



## Tadpole dance: the gymnastics of *Ololygon machadoi* larvae to feed on pollen

JÉSSICA S. KLOH<sup>2</sup>, CLEBER C. FIGUEREDO<sup>2</sup> & PAULA C. ETEROVICK<sup>1</sup>

<sup>1</sup>) Programa de Pós Graduação em Biologia de Vertebrados, Pontifícia Universidade Católica de Minas Gerais, 30535-610, Belo Horizonte, Brazil

<sup>2</sup>) Programa de Pós Graduação em Ecologia, Conservação e Manejo de Vida Silvestre, Universidade Federal de Minas Gerais, 31270-901, Belo Horizonte, Brazil; e-mail: cleberfigueredo@ufmg.br

Corresponding author: PAULA ETEROVICK, e-mail: pcterovick@gmail.com

Manuscript received: 12 June 2020

Accepted: 30 November 2020 by Arne Schulze

**Abstract.** A species diet offers information on its trophic level and its roles in the community. Tadpoles are known to be important components of aquatic food webs, however little is known about their feeding habits and food preferences. Tadpole ecomorphotypes have been shown to relate to their diet, but some consumed food resources are not available at preferred feeding microhabitats, such as pollen for benthic tadpoles. Pollen has high nutritive value, thus tadpoles may change their feeding behavior in order to explore it. We tested this hypothesis by offering fed or hungry tadpoles pollen on the water surface at three different depths, with or without an alternative food source. We noticed that pollen consumption decreased with increased depth, what is in accordance with optimal foraging theory, as the tadpoles have to make longer maneuvers to achieve the pollen from the bottom in deeper water. That is likely also the reason why tadpoles decreased pollen consumption when provided with alternative food on the bottom, where they usually forage. However, increased difficulty and alternative food sources did not prevent tadpoles to feed on pollen, showing that they can be flexible enough to change their feeding habits in order to opportunistically explore new nutritive food sources.

Key words. Amphibia, Anura, Hylidae, *Ololygon machadoi*, tadpole diet, feeding behaviour, buoyant food, optimum foraging.

### Introduction

A species diet indicates its role in the trophic web and reveals important connections among species in communities (DUTRA & CALLISTO 2005, SCHIESARI et al. 2009). Amphibian larvae have important roles in aquatic food chains and connect aquatic and terrestrial energy cycles (SEALE 1980, OSBORNE & MCLACHLAN 1985, ALTIG et al. 2007). However, knowledge on tadpole trophic ecology is relatively limited (ALTIG et al. 2007, MONTAÑA et al. 2019).

Contrary to old assumptions that considered tadpoles as non-selective feeders with little niche differentiation, tadpole diets are diverse (SCHIESARI et al. 2009) and can include algae, fungi, amoebae, eggs of aquatic invertebrates, vegetal debris, pollen, amphibian eggs and larvae (ALTIG et al. 2007, WELLS 2007, KLOH et al. 2019). Pollen from Gymnosperma and Angiosperma are rich in amino acids, vitamin, lipids, and thus a nutritive food resource (PALANIVELU & TSUKAMOTO 2011, FRIAS et al. 2016). Pollen enters aquatic food webs through pollen rains that deposit on water surface. It is an important allochthonous carbon and phosphorus source that boosts microbial growth and is ultimately transferred to zooplankton consumers (MAS-

CLAUX et al. 2013). Although it has received little attention as a component of tadpole diets, is commonly present in the diet of some species (WAGNER 1986, KLOH et al. 2018, 2019), such as *Ololygon machadoi* (BOKERMANN & SAZIMA 1973), which search for and intentionally eat pollen.

The tadpoles of *O. machadoi* are benthic (BOKERMANN & SAZIMA 1973) and would thus be expected to feed on the bottom (ALTIG & JOHNSTON 1989). They occur in rocky streams in montane meadows (“Campos Rupestres”) in southeastern Brazil, which are considered oligotrophic habitats (MENDES 2003). In that habitat, pollen could be an important nutritive resource for the tadpoles. However, because pollen may have bladders that make them buoyant (MASCLAUX et al. 2013), they may take time to sink and lose nutritious value with time (KELLER et al. 2005). To maximize the value of feeding on pollen, tadpoles may benefit from ingesting pollen while it is fresh on the water’s surface.

In this study, we explore the circumstances when *O. machadoi* tadpoles alter their benthic behavior in order to capture and profit from pollen on the water’s surface. We tested tadpoles in two developmental stages, and under satiety and hunger conditions. Tadpoles were subject to one

of three water levels and had an alternative food source or not during the trials. We expected larger tadpoles to eat more pollen than smaller tadpoles due to likely greater energy needs. We expected hungry tadpoles to consume more pollen, especially at low water level, where they needed to spend less energy to reach the water surface. We also expected tadpoles to eat less pollen when an alternative food source was available on the bottom, with easier access.

