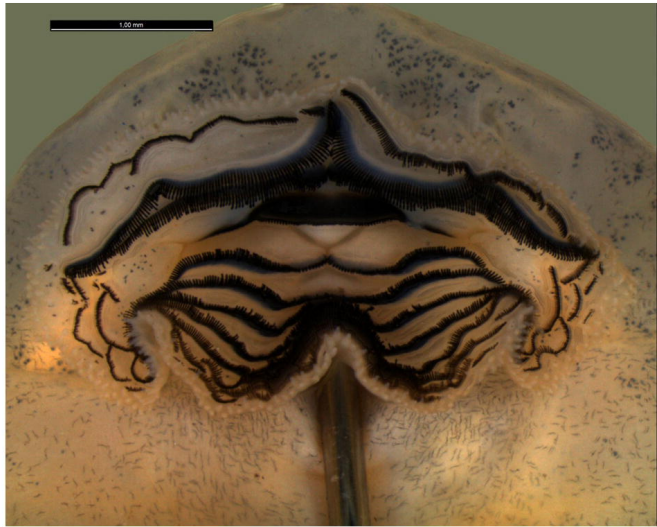
500 µm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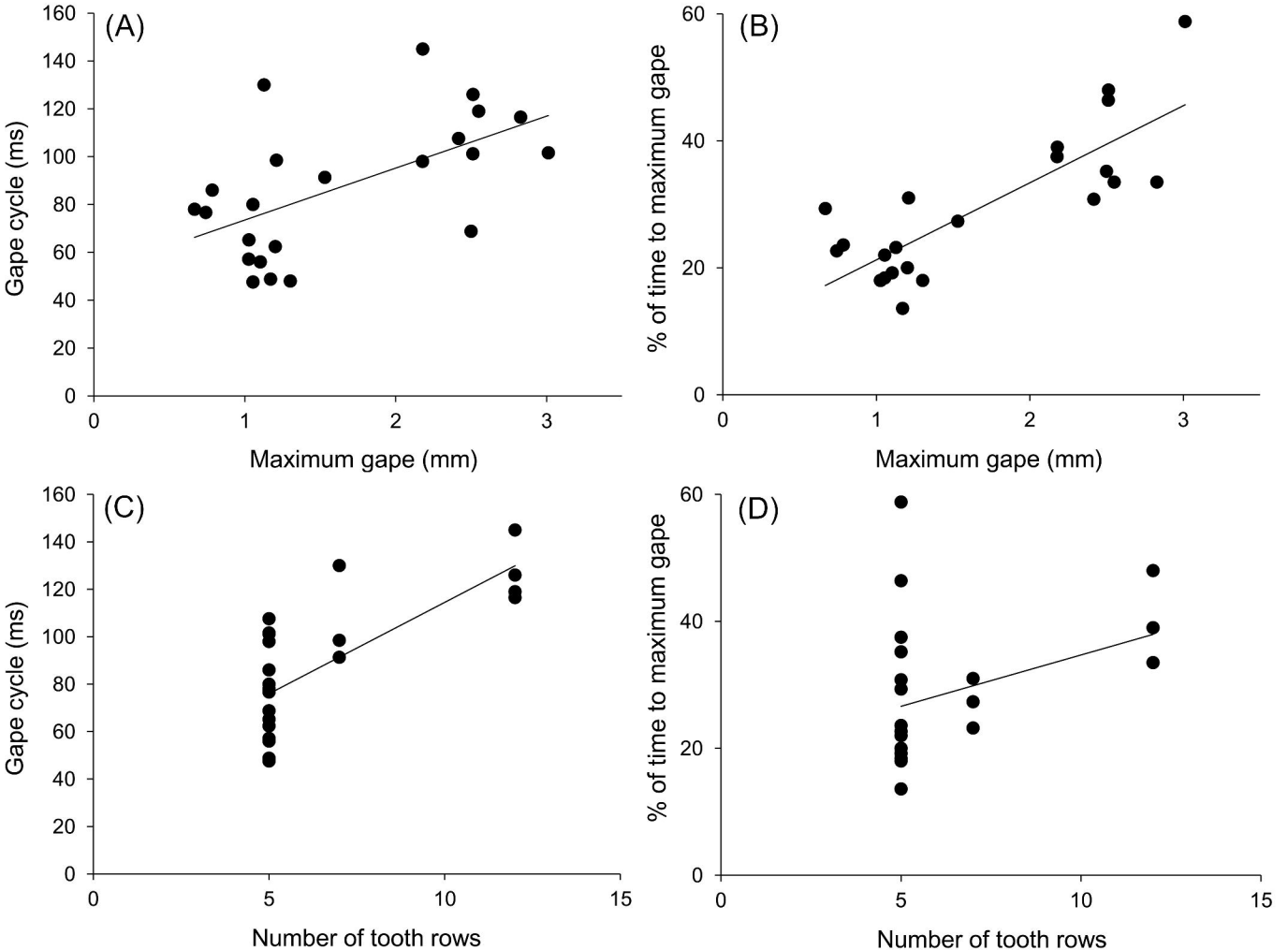
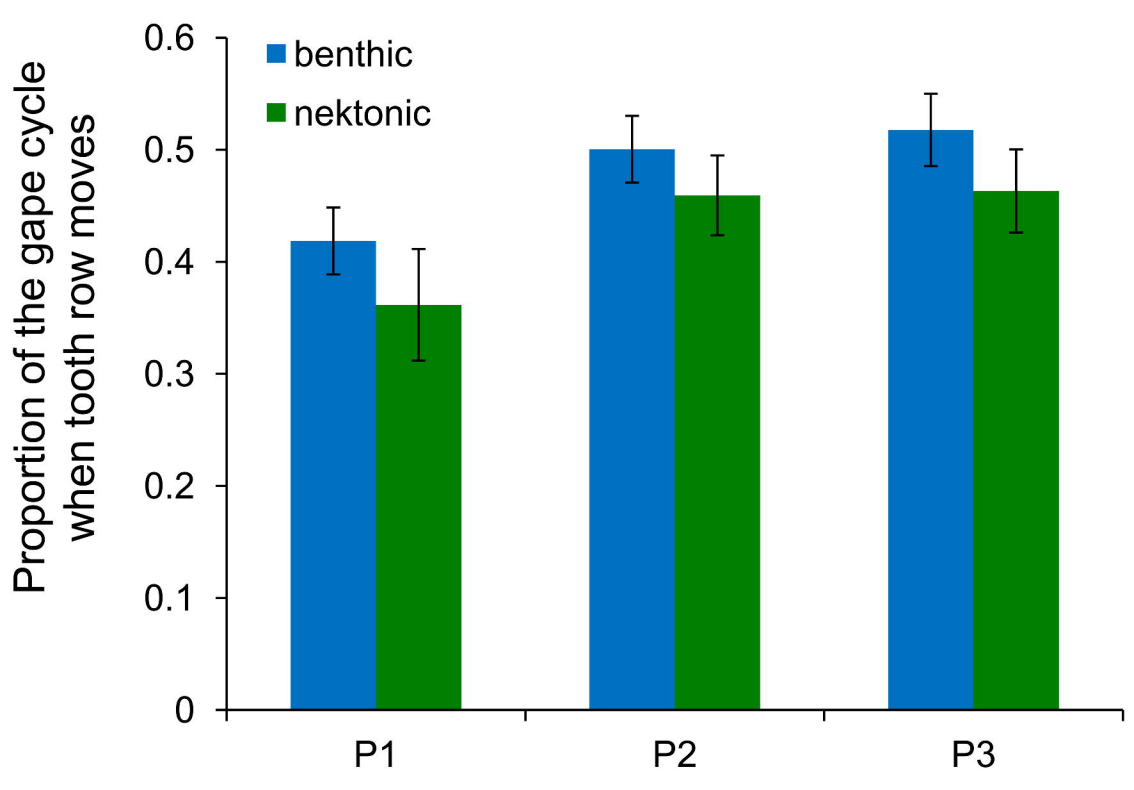
