



A Morphometric Analysis of Developing Cannibalistic Indian Bullfrog, *Hoplobatrachus tigerinus* (Daudin 1803), Tadpoles

Samadhan Phuge^{1,2*}, Andrea Sequeira¹, Sameer Padhye³, and Radhakrishna Pandit¹

¹Department of Zoology, Savitribai Phule Pune University, Ganeshkhind, Pune 411007, India

²Department of Education and Extension, Savitribai Phule Pune University, Ganeshkhind, Pune 411007, India (samadhanphuge@gmail.com)

³Systematics, Ecology & Conservation Lab, Zoo Outreach Organization, Coimbatore 641035, Tamil Nadu, India

Amphibian larval development reflects surrounding biotic and abiotic conditions, including the presence of predators and cannibalism. Phenotypes acquired by tadpoles in response to predation or cannibalism can enhance survival. For example, ‘predator tadpoles’ develop a widened gape and reduced gut length for efficient prey capture (Relyea and Auld 2004; Kishida et al. 2009), whereas ‘prey tadpoles’ develop a larger tail that helps them to escape predatory attacks (Van Buskirk et al. 1997; Lardner 2000). The expression of these traits in both ‘predator’ and ‘prey’ are limited due to the energy diverted from growth and development (Relyea 2002).

Cannibalism occurs when predators feed on conspecifics. This frequently is associated with constrained environments, such as a lack of food resources, high conspecific density, and other predation cues (Collins and Cheek 1983; Levis et al. 2015). Individual tadpoles within a population may develop morphological characters in response to cannibalism. Cannibalistic tadpoles generally have enlarged gape size and shorter guts (Pomeroy 1981; Kishida et al. 2009). However, even cannibalistic individuals must develop defensive phenotypes to avoid predation from other species or conspecifics, which can result in phenotypes intermediate between traits specialized for cannibalization and avoiding predation.

In the present study, we analyzed morphological traits of cannibalistic Indian Bullfrog (*Hoplobatrachus tigerinus*) tadpoles. These frogs breed explosively in ephemeral pools for two to three days during the first monsoon showers and lay large numbers of eggs in clutches, leading to a high density of tadpoles at the breeding site. The larval period is short (~25 days) and tadpoles often feed on conspecific and heterospecific individuals (Grosjean et al. 2004).

We monitored a breeding site on 28 and 29 July 2016 in a temporary pond in Sangavi, Pune, Maharashtra, India. We randomly collected 70 tadpoles from the same pond on 14 and 15 August 2016. We anesthetized tadpoles using 1%

Triclanemethanesulfonate (MS222), preserved them in 4% buffered formalin for 48 h, and washed them in tap water before transferring them to 70% ethanol. We measured eight morphometric characters of each tadpole: total length (TOL), body length (BL), tail length (TAL), body height (BH), maximum tail height (TAH), body width (BW), tail muscle thickness (TMT) and oral disc width/mouth width (OD), using Mitutoyo digital callipers to the nearest 0.1 mm (Fig. 1). We grouped these tadpoles into two groups based on Gosner (1960) stage of the development: Early-stage (ES; stages 25–33; n = 39) and late-stage (LS; stages 34–40; n = 31). For our morphometric analysis, we considered the following ratios: BL/TOL, TAL/TOL, BW/TOL, TMT/TAL, TMT/TAH, BH/BL, BH/TAH, TAH/TAL, OD/BL, and OD/BW.

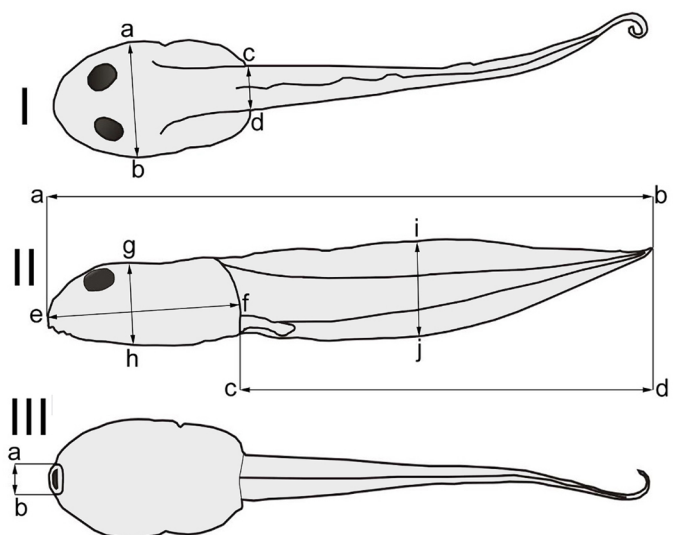


Figure 1. Schematic representation of morphometric measurements of the tadpoles taken for the analysis: (I) a–b = body width (BW), c–d = tail muscle thickness (TMT); (II) a–b = total length (TOL), c–d = tail length (TAL), e–f = body length (BL), g–h = body height (BH), i–j = maximum tail height (TAH); (III) a–b = oral disc width/mouth width (OD).