## Materials and methods

### Study system and sampling

Tadpoles of *Ololygon machadoi* were obtained for this study at a third order stream (*sensu* STRAHLER 1957) (19°16'3.35" S, 43°30'56.23" W, 1,000 m a.s.l.) from the Doce water basin located in the Parque Nacional (National Park) Serra do Cipó (PNSC), at the southern portion of the Espinhaço mountain range, in southeastern Brazil. The stream has low nutrient concentrations and can be considered as oligotrophic (MENDES 2003). The climate at the PNSC presents a dry, cold season from April to September and a wet, warm season from October to March. Mean monthly temperatures vary between 13 and 29°C and mean annual rainfall, between 1460 and 2490 mm (VIVEROS 2010).

*Ololygon machadoi* is endemic from the Espinhaço mountain range, a priority area for conservation in Brazil due to its great species diversity and levels of endemism for varied taxa (LEITE et al. 2008). It breeds year-round in permanent streams (ETEROVICK & SAZIMA 2004). The tadpoles have slow development, taking about five months to reach metamorphosis (ETEROVICK & SAZIMA 2004). They are easily recognizable due to their dark body crossed by bright yellow bars (ETEROVICK et al. 2018, Fig. 1A). They have an ovoid body, ventral mouth and dorsal eyes (Fig. 2A), being characterized as benthic (PIMENTA et al. 2014). They eat algae (Zygnematophyceae, Euglenophyceae, Bacillariophyceae, and Cyanophyceae), testate amoeba, vegetal debris, and pollen at the study site (KLOH et al. 2019), being normally seen at the bottom of streams (ETEROVICK & SAZIMA 2004). Their oral disc is not laterally emarginated, with biseriata marginal papillae around the whole perimeter, a keratinized beak, and labial tooth row formula (LTRF) 2(2)/3 (PEZZUTI 2019; Fig. 2B), characterizing a benthic scraper. It is likely adapted to microhabitats with little water flow due to the absence of attaching structures in the mouth (ALTIG et al. 2007). The oral disc is more prominent compared to other species with similar body features and biology, what could favor pollen capture on the water surface, when tadpoles can use their oral discs as a basal funnel. The marginal papillae could also aid to pollen sorting and capture.

Tadpoles of *O. machadoi* can be observed year-round, using varied types of microhabitats. We observed these tadpoles to swim to the surface and turn their ventral region upwards to capture floating pollen grains at sites with slow or no water flow close to flowering plants at the margins.

We collected *O. machadoi* tadpoles and pollen from *Tibouchina martiusiana* (DC.) Cogn. (Melastomataceae) for our experiments at the aforementioned stream in January 2019. *Tibouchina martiusiana* grows up to about 1.50 m high and flowers year-round. This shrub is adapted to high sun incidence at the “Campos rupestres” and is common alongside streams at the PNSC (MARTENS 2008). We collected 30 flowers for pollen from plants located up to 1 m from stream margins. We stored the flowers in plastic bags within polystyrene foam boxes with ice for transport to the laboratory (a 2-hour-drive). At the laboratory, we extracted pollen from the flowers with tweezers under a stereomicroscope and stored it at -4°C to avoid nutrient loss / degradation.

We captured tadpoles with dipnets while progressing upstream to avoid disturbing them with our movement in the water. We collected 120 tadpoles along a 150 m extension of the stream, including 60 tadpoles in developmental stage 25 and 60 in developmental stage 30 (*sensu* GOSNER 1960). We transported the tadpoles in plastic bags with aged mineral water, which were accommodated amidst ice packs within polystyrene foam boxes.

