

Taking tadpole biology into the 21st century: a consensus paper from the First Tadpoles International Workshop

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INTRODUCTION

Although tadpoles are common, frequently encountered vertebrates, their natural history is poorly known. Despite the fact that Brazil harbors the highest anuran diversity in the world, with almost a thousand known species (Segalla *et al.*, 2014), the larvae of many species remain undescribed. The problem is not unique to Brazil. At the turn of the century, approximately 2/3 of almost 3,300 anuran species with a larval stage lacked tadpole descriptions (McDiarmid & Altig, 1999). The proportion of neotropical anurans with free-swimming larvae that are not described is estimated to be around 40% (Provete *et al.*, 2012).

Since the larval phase of the anuran life cycle is particularly poorly studied in the Neotropics, there are large and important gaps in our knowledge of tadpole physiology, morphology, behavior, and ecology. The history of tadpole research in Brazil reveals a predominance of descriptive studies, focusing on morphology, natural history, and patterns of temporal and spatial distribution (Andrade *et al.*, 2007). Recently though studies on community processes have begun to include tadpoles (Prado & Rossa-Feres, 2014; Provete *et al.*, 2014; Almeida *et al.*, 2015) and phylogenetics studies increasingly included larval characters (Haas, 2003; Frost *et al.*, 2006)

Descriptive studies are at that base of all biological knowledge and underlie hypothesis about ecological and evolutionary processes. However Brazil has a large and heterogeneous landscape, whereas most descriptive studies about tadpole community structure are restricted to local scales (*e.g.*, Vasconcelos *et al.*, 2009; Provete *et al.*, 2014; Almeida *et al.*, 2015). In order to predict the impact on anurans of changes in land

use and land cover-and also climate change-on the larger scale we need, at first, to identify patterns in different biomes and associated ecosystems; *i.e.*, we need to understand and distinguish the natural variation from that originated by anthropic changes to the landscape. Experimental studies complement our knowledge of natural history by mean of hypothesis testing. However, experimental studies with tadpoles in Brazil are rare (Rossa-Feres *et al.*, 2011). This is despite the fact that tadpoles have been an important model-system in experimental ecology for close to half a century (reviewed in Wilbur, 1997).

To understand the fate of anurans in Brazil and protect their natural diversity, we need experimental studies that comprehensively explore ecological processes on several spatial scales. Such studies are fundamental to predicting ecology and establish effective conservation plans for anurans (Rossa-Feres *et al.*, 2011). Nevertheless, descriptive studies of anuran larvae continue to be needed because so little is known about basic tadpole biology.

With these facts in mind, the first “Tadpoles International Workshop” was held on 16 and 17 March 2015, UNESP in São José do Rio Preto. The purpose was to bring specialists on the larval phase of the anuran life cycle together to share information and insights through lectures and discussions. Certain major questions in tadpole biology were explored. These included: (1) How can knowledge about tadpoles help us understand anuran diversity in Brazil? (2) What is the relative intra- and interspecific morphological variation in tadpoles? (3) What is the relationship between morphology, phylogeny and ecology of tadpoles? (4) What hampers advances in experimental research with tadpoles in Brazil particularly on large geographic scales? (5) How do diseases, parasites, changes in land use and land

cover impact tadpole communities in Brazil? (6) What are the main evolutionary and ecological processes that structure tadpole communities in different Brazilian biomes? (7) How can this knowledge aid our ability to predict the impact of changes in climate and land use on anuran populations?

Obviously questions like these are complex and cross many disciplines. Thus our aim was to establish a forum to discuss concepts and ideas applied to tadpoles, which highlight the importance of this life stage for anurans to theoretical zoology and other sciences, such as ecology and biogeography. It is our hope that the discussion initiated at this Workshop will be a catalyst for multidiscipline research on tadpole biology. Following we present a brief summary of the Tadpoles Workshop' lectures.

LECTURE SUMMARIES

They don't look like frogs and they don't look like fish; so why are tadpoles built like that?

**Richard J. Wassersug,
Dalhousie University, Halifax, Canada**

In this talk I introduce tadpoles and tadpole diversity. I first explore common features of anuran larvae that distinguish them from both fish and frogs. I then examine adaptations of tadpoles for air-breathing, feeding, and swimming. These adaptations are explored in the context of the need for tadpoles to both function well in water, yet metamorphose rapidly into a terrestrial animal.

Tadpoles are obligatorily metamorphic and have a transient existence in the aquatic environment. They need to metamorphose rapidly since individuals in transition are neither efficient at swimming nor hopping. Having a tail without vertebrae permits rapid metamorphosis. It also makes the tadpoles very flexible. That means that they have high angular acceleration, which facilitates escaping from predators.

The tadpole oral disc functions not only in feeding, but in air-breathing. When a tadpole opens its mouths at the water's surface, the oral disc is concurrently projected forward, surrounding the mouth to prevent water from coming in. This helps tadpoles draw in air rapidly and reduce the amount of time they are at the surface and at risk of attack from aerial predators.

The labial teeth of tadpoles both anchor the mouth to a surface during grazing and help rack the surface to produce a suspension of material that is sucked into the mouth during feeding.

Although the tadpole shape, with a globose body and a flattened tail, intuitively looks less efficient than the more streamlined shape of fishes, fluid dynamic modelling shows that that shape is not necessarily inefficient. That shape allows for the development of hind limbs without a substantial increase in drag, which they would experience, if they were shaped like fishes and still had to develop hind limbs in preparation for a terrestrial existence. The characteristic position of the developing hind limbs, in the recess behind the body and at the base of

the tail, is a "dead water" zone. While it permits limb development without much added drag, it acts as a safety zone where aquatic parasites are difficult for tadpoles to shake off. This may explain why many trematode parasites enter tadpoles in that part of their body.