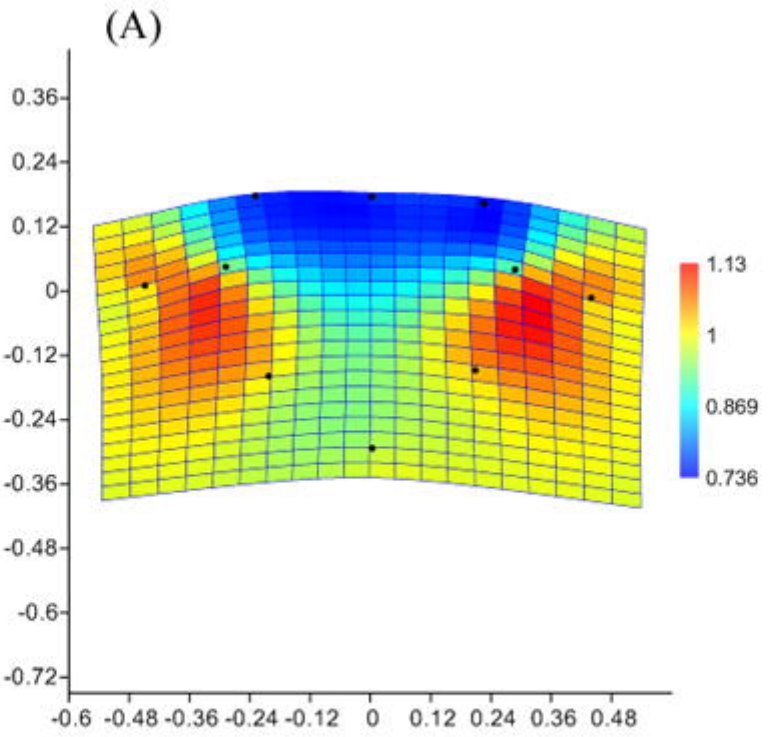
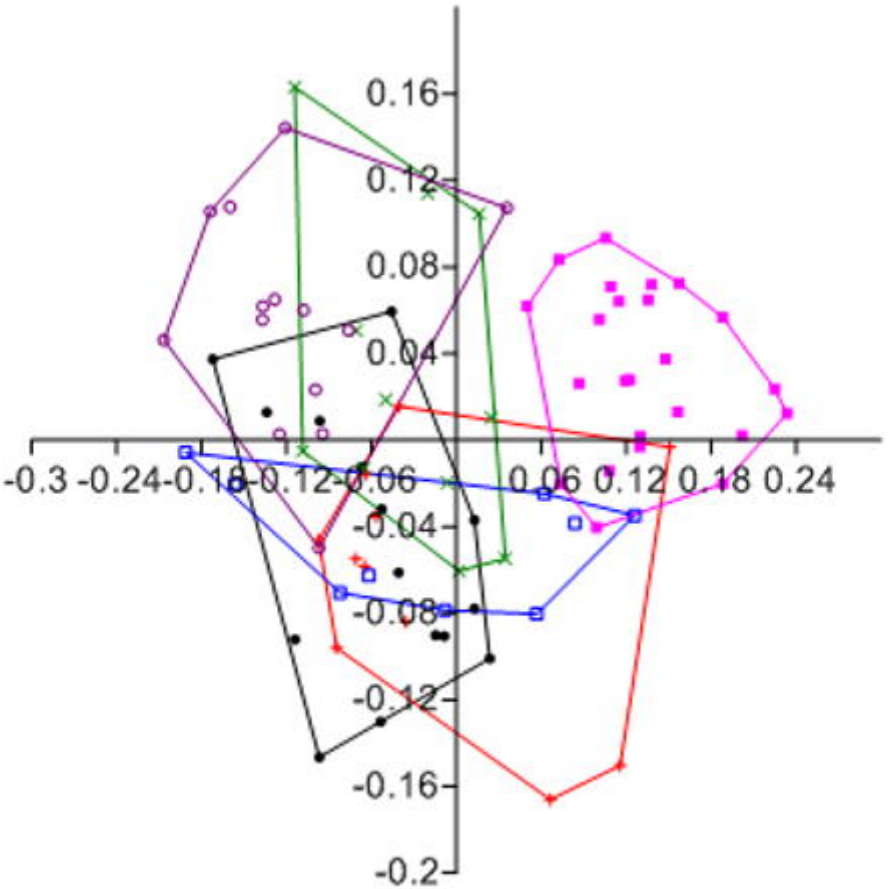