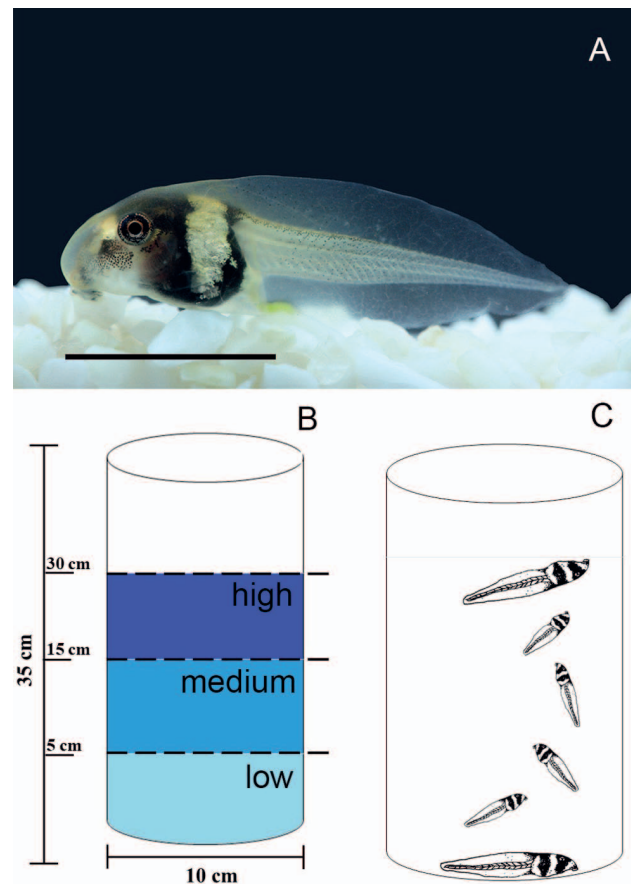


Figure 1. Tadpole of *Ololygon machadoi* in developmental stage 25 (A, photography by Tiago Leite Pezzuti), schematic representation of water levels in experimental containers (B) and tadpole maneuver to reach pollen on the water surface (C). The scale bar in A represents 10 mm. The tadpole in C is represented out of scale for greater detail.

### Experimental design

At the laboratory, tadpoles were individually allocated in polyethylene transparent containers (35 cm high, 10 cm diameter) with aged mineral water and randomly assigned to two experimental groups: one group received commercial fish food (Probetta Show<sup>®</sup>, 0.0215 g per individual every 3 hours) and the other received no food for 12 hours, before we started the experiments. Each group included 30 tadpoles in each of the two developmental stages. We have previously used this fish food to feed tadpoles in the laboratory and they showed normal growth during limited periods (up to a month). Colleagues have successfully used this and similar fish commercial foods for longer maintenance of tadpoles of other species (C. VINICIUS, C. LISBOA, I. F. MACHADO, T. KOHLSORF pers. comm.). We considered it to be a nutritive food, as it contains components from fungi, animal and vegetal origins (fish, shrimp, corn, soy protein, yeast, algae, spirulin, vitamins, and antioxidants according to the manufacturer).

For the experiments we used 120 of the same polyethylene containers with three different amounts of water to produce: 1) low water level (400 ml of water to fill the container up to 5 cm); 2) intermediate water level (1 liter of water, to 15 cm) and 3) high water level (2 liters of water, to 30 cm; Fig. 1B). We manipulated water depths based on depths of microhabitats where *O. machadoi* tadpoles normally occur within their natal stream (PCE, unpublished data). We assigned 20 tadpoles (10 of each developmental

stage) from the two dietary groups (tadpoles with or without food for 12 hours) to each water level treatment. The 10 tadpoles within the same developmental stage, previously determined hunger level, and water level were divided between two food treatments, that were introduced two minutes after tadpole placement in the recipient: (1) they either received five pollen grains ( $\pm 0.198$  g) and five fish food pellets ( $\pm 0.0215$  g) or (2) just five pollen grains ( $\pm 0.0198$  g). The pellets sink to the bottom, whereas the pollen remains on the water surface.

We then observed the tadpoles at a distance (2 m) during one hour and at the end, we counted the remaining pollen grains and fish food pellets at each recipient. No pollen grains sank during the experiment. At the end of the experiment, we euthanized the tadpoles with xylocain 10% and deposited them in the Amphibian Collection of the Museu de Ciências Naturais of the Pontifícia Universidade Católica de Minas Gerais (MCN-AM) as testimony material. We did not return the tadpoles to their original habitat after taking them to the laboratory to avoid any potential contamination with diseases, because we have no information about such risk.

We tested the 120 tadpoles (5 tadpoles  $\times$  2 developmental stages  $\times$  2 hunger levels  $\times$  3 water levels  $\times$  2 food treatments) simultaneously under natural day light at ambient temperature (22°C).

### Statistical analyses

We compared pollen consumption (i.e., number of pollen grains ingested as a dependent variable) among tadpoles from two developmental stages (25 and 30) subject to two previously determined hunger levels (hungry or fed), three water levels (low, medium, and high) and in the presence or absence of an alternative food source (i.e., fish food) as explanatory variables.

We built Generalized Linear Models (GLMs) with the packages car (FOX & WEISBERG 2011) and MASS (VENABLES & RIPLEY 2002) in R (R Core Team 2020). We then used the package MuMIn (BARTÓN 2019) for R (R Core Team 2020) to select the best models controlling the overall type I error rate (HOTHORN et al. 2008). Lastly, we conducted Tukey post hoc tests with the package emmeans in R (RUSSELL 2020).