Weird and wonderful things about tadpoles.

**Richard J. Wassersug,
Dalhousie University, Halifax, Canada**

It is not obvious how tadpoles can generate much thrust nor swim fast given how soft and flexible their tails are. Also tadpole swimming is characterized by a lot of lateral oscillation at the snout, which intuitively looks inefficient. However the flexible tails of tadpoles facilitate small turning radii and high angular acceleration. The high amplitude oscillations at the snout mean that tadpoles are essentially changing direction all the time while swimming in an overall straight line. Thus the lateral deflections of their snouts make their path unpredictable and aid in dodging predator attacks.

At modest speeds (< 5 Hz for large *Lithobates* larvae) the tail can act like a whip, when the tadpole swims in a straight line, and there need not be travelling waves of muscle contraction going down the full tail. However, at higher speeds (> 6 Hz) muscle activation is necessary to provide postural stiffness to control (*i.e.*, to limit) excessively lateral deflections. The tadpole tail tip thus assists in locomotion, but not because the small amount of muscle in it provides much thrust. Rather the tail tip can act passively, like the tail of a kite, to control excessive lateral deflection.

The tadpole tail tip can also act as a lure to draw predator attacks that are unavoidable away from the head where they would be lethal. Certain tadpoles can acquire brightly coloured tail tips when in water with high predator concentrations.

Lastly, tadpole tail fins are viscoelastic, which means that when they are pinched by sit-and-wait aquatic predators, such as a dragonfly larvae, they can stretch and tear easily allowing the tadpole to pull away with minimum tissue loss. Yet under other loading conditions, the fin can act stiff and inelastic. Biomechanical testing of the tail fins of North American *Lithobates* larvae show that the fin is stiffest at high speeds, just as one would expect it to be when the tadpole must escape being chased by a fast swimming predator.

How to build a tadpole: spatial and temporal variations during the early ontogeny of anurans.

**Maria Florencia Vera Candiotti,
CONICET, Tucumán, Argentina**

In the early development of anuran larvae structures appear that are distinct for tadpoles (*e.g.*, oral disc) but there are also exclusive transitory structures that disappear just after hatching and thus have no presence in the larval or adult body

plan. In this lecture results from comparative studies about these characters were presented. 1) Oral disc of Leiuperinae larvae. Our studies show that interspecific variation of oral configuration in larvae from this leptodactylid subfamily can be caused by novel development pathways as well as by heterochronic changes in shared developmental pathways; this results are compared with larvae from other families, and with larvae that exhibit smaller and larger labial formulae. 2) Structural variations and changes in developmental sequence of adhesive glands, hatching glands, external gills, and body ciliation. Our results show wide variations in these characters (e.g., gland morphogenetic types, pairs of external gills, persistence of body ciliation) at several taxonomic levels (e.g., interspecific, interfamilial), often related to ecological aspects (e.g., oviposition mode, site where embryos develop). 3) The tail in Brachycephaloidea. A few studies on the structure and ontogeny of the tail in embryos with direct development reveal differences in growth rates and in the spatial arrangement of the fins regarding the muscular axis of the tail, in species from at least two families. 4) Development of novelties. The comparative study the early ontogeny of characters that appear in convergence in some anuran lineages (e.g., abdominal suckers) may reveal functional and developmental restrictions, or alternatively identify different morphological solutions for similar functional needs.

The large variation in development of tadpole and embryonic structures elucidated to date indicates the need to investigate the variation in little-known groups, and suggests that further study of these structures in other taxa can help advance our understanding about the influence of phylogeny and ecology on the variation in the development of tadpoles.

Diversity of helminth fauna of amphibians and their implications for conservation studies.

**Luciano Alves dos Anjos,
UNESP, Ilha Solteira, PR**

Although the greatest diversity of biological species is situated in the tropics, biota in these regions is subsampled. In addition, the number of newly discovered organisms is decreasing faster than new organisms are described. Within the group of poorly sampled species are amphibian helminth parasites, which can be considered as an “invisible zoo”, since parasites represent a “hidden diversity” within the visible diversity of the “naked eye”. Only a small part of this diversity is of medical or veterinary importance. The rest however play an important role in regulating biodiversity of all ecosystems. Such parasites help maintain local diversity of hosts and ecosystem function. As well as being intimately related to the biology and ecology of their hosts, they act as regulators of amphibian populations and communities. Although Brazil has nearly a thousand anuran species, the associated helminth fauna is known for only 60 to 80 anuran species.

The parasite fauna associated with tadpoles is practically unknown in Brazil. Amphibian species that depend on water

bodies for reproduction or foraging at some stage of their life cycle are more susceptible to helminth infections, especially digenetic trematodes that infect tadpoles. Anuran biological and ecological features, such as reproductive modes, larvae type, and habitat are closely related to the associated helminth fauna. In turn, the helminth fauna depends on host local communities and environmental quality and conditions. The interaction between helminths and anuran larvae represents an excellent model for evaluating environmental stresses, species conservation, and historical and biogeographical aspects of the amphibians hosts.

Integrating organismal biology and population ecology to study amphibian chytridiomycosis.