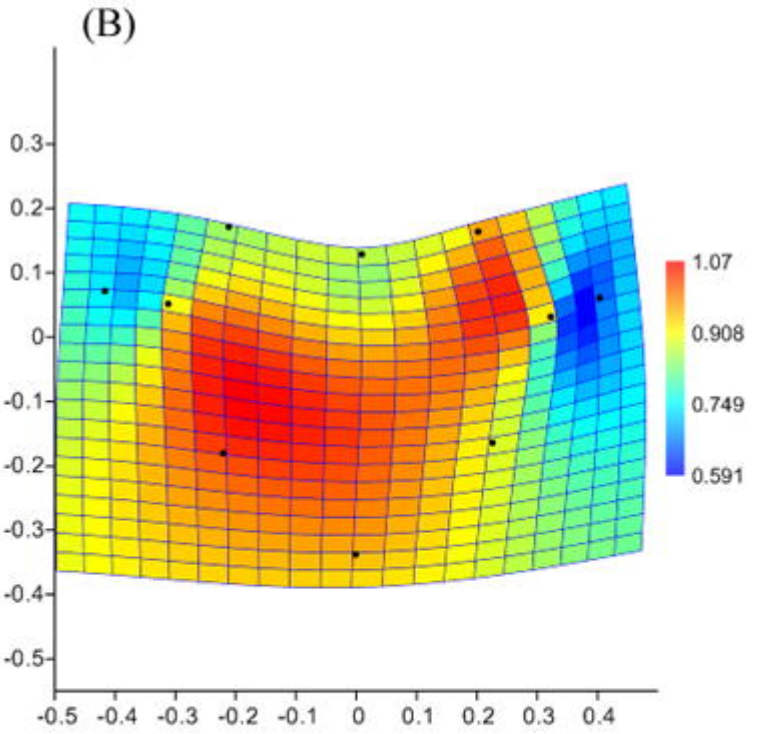
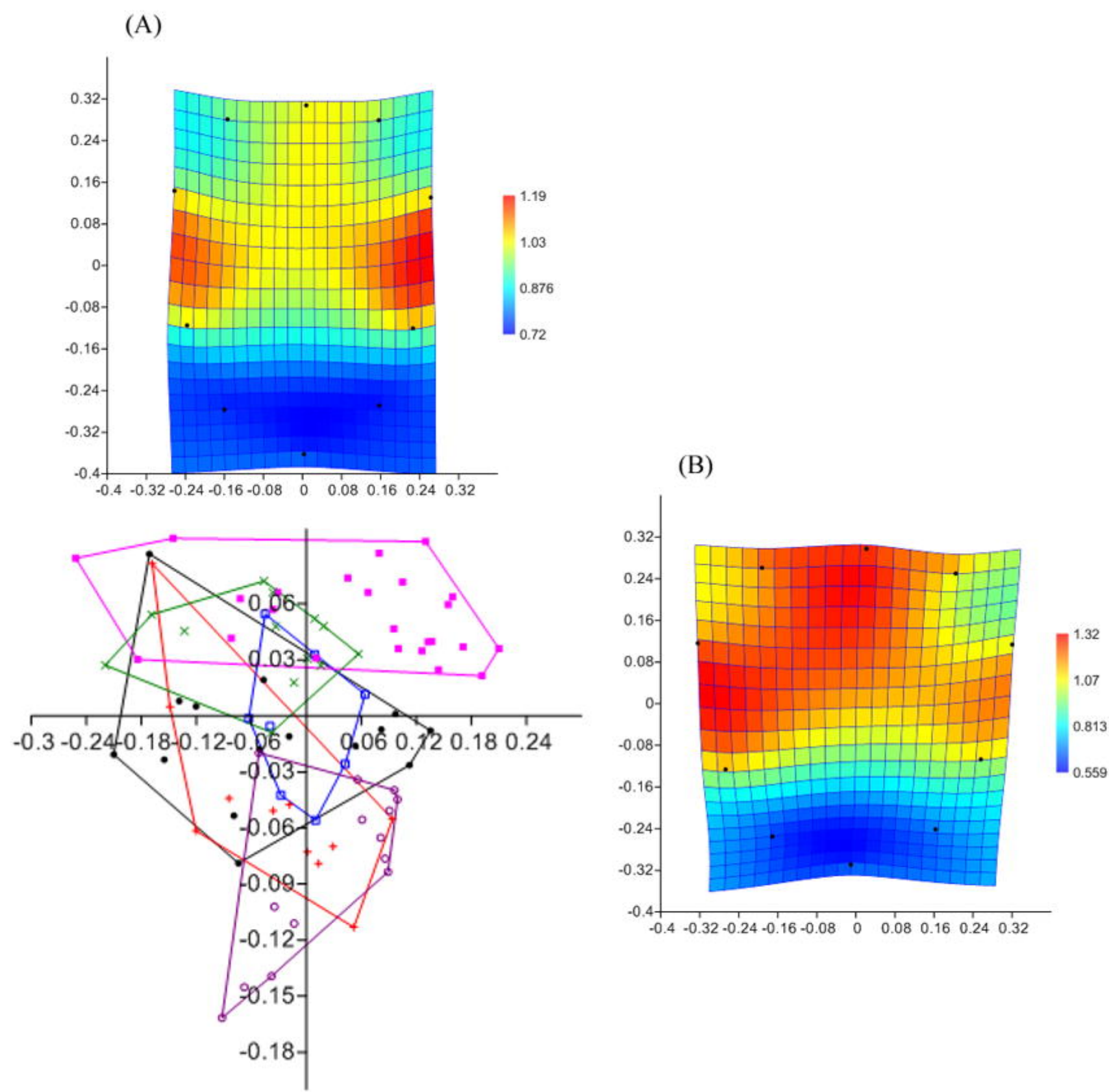