### Results

*Oligolygon machadoi* tadpoles fed on pollen under all experimental conditions, but in different amounts. When reaching for pollen (Fig. 1C), tadpoles initiate their movement turning their heads to the right (70% of individuals, Fig. 3) or to the left (30% of individuals). Afterwards they swim bending their bodies in pitch up (sensu FISH & LAUDER 2017) to the left or to the right respectively (depending on the side they initiated the movement) until they reach  $\frac{1}{3}$  of the distance to the surface. Then they turn to the right

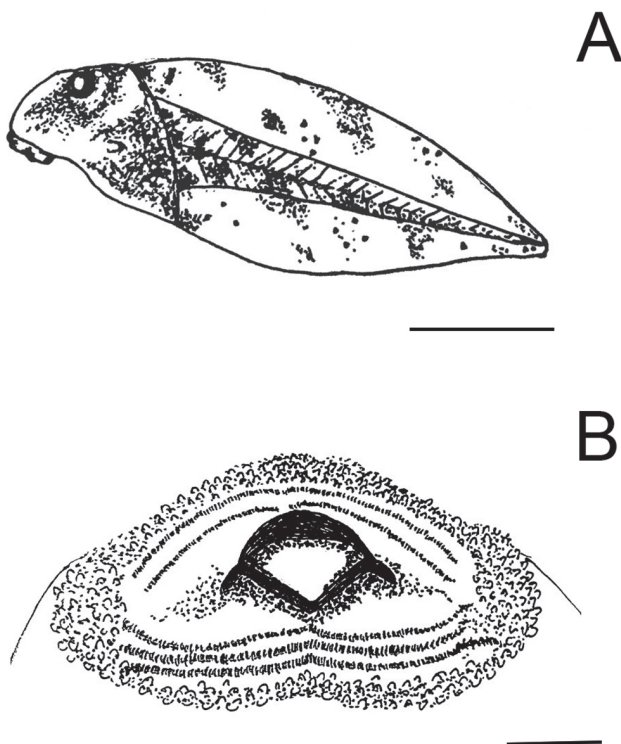


Figure 2. Lateral view (A) and details of the oral disc (B) of the tadpole of *Oligolygon machadoi*.

or to the left respectively, and continue in pitch up until they approach the surface, where they perform a pitch back movement turning their ventral region towards the surface at an angle of 30 degrees, their mouths touching the water surface. The tadpoles then perform continuous yaw movements, when their tails wag laterally, allowing them to move at the surface while they feed on pollen. When they are done feeding, they make a fast roll twisting the tail and turning the ventral region towards the bottom. During this movement, 80% of the tadpoles turn to the left, including all the ones that started the movement turning their heads to the right and some of the ones that started turning their heads to the left.

The best model to explain pollen consumption included all variables, but no interactions among them (Table 1). Tadpoles in stage 25 consumed more pollen ( $n = 81$  grains) than tadpoles in stage 30 ( $n = 28$  grains; deviance = 26.896,  $df = 1$ ,  $p < 0.001$ ). Tadpoles that were kept without food for 12 hours before the experimental trials ate more pollen ( $n = 84$  grains) than tadpoles that were fed during this period ( $n = 25$  grains; deviance = 33.713,  $df = 1$ ,  $p < 0.001$ ). Tadpoles that were offered fish food as an alternative food item during the experiments ate less pollen ( $n = 44$  grains) than those that received just pollen ( $n = 65$  grains; deviance = 4.071,  $df = 1$ ,  $p = 0.044$ ). Pollen consumption increased as water level decreased (deviance = 28.682,  $df = 2$ ,  $p < 0.001$ ). Pollen consumption at low water level ( $n = 63$  grains) was greater than both at medium ( $n = 27$  grains; Tukey estimate = 0.847,  $z = 3.684$ ,  $p < 0.001$ ) and high water levels ( $n = 19$  grains; Tukey estimate = -1.199,  $z = -4.580$ ,  $p < 0.001$ ). Consumption at medium and high water levels did not differ (Tukey estimate = -0.351,  $z = -1.173$ ,  $p = 0.469$ ; Fig. 2).

## Discussion

*Oloolygon machadoi* ate pollen under all experimental conditions, even when they suffered no food deprivation and

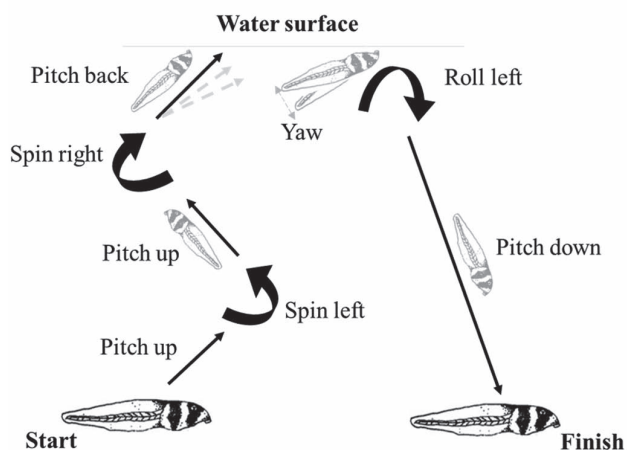


Figure 3. Schematic representation of *Oloolygon machadoi* tadpole maneuver to reach pollen at the water surface.

had an easily accessible alternative food source. Pollen can be very important in some freshwater food chains (MASCIAUX et al. 2013) and is considered an important nutrient source for several animals (KELLER et al. 2005, FRIAS et al. 2016). Pollen is estimated to provide 240–305 kcal per 100 g dry weight, which makes it an exceptional high caloric food compared to phytoplankton with 30–60 kcal for the same dry weight (PINTO-COELHO 2009). In bees, pollen consumption can increase life span, boost hypopharyngeal gland development and the immune system, making the insects more resistant to parasites and viruses (FRIAS et al. 2016). The effects of pollen on tadpoles is still unknown, but it may be an important and diverse food source, especially in the tropical region rich in pollen producing plant species (PALANIVELU & TSUKAMOTO 2011).