**Matthew D. Venesky,
Allegheny College, Meadville PA, USA**

Emerging infectious pathogens place tremendous burdens on wildlife, human health, and society. In particular, fungal pathogens have caused some of the largest mortality events in modern times and pose a disproportionately greater threat to plant and animal biodiversity than any other pathogen group. The amphibian chytrid fungus (*Batrachochytrium dendrobatidis*; “Bd”) is of great concern because it is a causal agent of hundreds of amphibian population declines and extinctions. In tadpoles, *Bd* only infects the keratinized mouthparts and causes mouthpart deformities and tooth loss, both of which can lead to a reduction in traits associated with survival (e.g., growth and developmental rates).

In benthic (*Bufo americanus*) and nektonic (*Hyla versicolor*) tadpoles, *Bd* infections, and the subsequent loss of teeth, cause the mouths of tadpoles to slip off the surface in which they are feeding, preventing them from efficiently foraging. This results in reduced food consumption in both species, which sometimes is evident throughout much of the tadpole’s developmental period. In addition, food consumption scales linearly with *Bd* infection intensity, where tadpoles with higher infection intensities consume less food than tadpoles with lower infections.

In communities of tadpoles, species should vary in their resistance to *Bd*, in part because species behave differently and they vary in the number of keratinized mouthparts they have. To test how this variation affects disease outcomes, I demonstrated that a filter-feeding tadpole (*Gastrophryne carolinensis*, which lacks keratinized mouthparts) remove *Bd* zoospores from the water whereas an algal-scraping tadpole (*Bufo terrestris*, which have many keratinized mouthparts) do not remove zoospores. In addition, tadpoles of *B. terrestris* carried the highest *Bd* infections. Because of these biological differences, when tadpoles of *G. carolinensis* were raised in two- or three-species communities, they diluted their *Bd* infection intensity. In contrast, whenever tadpoles of *B. terrestris* were raised with the same communities, they amplified *Bd* infection intensity. Field data of *Bd* prevalence from the continental United States of America reveal similar patterns as the laboratory experiments, indicating the results are robust and ecologically relevant.

Lastly, recent research on *Bd* in tadpoles indicates that disease outcomes are dependent on host condition. In a laboratory experiment, I demonstrated that the quantity of dietary protein can have profound impacts on host immunological traits and that tadpoles fed a low protein diet have poor resistance to *Bd*. These findings underscore the importance of testing disease outcomes under a variety of ecological settings and reinforce our need to link host condition with physiological/immunological parameters when we study infectious diseases.

Tadpoles diversity in Brazil.

Michel Varajão Garey,
Universidade Federal da Integração Latino-Americana,
Foz do Iguaçu, PR

What do we know about tadpole diversity in Brazil? Currently 1,026 amphibian species are recognized in Brazil of which 988 belong to the order Anura. Anuran richness in different biomes is not uniform. The Atlantic Forest biome harbors the highest anuran richness, followed by the Amazon, Cerrado, Caatinga, Pampas and, finally, Pantanal. In the biomes with the highest anuran richness, there is the highest richness of species with reproductive modes independent of water (*e.g.*, Brachycephalidae and Craugastoridae families). In Brazil 793 anuran species have aquatic tadpoles, but only 495 species, of which 62% are formally described.

What do we know about tadpole distribution in Brazil? Biotic and abiotic factors influence richness and the composition of tadpole assemblages. Abiotic factors, such as environment type (lentic or lotic), influence habitat selection; they act as ecological filters and, as a consequence, influence richness and the composition of tadpole communities in these environments. Several studies involving tadpole assemblages have highlighted how habitat structural characteristics influence tadpole assemblages. The main filters seem to be hydroperiod and canopy cover. However other factors, such as the amount of aquatic vegetation, microhabitats diversity and water properties, seem to be important for some assemblages. Besides, we know that spatial factors (*e.g.*, distance between water bodies) are also important in structuring assemblages, within the context of metacommunity theory. Studies in metacommunity theory that involve tadpole assemblages have increased significantly, and are important for comprehend the role factors related to niche and neutral processes have in controlling tadpole distribution.

Based on what was reviewed above, what is the future of tadpole assemblages studies? An important challenge that remains is to understand how processes-drift, selection, dispersion and speciation-generate and maintain species richness and composition patterns. Approaches grounded in functional ecology and phylogenetics are important, and research in those areas is increasing and may help us better understand what determines tadpole community structure. However there is a paucity of such studies applied to tadpole assemblages. Research in those areas combined with metacommunity analyses

holds the promise of advancing our understanding of tadpole community ecology.

Spatial and environmental factors mediating the structure of tadpole communities in central Amazonia: effects of habitat scale.

Marcelo Menin, Universidade Federal do Amazonas,
Manaus – AM

The structure of tadpole communities can be affected by biotic (*e.g.*, competition and predation), abiotic (*e.g.*, water quality, pond structure, hydroperiod), temporal (*e.g.*, seasonality of climatic variables), spatial (the location and distance of water bodies), and historical (biogeographic processes, which form the regional pool of species) factors. Water quality (*e.g.*, pH, electrical conductivity, and dissolved oxygen) is considered an important determinant of for aquatic communities in general and specifically influences tadpole assemblages. Electrical conductivity and temperature were found to affect tadpoles living among floating meadows macrophytes in a lake. The number of ponds and the valley width (the floodplain area along the margins of streams) strongly relate to tadpole species richness in a more recent study.

Since tadpoles are prey for a variety of vertebrate and invertebrate species, predators can determine the structure of tadpole assemblages in different aquatic habitats. In Central Amazonia, studies conducted in streamside ponds and isolated ponds indicate that tadpole assemblages are often regulated by predator assemblages. Conversely, predator density was not a major biotic factor when other studies were conducted on a broader spatial scale, either in upland forests ponds or in lakes. According to studies conducted in Central Amazonia, the influence of predators on species richness and the composition of tadpole assemblages remains unclear, but may relate to spatial scale and differences between habitats. Integrative approaches in phylogenetics and functional diversity can help us understand the importance of species composition in terms of evolutionary history and ecological convergence. This, in turn, may increase the chances that conservation measures, based on this knowledge, will be effective in protecting amphibian diversity in the Amazon forest.