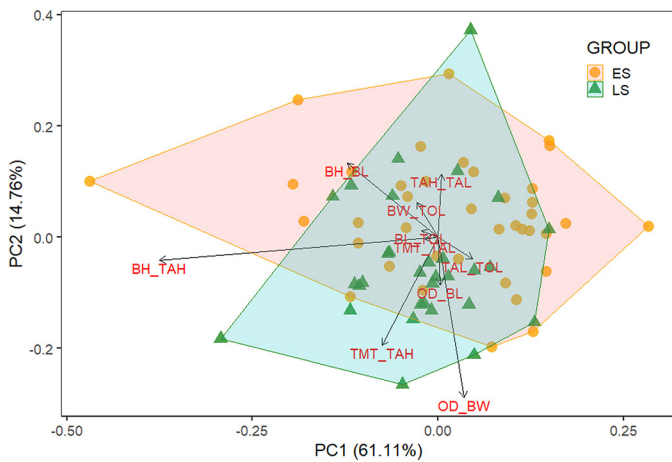


Figure 2. Principal component analysis (PCA) of 10 morphometric ratios. Scatter plot showing partial separation of the two groups on PC1 and PC2; ES = early stages, LS = later stages.

Table 1. Factors loadings and variation explained on two axis of principal component analysis (PCA) of two groups using ten morphometric ratios.

	PC1	PC2
BL/TOL	-0.055	0.030
TAL/TOL	0.115	-0.095
BW/TOL	-0.069	0.155
TMT/TAL	-0.043	-0.008
TMT/TAH	-0.184	-0.477
BH/BL	-0.300	0.327
BH/TAH	-0.920	-0.100
TAH/TAL	0.012	0.278
OD/BL	0.007	-0.210
OD/BW	0.086	-0.707
Explained variance (%)	61.18	14.76

We initially performed a principal component analysis (PCA) to explore the association of morphometric ratios in both groups. Data were not scaled because they were ratios. Ratios with a loading of $\geq \pm 0.15$ on the first two PCA axes (suggesting a higher association with the axes) were selected for further analysis. Multivariate normality of these selected ratios was assessed using the Shapiro Wilk test from the ‘MVN’ package (Korkmaz et al. 2014) and the homogeneity of variance was checked using the Bartlett test from the ‘heplots’ package (Fox et al. 2018) in R (R Developmental Core Team 2008). Using SPSS 19, we performed a one-way MANOVA to analyze the statistical significance (if any) between the groups using the selected ratios as response variables before applying a one-way ANOVA using each of the ratios as dependent variables between the same groups.

The first two axes of the PCA explained more than 75% of the total variation between the ES and LS groups (Fig. 2; Table 1), and seven of the ten ratios had loading values of ≥ 0.15 (Table 1). The PCA revealed that ES and LS could only be partially separated based on BH/TAH on the first axis and OD/BW, TMT/TAH, BH/BL, and TAH/TAL on the second axis (Fig. 2; Table 1).

MANOVA results showed that the ratios differed significantly between the two groups (Wilks $\lambda_{7,62} = 4.31$, $P < 0.001$). Post-hoc analysis also revealed a significant difference in BW/TOL and OD/BW between the two groups, which was suggestive of increased body and oral disc width in LS tadpoles (Table 2). However, other ratios contributing to separation along PC1 and PC2 did not differ between groups (Table 2).

Although cannibalistic/predatory, *H. tigrinus* tadpoles must protect themselves from conspecifics and other predators. The present study revealed that the tadpoles of *H. tigrinus* develop a wide oral cavity and increase in body width over the course of their development. Increased width of the oral cavity in LS tadpoles is a typical characteristic of ‘predators’ as evidenced by the other studies on amphibians (Relyea and Auld 2004; Kishida et al. 2009). A wide oral cavity facilitates capture and ingestion of large prey.

The increased body width of LS tadpoles fits a trade-off scenario in cannibalistic species. Carnivorous predatory tadpoles generally have large bodies and stomachs for accepting and easily digesting large prey (Martin and Pfennig 2009; Haas et al. 2014). On the other hand, tadpoles of some species that serve as prey also develop bulkier bodies in response to predation to avoid predation from gape-limited preda-

Table 2. Comparison of ten ratios between early-stage (ES) and late-stage (LS) tadpoles using posthoc ANOVA. P-values ≤ 0.05 (in bold type) denote significant differences between the two groups. BW = body width, TMT = tail muscle thickness, TOL = total length, TAL = tail length, BL = body length, BH = body height, TAH = maximum tail height, OD = oral disc width/mouth width.