The tadpoles of *O. machadoi* exhibited lateralization in their maneuvers to reach for pollen. Lateralization has already been described to occur in fish, amphibians, and reptiles and related to feeding behavior (SOVRANO 2007, STANCHER et al. 2018). Most individuals of *O. machadoi* turned to the left during spin and roll movements. This could be related to the presence of the spiracle at the left side (GOREE & WASSERSUG 2001), what could optimize oxygen flow during the movements (GOREE & WASSERSUG 2001, STANCHER et al. 2018). However, some studies suggest that side preferences would be more strongly related to phylogeny than to external morphology (WASSERSUG et al. 1999). Lateralization studies are still incipient in tadpoles, with most studies on amphibians focusing on post-metamorphic individuals (WASSERSUG et al. 1999).

Contrary to our expectations, tadpoles in developmental stage 25 ate more pollen than larger tadpoles in developmental stage 30. Tadpoles of *O. machadoi* hatch at stage 25 and are susceptible to predators due to their small size. Tadpoles have a great nutrient need in order to be active and grow quickly (WASSERSUG, 1975). Tadpoles in developmental stage 30 are heavier and likely must expend more energy to turn and move their bodies up to the surface, increasing the energetic costs of pollen acquisition. This is in accordance with the optimal foraging theory (MACARTHUR & PINANKA 1966), which predicts that foragers should invest in food items that provide the greatest nutritional advantage (energy gain) with the lowest costs (energy expenditure for food acquisition). This theory is also compatible with the decrease of pollen consumption as water level increased, representing a longer distance upwards to be covered by tadpoles trying to feed on pollen at the surface. Greater pollen proximity provided by lower water levels may also have intensified the cues used by tadpoles to detect it, encouraging its pursue.

In *O. machadoi* natural habitats, food abundance varies spatially (KLOH et al. 2019) and likely also temporally. The periphyton algal community, for instance, can be influenced by water flow, that depends on rainfall and discharge (NECCHI-JR. et al. 1991). Pollen availability on the water surface may be influenced by both water flow and pollen production as a result of plant reproductive cycles. *Tibouchina martusiana* flowers year-round, but an in-

crease in the number of flowers can be noticed during the rainy season, also coinciding with the presence of the highest numbers of newly hatched *O. machadoi* tadpoles (JSK, pers. obs.). Thus, pollen is likely to represent an important food source for initial tadpole growth, corroborating our results of higher likelihood of smaller tadpoles to feed on pollen.

Different feeding strategies are linked to different phenotypes (BEGON et al. 2007), and this also applies to tadpoles (CANDIOTI 2007). Being benthic, tadpoles of *O. machadoi* are adapted to feed on the bottom, but their behavioural plasticity demonstrated here corroborates the importance of flexible feeding strategies for better food harvesting. Waiting for pollen grains to sink would not be