Tadpoles from semiarid ecotones.

Flora Acuña Juncá,
Universidade Estadual de Feira de Santana,
Feira de Santana, BA

Brazil's semiarid region has an area of over 982,000 km² and covers 1,133 cities. Vegetation includes forest areas, rocky grasslands, and Cerrado, but the principal vegetation is Caatinga. Annual precipitation in these areas does not exceed 800 mm. In Bahia state, with an extension of almost 560,000 km², semiarid represents the main portion, although

a percentage of Atlantic Forest remains in the east. In both formations, Caatinga or Atlantic Forest, knowledge of anuran larval diversity can be used as a differential to recognize species richness. In Atlantic Forest, adults of most species live in canopy cover, hampering the collection of individuals, while in Caatinga many species have explosive reproduction so, adults disappear just after they reproduce. On the other hand, tadpoles are easily accessed in both regions. However, taxonomic recognition of anuran larvae is still incipient.

In another context, in semiarid environments of Northeastern region of Brazil, the unpredictability and heterogeneity of aquatic environments stand out. These characteristics could determine species richness and associated tadpole ecomorphotypes. So, in order to increase taxonomic knowledge and, at the same time, to initiate studies on ecomorphotypes and ecological conditions associated to tadpoles from semiarid environments, a study was conducted in Maracás, Bahia.

Tadpoles and their predators were collected in thirty aquatic habitats. Methodologies for collecting and morphological measurements were standardized following the SISBIOTA-Tadpoles protocols (FAPESP/CNPq). Eighteen measurements were taken from at least five tadpoles from each species in stages 35-38. There were 24 species from 6 anuran families identified overall. Through a principal component analysis (PCA), 7 ecomorphotypes were recognized: carnivorous (1 species), macrophage (3 species), nektonic (6 species), suspension-rasper (2 species), rasper (2 species), suspension-filter (1 species) and benthic (10 species). As benthic was the most diverse ecomorphotype, it was possible to discriminate, through PCA, 4 additional subtypes. These were determined by the width of the tail musculature, length of the tail, and tail fin height. Two subtypes were related to shallow environment, one subtype to deep ponds and a third subtype to running water. The number of species varied from 1 to 8 and the number of ecomorphotypes from 1 to 6 in all 30 sampled habitats. However vegetation heterogeneity, number of predators, water body depth and area did not explain the species richness or the number of ecomorphotypes found. Thus other factors may be involved in determining species assemblages.

Isolated frogs in a crowded world: how much human occupancy can they stand?

**Paula Cabral Eterovick,
Pontifícia Universidade Católica de Minas Gerais,
Belo Horizonte, MG**

Amphibian population declines are widespread; the main causes are human related and include habitat fragmentation due to agriculture, mining, fires, and urban development. Brazil is the richest country in the world in terms of species of amphibians and Brazilian regions with the greatest amphibian diversity are experiencing relatively high rates of habitat destruction. Yet so far there are relatively few reports of amphibian declines. It is important, though, to have research methods able to detect deterioration in population health before severe declines occur.

In this regard, we examined fluctuating asymmetry (FA) of anuran larvae and adults, as well as heterozygosity estimates to detect anthropogenic stress. We hypothesized that greater human occupancy in the landscape might result in more stressful conditions for amphibians. We conducted this study at the Espinhaço mountain range in southeastern Brazil, using as a model an endemic hyliid species, *Bokermannohyla saxicola*.

We found tadpole and adult frog FA levels to differ among localities, but no relationship between human modification of the landscape and FA levels. However, heterozygosity was inversely related to FA in adult frogs, and heterozygosity tended to decrease with increasing human occupancy in the landscape. Our major finding was that reduced heterozygosity could be caused by human occupancy (among other causes) and related to increased fluctuating asymmetry, indicating higher levels of stress in populations of *Bokermannohyla saxicola*.

Tadpoles as model organisms for understanding the consequences of land use and land management on biodiversity.

**Luis Cesar Schiesari,
Universidade de São Paulo, São Paulo, SP**

Reconciling agricultural production and biodiversity conservation will depend on increasing the value of productive landscapes as habitat and/or dispersal matrix for biodiversity. We therefore need a better understanding of how agricultural land use and land management influences biodiversity. We argue that tadpoles (and organisms surrounding tadpoles in freshwater food webs) constitute an excellent study system for assessing the impact of land use and land management on biodiversity. In fact, for over 40 years amphibian larvae have been used extensively as model systems in community ecology due to their tractability and amenability to realistic manipulations in laboratory, mesocosms, and field conditions. More recently amphibian larvae have become model organisms in ecotoxicological studies (e.g., FETAX), and in studies at the interface between ecology and ecotoxicology.