	Mean Early-stages	Mean Late-stages	F-statistic (df = 1.68)	P-value
BL_TOL	0.323	0.328	1.50	0.22
TAL_TOL	0.675	0.672	0.36	0.54
BW_TOL	0.190	0.182	5.73	0.01
TMT_TAL	0.104	0.103	0.32	0.56
TMT_TAH	0.409	0.421	2.24	0.13
BH_TAH	0.835	0.870	3.65	0.06
OD_BL	0.249	0.246	0.42	0.51
OD_BW	0.424	0.444	5.42	0.02
BH_BL	0.441	0.435	0.74	0.39
TAH_TAL	0.254	0.245	3.23	0.07

tors (Kishida and Nishimura 2005; Kishida et al. 2009). So, increased body width appears to be helpful when *H. tigerinus* tadpoles engulf and digest large prey but also can prevent them from being eaten by conspecifics. In addition, tadpoles of *H. tigerinus* possess keratinized beaks and spurs in the sub-terminal mouth, both of which are adaptations for carnivorous feeding (Grosjean et al. 2004).

We did not observe any changes in the tail morphology between ES and LS tadpoles except for a reduction in tail height when compared to body width. Tadpoles exposed to predation cues increase tail length, depth, and musculature to avoid predation (Kishida and Nishimura 2005; Johnson et al. 2015), although *H. tigerinus* tadpoles appear to compensate for increased body width with tail height.

Acknowledgements

We thank Maharashtra State Biodiversity Board (MSBB/Desk-5/Appl/NOC/CR-275/1362/2015-16) for permission to collect animals. SKP is thankful to the SPPU Authority for providing research facilities. SMP thanks Sanjay Molur, Zoo Outreach Organization, for his support.

Literature Cited

- Collins, J.P. and J.E. Cheek. 1983. Effect of food and density on development of typical and cannibalistic salamander larvae in *Ambystoma tigrinum nebulosum*. *American Zoologist* 23: 77–84. <https://doi.org/10.1093/icb/23.1.77>.
- Fox, J., M. Friendly, and G. Monette. 2018. Heplots: Visualizing tests in multivariate linear models. *R package version 1.3-5*. <<https://CRAN.R-project.org/package=heplots>>.
- Gosner, K.L. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* 16: 183–190.
- Grosjean, S., M. Vences, and A. Dubois. 2004. Evolutionary significance of oral morphology in the carnivorous tadpoles of tiger frogs, genus *Hoplobatrachus* (Ranidae). *Biological Journal of the Linnean Society* 81: 171–181. <https://doi.org/10.1111/j.1095-8312.2003.00272.x>.
- Haas, A., J. Pohlmeier, D.S. McLeod, T. Kleinteich, S.T. Hertwig, I. Das, and D.R. Buchholz. 2014. Extreme tadpoles II: the highly derived larval anatomy of *Occidozyga baluensis* (Boulenger, 1896), an obligate carnivorous tadpole. *Zoomorphology* 133: 321–342. <https://doi.org/10.1007/s00435-014-0226-7>.
- Johnson, J.B., D. Saenz, C.K. Adams, and T.J. Hibbitts. 2015. Naturally occurring variation in tadpole morphology and performance linked to predator regime. *Ecology and Evolution* 5: 2991–3002. <https://doi.org/10.1002/ece3.1538>.
- Kishida, O. and K. Nishimura. 2005. Multiple inducible defences against multiple predators in the anuran tadpole, *Rana pirica*. *Evolutionary Ecology Research* 7: 619–631.
- Kishida, O., G.C. Trussell, and K. Nishimura. 2009. Top-down effects on antagonistic inducible defence and offense. *Ecology* 90: 1217–1226. <https://doi.org/10.1890/08-0238.1>.
- Korkmaz, S., D. Goksuluk, and G. Zararsiz. 2014. MVN: An R package for assessing multivariate normality. *The R Journal* 6: 151–162.
- Lardner, B. 2000. Morphological and life history responses to predators in larvae of seven anurans. *Oikos* 88: 169–180. <https://doi.org/10.1034/j.1600-0706.2000.880119.x>.
- Levis, N.A., S.S. Buzón, and D.W. Pfennig. 2015. An inducible offense: carnivore morph tadpoles induced by tadpole carnivory. *Ecology and Evolution* 5: 1405–1411. <https://doi.org/10.1002/ece3.1448>.
- Martin, R.A. and D.W. Pfennig. 2009. Disruptive selection in natural populations: The roles of ecological specialization and resource competition. *The American Naturalist* 174: 268–281. <https://doi.org/10.1086/600090>.
- Pomeroy, L.V. 1981. Developmental Polymorphism in the Tadpoles of the Spadefoot Toad *Scaphiopus multiplicatus*. Unpublished Ph.D. Dissertation, University of California, Riverside, California, USA.
- R Development Core Team. 2008. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. <<http://www.R-project.org>>.
- Relyea, R.A. 2002. Costs of phenotypic plasticity. *The American Naturalist* 159: 272–282. <https://doi.org/10.1086/338540>.
- Relyea, R.A. and J.R. Auld. 2004. Having the guts to compete: how intestinal plasticity explains costs of inducible defences. *Ecology Letters* 7: 869–875. <https://doi.org/10.1111/j.1461-0248.2004.00645.x>.
- Van Buskirk, J., S. S. McCollum, and E.E. Werner. 1997. Natural selection for environmentally-induced phenotypes in tadpoles. *Evolution* 52: 1983–1992. <https://doi.org/10.1111/j.1558-5646.1997.tb05119.x>.