(A)
Gape cycle (ms)
Maximum gape (mm)
(B)
% of time to maximum gape
Maximum gape (mm)
(C)
Gape cycle (ms)
Number of tooth rows
(D)
% of time to maximum gape
Number of tooth rows


Proportion of the gape cycle when tooth row moves
0.6
0.5
0.4
0.3
0.2
0.1
0
benthic
nektonic
P1
P2
P3

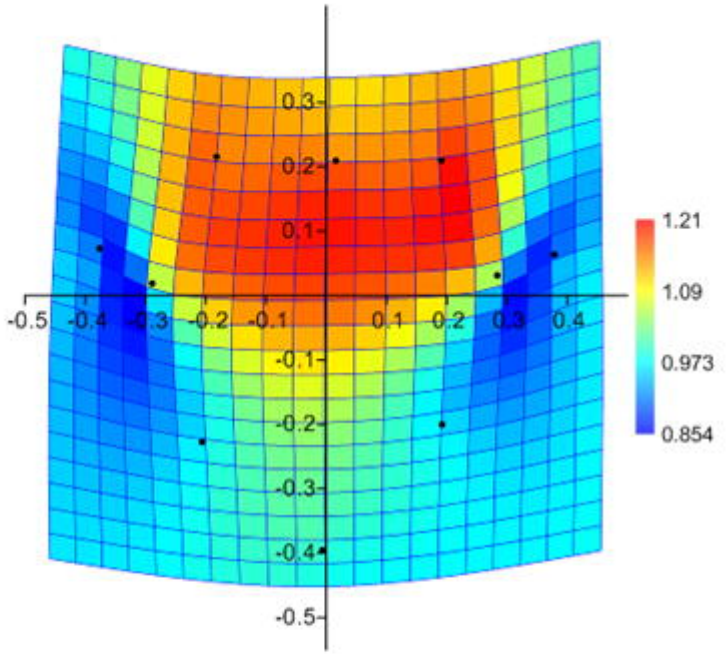

(A)
0.36
0.24
0.12
0
-0.12
-0.24
-0.36
-0.48
-0.6
-0.72
-0.6 -0.48 -0.36 -0.24 -0.12 0 0.12 0.24 0.36 0.48
1.13
1
0.869
0.736