We conducted biodiversity and habitat surveys of lentic water bodies distributed across gradients in land use intensity in the rural state of São Paulo (i.e., seasonal Atlantic Forest/cerrado forest < pastures < sugarcane plantations) and in the upper Xingu Basin (transitional Amazonian broadleaf/cerrado forest < pastures < soybean plantations). The study system was comprised of algae as producers, amphibian larvae as consumers and the focal organisms, plus fishes and aquatic insects as predators. Simultaneously, we conducted experiments in the laboratory, mesocosms, and field. In those settings we manipulated the same study system to test what environmental drivers might be mediating biodiversity changes observed in the field. We documented a strong signal of land use and land management on tadpole biodiversity. However, this signal was strikingly different in lands converted to sugarcane and soybean. Sugarcane fields were impoverished relative to cerrados, but supported tadpoles and insects from several species. This

confirmed that those landscapes were permeable and functioning as habitat for some freshwater fauna. In contrast, relative to forest, soybean fields were enriched in amphibians, but devoid of insects. This occurred because land conversion effectively creates lentic habitats by increasing soil compaction, but heavy pesticide application affects invertebrate predators more than amphibians. Nevertheless, laboratory experiments manipulating eggs, embryos, and larvae demonstrated lethal and sublethal effects of pesticides and nitrogen fertilizers even at application doses recommended by the manufacturer.

The response of the amphibian fauna to land use seems to be influenced by: 1) environmental attributes at the regional level, such as phytophysiognomy, soil structure and seasonality, 2) environmental attributes at the landscape level, such as landscape structure and type, and intensity of land management, and 3) the identity and traits of the species represented in the community.

Ecomorphology of tadpoles and geometric morphometry.

Fausto Nomura,
Universidade Federal de Goiás, Goiânia, GO

The origin and meaning of morphological diversity in tadpoles has aroused curiosity of herpetologists since studies by Orton (1943), Starrett (1973) and others. However, only in 1986 was the ecomorphology concept introduced in the vocabulary of researchers of tadpoles (following Altig and Johnston (1989). Those authors defined several morphological guilds that are currently used to classify morphological types in tadpoles. However the proper matching of form with feeding, habitats, or other aspects are not always well established. This may result in mistaken suppositions about tadpole habits (such as feeding or occurrence) and, as a consequence, may affect studies that try to find ecology and biogeography patterns.

There are various approaches to exploring ecomorphological relationships among organisms; in this lecture the focus was on those that emphasize morphological variation among individuals, populations, and species. Traditional morphometry (e.g., counting, measuring dimensions), ecomorphological indices (e.g., proportion between lengths) and geometric morphometry (set of Cartesian coordinates that represent the shape configuration). Traditional morphometry tends to overestimate the influence of phylogenetic effects, whereas geometric morphometry is more precise. However, geometric morphometry is sensitive to morphological variation so, it is more precise when variation is low (for example, the twist of the mouth in *Phasmahyla*, the absence of nostrils in Microhylidae, or having spiracles in the ventral position). Ecomorphological indices are intermediate to these two.

Regardless of the techniques used to assess morphological variation, there remain difficulties in generating testable hypothesis with ecomorphology as a basis. Challenges include representing phenotypic plasticity and behaviors for which there are no specialized morphological structures.

Tadpoles in the Tropics: What do we know and what can we learn in the next decade or so?

Considering our current knowledge about Neotropical tadpoles, the Workshop concluded with an Open Discussion titled: "Knowledge gaps about Neotropical tadpoles: What are the most important questions that should be answered in the next 10 years?" In this Discussion the speakers and the Workshop attendees (Fig. 1) aimed to identify the important questions that could guide and stimulate studies with tadpoles in the next 10 years. Two general themes were selected: "Tadpoles Biology" and "Community Ecology". Each one was discussed separately and independently by two groups, which then came together to identify the strongest and commonest themes that had emerged. At the end, the full group convened to review the recommendations.

The questions presented were identified as of fundamental importance to advancing our knowledge of tadpole biology and community ecology in Brazil. Aiming to provide a basic grounding to the problems detected, we present a succinct framework/scientific background associated with questions and problems that we identified in each theme.

Tadpoles Biology Theme

Background

The comparative study of development, undertaken to understand the relationship between vertebrate taxa, has a history going back some 150 years (i.e., Haeckel, 1874). However this comparative approach plateaued in recent decades as developmental biology increasingly focused in on model species (e.g., "the chicken", the "zebra fish"). Since the 1930s *Xenopus laevis* (e.g., Dickinson & Sive, 2006; Nieuwkoop & Faber, 1956; Weisz, 1945) has been the "model frog" and its developmental biology better known by far than that of any other species.

Thanks to renewed interest in the relation between evolution and development (e.g., Gould, 1977; Hanken & Thorogood, 1993) changes in developmental timing (heterochrony) are now recognized as a determinant of morphological variation among species (reviewed in Richardson, 1995). Indeed there is now a growing appreciation that the morphological diversity among tadpoles of different species can be traced to changes in their ontogenetic trajectories. Sadly however, the early development of frogs is well studied for but a handful of species and attention has been given to a very limited number of anatomical systems (e.g., Bell & Wassersug, 2003; De Bavay, 1993; Duellmann & Trueb, 1986; Dziminski & Anstis, 2004; Hall *et al.*, 1997). Most investigations have understandably been on conspicuous external morphological traits (e.g., Nokhbatolfoghahai & Downie, 2005, 2007, 2008; Nokhbatolfoghahai *et al.*, 2006; Chipman *et al.*, 2000). We now know that interspecific variation in, for example, oral disc configurations and tail structure can be the product of novel developmental pathways, as well as of heterochronic changes (Salica *et al.*, 2011; Vera Candiotti *et al.*, 2011; Goldberg *et al.*, 2012; Goldberg & Vera Candiotti, in press). Additionally, we have become increasingly aware of

an enormous amounts of anatomical complexity in internal structures, for instance, in the buccal cavity and chondrocrania of anuran larvae. This has expanded the list of anatomical features that can be used to distinguish tadpoles of different taxa, which, in turn, are amenable to developmental study.