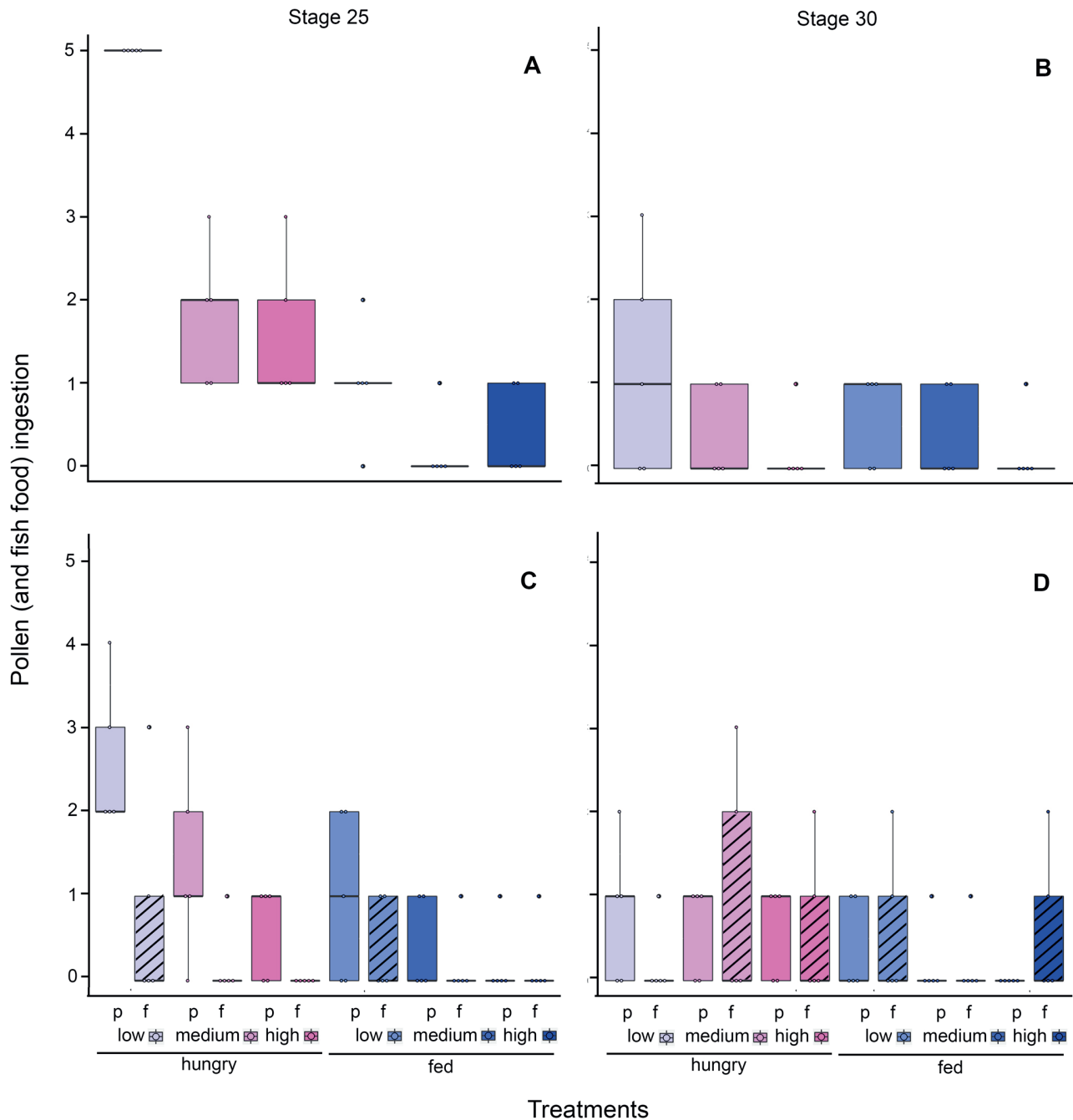


Figure 4. Box plots representing ingestion of pollen (p, plain bars) and fish food pellets (f, dashed bars) by *Ololygon machadoi* tadpoles in developmental stages 25 (A, C) and 30 (B, D) offered just pollen (A, B) or pollen and fish food (C, D). Tadpoles were submitted to two previous feeding regimes that consisted in receiving no food (hungry) or fish food (fed) for 12 hours before the experimental trials and three water levels (low, medium, and high) in the experimental containers.

so profitable, as pollen loses its nutritional value over time and it can be carried away by the water flow before it sinks (KELLER et al. 2005). *Pseudacris regilla* (BAIRD & GIRARD, 1852) tadpoles are also able to somehow detect pollen on the water surface and evaluate its quality. These tadpoles adjust their feeding behavior during gymnosperm pollen dispersion, when they elect pollen as a preferential food resource (WAGNER 1986).

This study demonstrates that tadpoles can actively select their food to the point of altering their feeding behavior in order to opportunistically acquire nutritive food items with more difficult access. The accessibility of food for tadpoles is intimately dependent on their morphological attributes (ANNIBALE et al. 2019), and in our study we have shown that the tadpoles of *O. machadoi* can overcome morphological limitations (i.e., the ventral position of the oral disc as an unfavorable feature for surface feeding) with behavioural maneuvers in order to reach a profitable resource. Our study represents an example of optimal foraging theory, in which the energetic costs of food acquisition may be compensated by its nutritional value. Tadpoles are under strong pressure to acquire nutrients for growth and development, and here we have shown that they can be flexible and adapt to explore occasional profitable food sources via behavioural plasticity.

#### Acknowledgements

We are grateful to TIAGO PEZZUTI for kindly giving us the tadpole picture (Fig. 1A), to RICHARD WASSERSUG and an anonymous reviewer for helpful suggestions in a previous version of the manuscript, to the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (Fapemig) for financial support; and to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for a Research Productivity grant (310162/2017-3) provided to P. C. ETEROVICK. Collection permit was provided by Sisbio/ICMBio (64500).