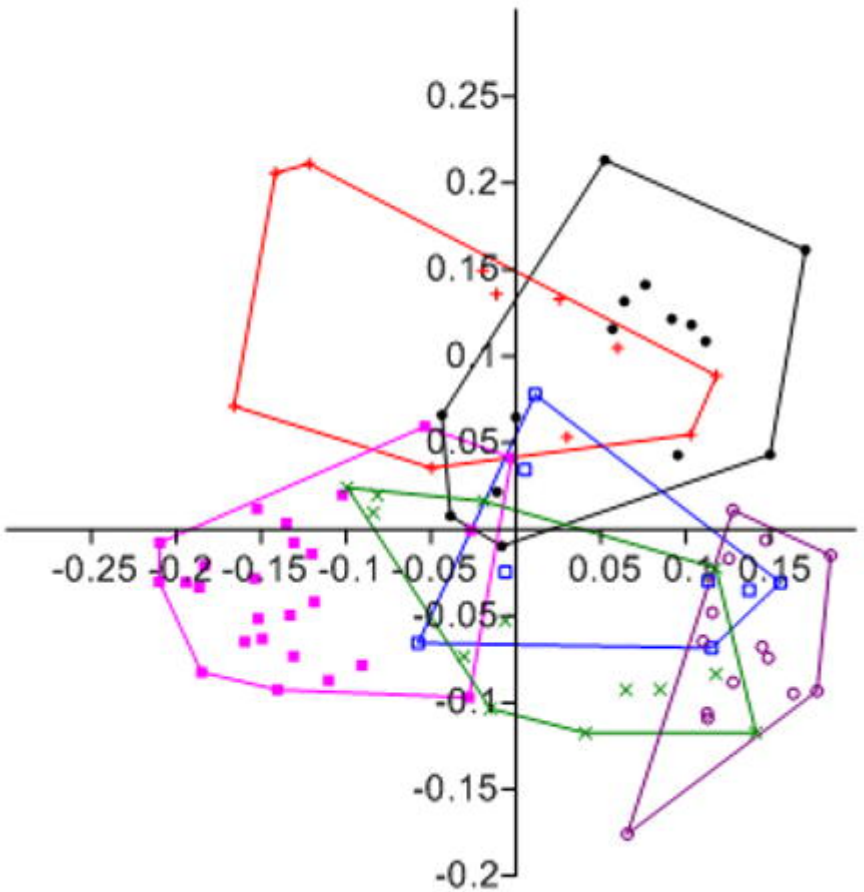

0.16
0.12
0.08
0.04
-0.3 -0.24 -0.18 -0.12 -0.06 0.06 0.12 0.18 0.24
-0.04
-0.08
-0.12
-0.16
-0.2


(B)
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
-0.4
-0.5
-0.5 -0.4 -0.3 -0.2 -0.1 0 0.1 0.2 0.3 0.4
1.07
0.908
0.749
0.591

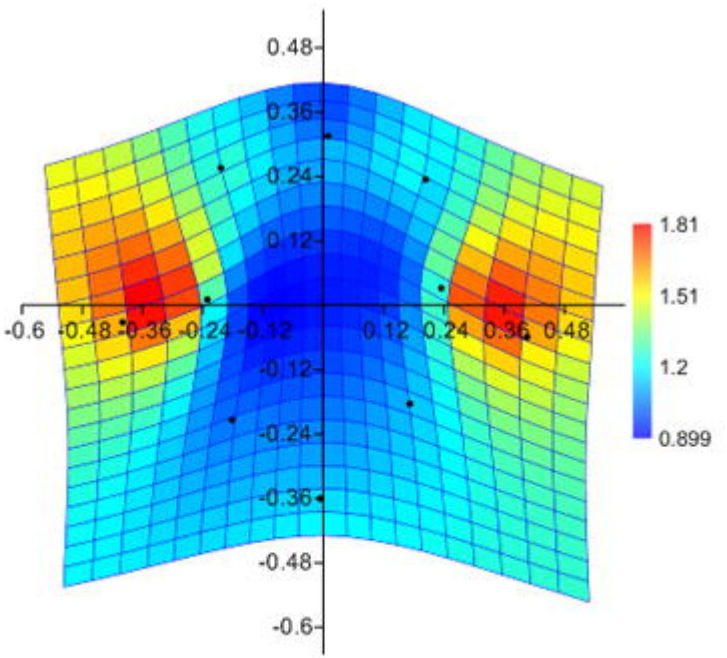

(A)
0.32
0.24
0.16
0.08
0
-0.08
-0.16
-0.24
-0.32
-0.4
-0.4 -0.32 -0.24 -0.16 -0.08 0 0.08 0.16 0.24 0.32
1.19
1.03
0.876
0.72
0.06
0.03
-0.3 -0.24 -0.18 -0.12 -0.06 0.06 0.12 0.18 0.24
-0.03
-0.06
-0.09
-0.12
-0.15
-0.18
(B)
0.32
0.24
0.16
0.08
0
-0.08
-0.16
-0.24
-0.32
-0.4
-0.4 -0.32 -0.24 -0.16 -0.08 0 0.08 0.16 0.24 0.32
1.32
1.07
0.813
0.559

(A)


0.3
0.2
0.1
-0.5 -0.4 -0.3 -0.2 -0.1 0.1 0.2 0.3 0.4
-0.1
-0.2
-0.3
-0.4
-0.5
1.21
1.09
0.973
0.854


0.25
0.2
0.15
0.1
0.05
-0.25 -0.2 -0.15 -0.1 -0.05 0.05 0.1 0.15
-0.05
-0.1
-0.15
-0.2

(B)


0.48
0.36
0.24
0.12
-0.6 -0.48 -0.36 -0.24 -0.12 0.12 0.24 0.36 0.48
-0.12
-0.24
-0.36
-0.48
-0.6
1.81
1.51
1.2
0.899