The classic “character by character” approach to comparative studies in tadpole development (e.g., “This species has a truncated development of the oral disc.”) can now be extended into integrated studies that use a multivariate approach to reveal the developmental trajectory of overall shape of parts, if not all, of tadpoles. With this approach it is now possible to ask, for example, if there is concordant truncation in other larval features that match with changes in, say, the oral disc. One can similarly now quantify the relationship of changes in shape with changes in the size and age of tadpoles through their development (e.g., asking “Are truncated larvae also smaller or younger?”).

Additional new and sophisticated techniques, such as 3D reconstructions and CTscan, are available to complement dissections (e.g., Haas *et al.*, 2014). This contemporary evo-devo approach, and the new analytical tools for studying change in shape, can be applied not only to early anuran development, but also to metamorphosis. As such, it is increasingly possible to explore how the early ontogeny of larvae leads to, not just the features of mature tadpoles, but also the features of adult anurans (e.g., Fabrezi & Quinzio, 2008).

What stands out as an area in need of much greater attention is the link between the anatomical structure of anuran larvae and their function. Because of their small size and the speed at which tadpoles move, the behavior of most anuran larvae has been hard to document, not just in the field (where many are cryptically colored and hidden in vegetation), but even in the laboratory. However recently tools, such as high-speed video-graphic equipment, have become more than an order of magnitude cheaper than just a decade ago. This means that more researchers can afford such equipment. It is increasing feasible for biologists to capture and study the intricate actions of, for example, tadpole mouthparts that move too fast to be witnessed by the naked eye (Deban & Olson, 2002; Venesky *et al.*, 2013).

Although tadpoles of different species are often categorized by ecomorphological type (McDiarmid & Altig, 1999), this classificatory scheme is not particularly refined and is based on rather limited ecological data, such as whether the tadpoles are found on the bottom or in the water column of a pond. Tadpoles of too many species fall into the same ecomorphological type. This gives us little understanding about how tadpole of different species partitioning the aquatic environment. More needs to be done to understand tadpole behavioral ecology in the field. For this to happen, we need to know more about the fundamental natural history of tadpoles. For example, we cannot continue to assume that where tadpoles are found in a



Attendees of the first “Tadpoles International Workshop”, held in 16 and 17 March, 2015, at the UNESP, São José do Rio Preto, SP, Brazil.

pond is necessarily their optimal habitat. Rather predator pressure may have pushed them into marginal habitats and feeding activities and locations may vary across a daily cycle, as has been suggested by Warkentin (1992).

We have learned in recent years that many species exhibit phenotypic plasticity in the presence of stressors such as parasites, toxins, and predators. The larvae of some species can change their shape and/or color, as well as their behavior when exposed to environmental stressors (Relyea, 2001). This has led to the questions listed here about tadpole sensory physiology. What is clear is that not all species have the same sensitivity or responsiveness to various stressors. To sort this out and understand why some species are more sensitive and more plastic, we need far more comparative studies in tadpole sensory physiology, functional morphology, toxicology, and disease biology.

Brazil, with its luxurious diversity of pre and post metamorphic anurans, stands out as one of the best places on the planet for studying not just the diversity of anuran development about the diversity of their ecology in the context of the growing list of stressors that are threatening anurans globally.

Problem 1: Lack of information about the developmental pathways that account for tadpole morphological diversity and the features of tadpoles that influence their functional performance.

- 1) How do developmental pathways explain/determine morphological diversity in tadpoles?
- 2) How does morphological diversity of tadpoles relate to their functional performance?
- 3) How can we improve taxonomic identification of larval stages, and how can we standardize and make descriptions complete?
- 4) Which ecomorphological systems are most subject to plasticity? What are the biological factors that account for why tadpoles of some species to be more plastic than others?

Problem 2: Lack of knowledge on tadpole physiological performance and response to different stressors.

- 5) How does environmental change impact tadpole performance and through which physiological mechanisms?
- 6) What is the synergistic interaction among different stressors that affect tadpoles?
- 7) Why do species respond differently to stressful conditions and why are tadpoles of some species threatened whereas others are resistant?
- 8) What are the sensory mechanisms used by tadpoles to detect and avoid stressors?

Community Ecology Theme

Background

Until around the late 1990s, studies in community ecology were based on the niche perspective as the only set of processes

structuring communities, and this perspective was created and supported by the limiting similarity principle (e.g., MacArthur & Levins, 1967; Diamond, 1975). Two new perspectives expanded the scope and approaches in this area (see Cavender-Bares *et al.*, 2009 and HilleRisLambers *et al.*, 2012): progress in obtaining and providing phylogenetic data (e.g., Cadle & Greene, 1993; Webb *et al.*, 2002) and the neutral theory of biodiversity (Hubbell, 2001). The niche theory (Hutchinson, 1957; MacArthur & Levins, 1967; Chase & Leibold, 2003) includes biotic and abiotic interactions such as competitive ability of species, predation and habitat variables effects acting as environmental filters (Keddy, 1992; Weiher & Keddy, 1995). While our comprehension of community assembly was restricted to the niche based processes, the results were contingent to organisms and/or specific environments (Lawton, 1999; Vellend, 2010). The inclusion of phylogenetic data, considering speciation, adaptation, extinction, and dispersion events, which together gave rise to the lineages represented in communities, introduced an evolutionary perspective to the structure communities (Cadle & Greene, 1997; Leibold *et al.*, 2010). The neutral theory of biodiversity (Hubbell, 2001) expanded such studies beyond the local habitat, taking into consideration dispersion among habitats and stochastic events, which could be analyzed only on large spatial scales.