#### References

- ALTIG, R. & G. F. JOHNSTON (1989): Guilds of anuran larvae: relationships among developmental modes, morphologies, and habitats. – *Herpetological Monographs*, **3**: 81–109.
- ALTIG, R., M. R. WHILES & C. L. TAYLOR (2007): What do tadpoles really eat? Assessing the trophic status of an understudied and imperiled group of consumers in freshwater habitats. – *Freshwater Biology*, **52**: 386–395.
- ANNIBALE, F. S., V. T. T. SOUSA, C. E. SOUSA, M. D. VENESKY, D. C. ROSSA-FERES, F. NOMURA, R. J. WASSERSUG (2019): Influence of substrate orientation on tadpoles' feeding efficiency. *Biology Open*, **8**: bio037598, DOI:10.1242/bio.037598.
- BAIRD, S. F. & C. GIRARD (1852): Descriptions of new species of reptiles, collected by the U.S. Exploring Expedition under the command of Capt. Charles Wilkes, U.S.N., First Part – including the species from the western part of America. – *Proceedings of the Academy of Natural Sciences of Philadelphia*, **6**: 174–177.
- BARTOŃ, K. (2019): MuMIn: Multi-Model Inference. R package version 1.43.6. <https://CRAN.R-project.org/package=MuMIn>.
- BEGON, M. C. R., E. TOWNSEND & J. L. HARPER (2007): *Ecologia: de indivíduos a ecossistemas*. 4<sup>th</sup> ed. – Artmed, Porto Alegre, Brazil.
- BOKERMANN, W. C. A. & I. SAZIMA (1973): Anfíbios da Serra do Cipó, Minas Gerais, Brasil. 1: Duas espécies novas de “*Hyla*” (Anura, Hylidae). – *Revista Brasileira de Biologia*, **33**: 521–528.
- CANDIOTI, M. F. V. (2007): Anatomy of anuran tadpoles from lentic water bodies: systematic relevance and correlation with feeding habits. – *Zootaxa*, **1600**: 1–175.
- DUTRA, S. L. & M. CALLISTO (2005): Macroinvertebrates as tadpole food: importance and body size relationships. – *Revista Brasileira de Zoologia*, **22**: 923–927.
- ETEROVICK, P. C. & I. SAZIMA (2004): Amphibians from the Serra do Cipó, Minas Gerais, Brazil. – Editora PUC Minas, Belo Horizonte, Brazil.
- ETEROVICK, P. C., I. S. MENDES, J. S. KLOH, L. T. PINHEIRO, A. B. H. P. VÁCLAV, T. SANTOS & A. S. B. GONTIJO (2018): Tadpoles respond to background colour under threat. – *Scientific Reports*, **8**: 4085, DOI:10.1038/s41598-018-22315-8.
- FISH, F. E. & G. V. LAUDER (2017): Control surfaces of aquatic vertebrates: active and passive design and function. – *Journal of Experimental Biology*, **220**: 4363–4351.
- FOX, J. & S. WEISBERG (2011): *An {R} Companion to Applied Regression*, 2<sup>nd</sup> ed. – Thousand Oaks CA, Sage. <http://socserv.socsci.mcmaster.ca/jfox/Books/Companion>.
- FRIAS, B. E., C. D. BARBOSA & A. P. LOURENÇO (2016): Pollen nutrition in honey bees (*Apis mellifera*): impact on adult health. – *Apidologie*, **47**: 15–25.
- GOREE, B. D. & R. J. WASSERSUG (2001): Are archeobatrachian anurans ambidextrous? Assessing handedness in *Bombina orientalis*. – *Journal of Herpetology*, **35**: 538–541.
- GOSNER, K. L. (1960): A simplified table for staging anuran embryos and larvae with notes on identification. – *Herpetologica*, **16**: 183–190.
- HOTHORN, T., F. BRETZ & P. WESTFALL. (2008): Simultaneous inference in General Parametric Models. – *Biometrical Journal*, **50**: 346–363. doi: 10.1002/bimj.200810425.
- KELLER I., P. FLURI & A. IMDORF (2005): Pollen nutrition and colony development in honey bees – Part II. – *Bee World*, **86**: 27–34.
- KLOH, J. S., C. C. FIGUEREDO & P. C. ETEROVICK (2018): You are what, where, and when you eat: seasonal and ontogenetic changes in a tropical tadpole's diet. – *Amphibia-Reptilia*, **39**: 445–456.
- KLOH, J. S., C. C. FIGUEREDO & P. C. ETEROVICK (2019): How close is microhabitat and diet association in aquatic ecomorphotypes? A test with tadpoles of syntopic species. – *Hydrobiologia*, **828**: 271–285.
- LEITE, F. S. F., F. A. JUNCÁ & P. C. ETEROVICK (2008): Status do conhecimento, endemismo e conservação de anfíbios anuros da Cadeia do Espinhaço, Brasil. – *Megadiversidade*, **4**: 182–200.
- MACARTHUR, R. H. & E. R. PIANKA (1966). On optimal use of a patchy environment. *American Naturalist*, **100**: 603–609.
- MARTENS, L. A. (2008): *Flores da Serra da Calçada*, Editora UFMG, Belo Horizonte, Brazil.
- MASCLAUX, H., M. E. PERGA, M. KAGAMI, C. DESVILLETES, G. BOURDIER & A. BEC (2013): How pollen organic matter enters freshwater food webs. – *Limnology and Oceanography*, **58**: 1185–1195.