This progress was followed by the development of several new statistical tools (e.g., Leibold & Mikkelsen, 2002; Webb *et al.*, 2008; Presley *et al.*, 2010; Maddison & Maddison, 2011; Pavoine *et al.*, 2011; Ulrich, 2012; Ulrich *et al.*, 2012; Rangel *et al.*, 2010; Dallas, 2014). These tools, in turn, enabled researchers to apply new theories to empirical studies and evaluate the relative influence of historical, neutral, and niche-based processes at the center of community ecology (e.g., Cavender-Bares *et al.*, 2009; Howeth & Leibold, 2010; HilleRisLambers *et al.*, 2012). In addition to the strong theoretical nature, which illuminates rules for community assembly (Diamond, 1975; Chase & Leibold, 2003; Ulrich *et al.*, 2010; Pavoine *et al.*, 2011), this approach also provides fundamental information to management and conservation plans, guiding the focus of actions to local, landscape or macroecological scales.

However, even applying these frameworks, no pattern on the relative influence of niche-based, phylogenetic and neutral processes in community assembly has been detected and, at least for anurans, the results of studies are conflicting (e.g., Parris, 2004; Iop *et al.*, 2013; Melo *et al.*, 2014; Prado & Rossa-Feres, 2014; Provete *et al.*, 2014; Almeida *et al.*, 2015). Despite few studies about communities assembling had been developed in Brazil, the different results obtained can indicate a contingent association of determined set of processes with biomes or specific ecological systems (e.g., streams or ponds, preserved or modified areas, open fields or forests) or still can be a matter of spatial scale (Leibold *et al.*, 2004; Swenson *et al.*, 2007). On a restricted spatial scale, the main processes regulating species distributions are related to niche (Hutchinson, 1957) and neutral processes, mainly related to dispersion ability of species (Hubbell, 2001). However, with an increase in spatial scale, the influence of dispersion processes in structuring community is higher, once distance limits species dispersion (Hubbell, 2001; Ng *et al.*, 2009; Astorga *et al.*, 2012).

As important as looking for patterns in processes that regulate communities, is the perception that, despite the growing complexity and refining of analytical frameworks, the interpretation of these results is strongly based on the biological knowledge of a researcher. Far from being a failure, this emphasizes the importance of decreasing the information gaps in natural history and biology of tropical anurans.

In fact, the scarcity of information on species natural history is a major caveat for understanding Neotropical anuran community ecology (as well as conservation studies) (Verdade *et al.*, 2012; Toledo *et al.*, 2014), especially in the larval stage. Data digitization has accelerated research progress and created opportunities through linking information on topics, such as geographical location, species conservation status, and genetic sequences (*e.g.*, Hajibabaei *et al.*, 2007; IUCN, 2015; Ellwood *et al.*, 2015). Amphibian natural history information is widespread in the literature (*e.g.*, Lima & Eterovick, 2003; Lima *et al.*, 2014a, b) but a specific electronic database to organize, standardize, and compare natural history data is missing. As well as natural history data on larvae, data on spatial distribution of species is particularly important for predicting how likely species are to be affected by current threats (Hof *et al.*, 2011).

One of the main causes of death of animal populations is the emerging infectious diseases (Daszak *et al.*, 2000). These diseases are a concern especially for species that have declining population, declining in habitat quality or availability, or small distribution range (Daszak *et al.*, 2000). Particularly, these characteristics make the amphibians' populations more vulnerable to emerging diseases, once many species have small geographic distribution and are experiencing a reduction in both habitat quality and extension (Duellman & Trueb, 1986). As a result, the amphibians are considered as the more threatened vertebrate group, being a major concern for conservation efforts.

Clearly, this is not the result only of emerging infectious diseases, but a conjunction of many causes, including habitat loss and modification, which effects are difficult to isolate. Because amphibians, due to its biphasic life cycle, are exposed to land and aquatic stressors, the tadpoles are an important link to understand population declines and biodiversity loss. Without understanding how the threats interact with each other and with the amphibians life stages we are unable to generate a better ecological theories that could explain and predict patterns of species loss.

This complexity of causes that driven the global decline of amphibian populations is already recognize but few research programs are planned to investigate the amphibians threats in a multivariate approach, in general focusing in a single factor (Blaustein & Kiesecker, 2002). The univariate approach for studying causal factors of amphibian diversity threats provided little predictive insights (Blaustein & Kiesecker, 2002). In this context, species distribution models are useful to predict ecologically suitable areas for different species and for different purposes, as the establishment of invasive species (*e.g.*, Franklin, 2010; Nori *et al.*, 2011), and the effects of pathogens and of climate change on the distribution of species.

So, understand the spatial distribution of species is critical because its distribution is closely linked both to the distribution of appropriate habitats and to frog dispersion and migration abilities (Toledo *et al.*, 2014), which vary between larval and adult stages (Eterovick *et al.*, 2009). However, there are few studies determining the specific components of habitat (such as a specific microhabitat for the development of tadpole), which are recognized only at very small scales (Sinclair *et al.*, 2010). Similarly, studies on genetic structure of Neotropical anuran populations are still incipient (*e.g.*, Eterovick *et al.*, 2009; Nascimento, 2013), as well as the understanding of the roles of both adults and tadpoles in gene flow (Eterovick *et al.*, 2009). This precludes the comprehension of the effects of different human activities on frog populations via interference on migration patterns.

Finally, a topic that has been fascinating ecologists and evolutionary biologists for decades is the phenotypic plasticity of tadpoles (see Tadpoles Biology Theme Section; Via *et al.*, 1995). For tadpole biologists, the study of phenotypic plasticity gained traction with Wilbur & Collins' (1973) seminal paper on factors that influence larval amphibian growth, development, and ultimately their metamorphosis. Since then, plasticity in tadpole phenotypes is well documented and can occur in response to a variety of environmental conditions, including pond hydroperiod (Denver *et al.*, 1998), competitors (Relyea, 2002a), predation (Relyea, 2002b), parasites (Venesky *et al.*, 2013a), and in food availability (de Sousa *et al.*, 2014). Thus, the plasticity in these, and other, traits among tadpoles begs the questions: Do tadpoles fit neatly into eco-morphological guilds (Altig & Johnson, 1989) and do tadpoles exhibit more flexibility to thrive in various environmental conditions that what we currently understand?

In South America, particularly in Brazil, tadpoles exhibit tremendous diversity in their morphology as well as the habitats in which they develop, which include ponds, swamps and streams in open or forested areas. Within such habitats, tadpoles can occupy different positions along the water column, they can also thrive in water accumulated in epiphytes (*e.g.*, *Phyllodytes luteolus*, Eterovick, 1999), buried in stream sandy bottoms (*e.g.*, *Vitreorana uranoscopa*, Heyer, 1985), live in tree trunk phytotelmata (*e.g.*, *Osteocephalus oophagus*, Schiesari *et al.*, 1996), or even develop in the thin water film flowing over rocks by streams, such as the semiterrestrial tadpoles of the genus *Thoropa* (Bokermann, 1965). Yet, we have few comparative studies on the degree of plasticity that exists among South American tadpoles, how that influences their selection of a habitat (or micro-habitat), and the consequences of developing in a "sub-optimal" habitat.

Understanding these and other questions related to tadpole phenotypic plasticity and habitat preference will first require an understanding of the function of the diverse phenotypes within and between species. After we have a better understanding of a particular tadpole phenotype, manipulative experiments will be useful in understanding the extent to which these phenotypes change in particular environments. Eventually researchers will need to measure the fitness consequences of plastic traits as they correlate to the heterogeneous environments in which tadpoles develop.

This framework is now being applied to the study of tadpole feeding. In the first series of experiments on tadpole feeding, Venesky *et al.* (2013b) used a standardized approach to describe the diversity of oral kinematics in six hylid tadpoles that varied in their feeding structures and also in their feeding guilds. Building upon these initial findings, this research group manipulated the position of the food within the water column (de Sousa *et al.*, 2014) and also the temperature at which tadpoles were studied (de Sousa *et al.*, 2015) to understand the plasticity in the feeding kinematics. The fact that tadpoles have flexibility in their feeding coupled with the fact that they also perform better in certain environments (de Sousa *et al.*, 2014; de Sousa *et al.*, 2015) suggests that they should have higher fitness Blaustein in some environments than others. Research that explicitly tests these hypotheses is needed to fully understand the relationship between tadpole phenotypic plasticity and habitat choice.

Problem 1: Lack of information on (1) the natural history of anurans – especially in the larval stage – and (2) the best ways to maintain tadpoles in captivity, need to hamper the development of standardized protocols for tadpole research.

- 1) What is the best way to make natural history data more accessible?
- 2) Can we create a national/international natural history database linked to museum specimens that included data on breeding period, number of eggs, time to metamorphosis, trophic relationships, dispersion abilities etc.?
- 3) What is the dispersal ability of Neotropical frogs? [Although dispersal occurs mainly in the post-metamorphic stage, such information is fundamental to understand processes that shape tadpole assemblages and the conservation of species.]

Problem 2: Conflicts in ecological data for tadpoles in South America and lack of information on the drivers of habitat choice.

- 4) There appear to be different community assembly drivers in different locations and biogeographical regions. Are there any general rules that apply at all scales?
- 5) Is it possible to connect behavior and morphology? When it comes to behavior, what really matters: species or functional groups?
- 6) Why do amphibians choose the habitat in which they occur? What determines the preference for habitats?
- 7) What are the consequences for anurans of choosing a sub-optimal habitat for their larvae?

Possible pathways to answer the questions listed above

The set of questions raised during the Open Discussion indicates the need for developing:

1. A database about natural history, associated with a verification and validation of such information, available to the scientific community (*i.e.*, following an open access philosophy). We suggest an online dataset where researchers are allowed to insert information including video clips (published or personal data) expanding upon the AmphibianWeb and Encyclopedia of Life models. In this case, though, the website would be focused on natural history information, that should be gathered, standardized and validated by a team before open posting.
2. A program in data acquisition to identify community assembly rules along many spatial scales. The focus would be on standardizing collection protocols and experimental designs to look at assembly rules in different biomes so that meaningful comparisons can be made between tadpole communities.
3. More controlled experimental research on the biological interactions among tropical species, employing standardized protocols for maintenance tadpoles of different families in captivity, to test predictions, since much of the theory on that subject is based solely on data from temperate systems.

This is clearly not a complete list of research programs that can be done with tadpoles in Brazil in the next decade. But collectively the attendees at the Workshop felt that substantive progress could be made in all of these areas within ten years. We recognize that the questions we are asking here are broad-based and we can't realistically expect them to be answered for anurans in Brazil in just a decade. However, they are clearly focal questions where, if attention is directed, substantial advances could easily be made within the next ten years.

Research in these areas could do much to help us understand and preserve Brazil's anurans. But with limited funds for amphibian research, individuals and teams will need to collaborate to address these complex and comprehensive topics. The Tadpoles International Workshop was a step toward integrating tadpole research in Brazil to address these important areas for future research.

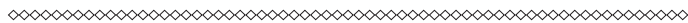
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Uranoscodon superciliaris, Oriximiná, PA - Foto: Magno Segalla.