- MENDES, R. S. (2003): Experimentos de enriquecimento in situ: Respostas de algas bentônicas a adição de nutrientes em um córrego de Altitude da Serra do Cipó – MG. – Masters Thesis, Federal University of Minas Gerais, Brazil.
- MONTAÑA, C. G., S. D. G. T. M. SILVA, D. HAGYARI, J. WAGER, L. TIEGS, C. SADEGHIAN, T. A. SCHRIEVER & C. M. SCHALK (2019): Revisiting “what do tadpoles really eat?” A 10-year perspective. – *Freshwater Biology*, **64**: 2269–2282.
- NECCHI-JR., O., M. R. DIP & R. M. GOÉS (1991): Macroalgae of a stream in southeastern Brazil: composition, seasonal variation and relation to physical and chemical variables. – *Hydrobiologia*, **213**: 241–250.
- OSBORNE, P. L. & A. J. MCLACHLAN (1985): The effect of tadpoles on algal growth in temporary, rain-filled rock pools. – *Freshwater Biology*, **15**: 77–87.
- PALANIVELU, R. & T. TSUKAMOTO (2011): Pathfinding in angiosperm reproduction: Pollen tube guidance by pistils ensures successful double fertilization. – *WIREs Developmental Biology*, **1**: 96–113.
- PEZZUTI, T. L., F. S. F. LEITE, P. C. A. GARCIA (2019): Chave de identificação interativa para os girinos do Quadrilátero Ferrífero, Minas Gerais, Sudeste do Brasil. Version 1.0. – Universidade Federal de Viçosa, Campus Florestal, Brazil. Available at <http://biodiversus.com.br/saglab/aqf/chave/girinos/>, accessed on 23 September 2020.
- PIMENTA, B. V. S, D. COSTA, R. MURTA-FONSECA & T. PEZZUTI (2014): Anfíbios: Alvorada de Minas, Conceição do Mato Dentro, Dom Joaquim – Minas Gerais. Bicho do Mato, Belo Horizonte, Brazil.
- PINTO-COELHO, R. M. (2009): Fundamentos de Ecologia. Artmed Editora, Belo Horizonte, Brazil.
- R Core Team (2020): R: A language and environment for statistical computing. – R Foundation for Statistical Computing. Version 3.6.3, Vienna, Austria. <https://cran.r-project.org/>.
- RUSSELL, L. (2020): emmeans: Estimated Marginal Means, aka Least-Squares Means. – R package version 1.4.5. <https://CRAN.R-project.org/package=emmeans>
- SCHIESARI, L., E. E. WERNER & G. W. KLING (2009): Carnivory and resource-based niche differentiation in anuran larvae: implications for food web and experimental ecology. – *Freshwater Biology*, **54**: 572–586.
- SEALE, D. B. (1980): Influence of amphibian larvae on primary production, nutrient flux, and competition in a pond ecosystem. – *Ecology* **61**: 1531–1550.
- SOVRANO, A. V. (2007): A note on asymmetric use of the forelimbs during feeding in the European green toad (*Bufo viridis*). – *Laterality*, **12**: 458–463.
- STANCHER, G., A. V. SOVRANO & G. VALLORTIGARA (2018): Motor asymmetries in fishes, amphibians, and reptiles. – pp. 33–56 in: FORRESTER G. S., W. D. HOPKINS, K. HUDRY & A. LINDELL (eds): Progress in brain research, Vol. 238. Cerebral lateralization and cognition: Evolutionary and developmental investigations of behavioral biases. – Elsevier Academic Press.
- STRAHLER, A. N. (1957): Quantitative analysis of watershed morphology. – *Transactions of the American Geophysical Union*, **38**: 913–920.
- VENABLES, W. N. & B. D. RIPLEY (2002): Modern applied statistics with S. 4<sup>th</sup> ed. – Springer, New York.
- VIVEROS, R. S. (2010). Pteridófitas da Serra do Caraça, Minas Gerais, Brasil. – Master Thesis, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil.
- WAGNER, W. E. (1986): Tadpoles and pollen: observations on the feeding behavior of *Hyla regilla* larvae. – *Copeia*, **1986**: 802–804.
- WELLS, K. D. (2007): The ecology and behavior of amphibians. – The University of Chicago Press, Chicago, U.S.A.
- WASSERSUG, R. J. (1975): The adaptive significance of the tadpole stage with comments on the maintenance of complex life cycles in anurans. – *American Zoologist*, **15**: 404–417.
- WASSERSUG, R. J., T. NAITOH & YAMASHITA, M. (1999): Turning bias in tadpoles. – *Journal of Herpetology*, **33**: 543–548.