

UNIVERSIDADE FEDERAL DE GOIÁS
INSTITUTO DE CIÊNCIAS BIOLÓGICAS
PROGRAMA DE PÓS GRADUAÇÃO EM ECOLOGIA E EVOLUÇÃO

VERÔNICA THIEMI TSUTAE DE SOUSA

Tese de Doutorado

BIOMECÂNICA DO COMPORTAMENTO ALIMENTAR DE
GIRINOS DE ANFÍBIOS ANUROS

Orientador: Prof. Dr. Fausto Nomura

Co-orientador: Prof. Dr. Matthew Venesky

Goiânia – GO

Março, 2016

VERÔNICA THIEMI TSUTAE DE SOUSA

Tese de Doutorado

**BIOMECÂNICA DO COMPORTAMENTO ALIMENTAR DE
GIRINOS DE ANFÍBIOS ANUROS**

Orientador: Prof. Dr. Fausto Nomura

Co-orientador: Prof. Dr. Matthew Venesky

Goiânia – GO

Março, 2016

TERMO DE CIÊNCIA E DE AUTORIZAÇÃO PARA DISPONIBILIZAR AS TESES E DISSERTAÇÕES ELETRÔNICAS (TEDE) NA BIBLIOTECA DIGITAL DA UFG

Na qualidade de titular dos direitos de autor, autorizo a Universidade Federal de Goiás (UFG) a disponibilizar, gratuitamente, por meio da Biblioteca Digital de Teses e Dissertações (BDTD/UFG), sem ressarcimento dos direitos autorais, de acordo com a Lei nº 9610/98, o documento conforme permissões assinaladas abaixo, para fins de leitura, impressão e/ou *download*, a título de divulgação da produção científica brasileira, a partir desta data.

1. Identificação do material bibliográfico: **Dissertação** **Tese**

1

2. Identificação da Tese ou Dissertação

Autor (a):	Verônica Thiemi Tsutae de Sousa		
E-mail:	veronicathiemi@outlook.com		
Seu e-mail pode ser disponibilizado na página?	<input checked="" type="checkbox"/> Sim	<input type="checkbox"/> Não	
Vínculo empregatício do autor	Livre		
Agência de fomento:	REUNI de Assistência ao Ensino; Coordenação de Aperfeiçoamento de Pessoal de Nível Superior	Sigla:	REUNI, CAPES
País:	Brasil	UF:	GO CNPJ: 00889834/0001-08
Título:	Biomecânica do comportamento alimentar de girinos de anfíbios anuros		
Palavras-chave:	Anura, cinemática, plasticidade comportamental, morfometria geométrica, morfologia oral, guildas ecomorfológicas, ecologia, phylogeny		
Título em outra língua:	Biomechanics of tadpoles feeding behavior		
Palavras-chave em outra língua:	Anura, kinematics, behavioral plasticity, geometric morphometrics, oral morphology, ecomorphological guilds, ecology, phylogeny		
Área de concentração:	Ecologia e Evolução		
Data defesa: (dd/mm/aaaa)	22/03/2016		
Programa de Pós-Graduação:	Ecologia e Evolução		
Orientador (a):	Prof. Dr. Fausto Nomura		
E-mail:	fausto_nomura@yahoo.com.br		
Co-orientador (a):*	Prof. Dr. Matthew D. Venesky		
E-mail:	mvenesky@gmail.com		

*Necessita do CPF quando não constar no SisPG

3. Informações de acesso ao documento:

Concorda com a liberação total do documento SIM NÃO¹

Havendo concordância com a disponibilização eletrônica, torna-se imprescindível o envio do(s) arquivo(s) em formato digital PDF ou DOC da tese ou dissertação.

O sistema da Biblioteca Digital de Teses e Dissertações garante aos autores, que os arquivos contendo eletronicamente as teses e ou dissertações, antes de sua disponibilização, receberão procedimentos de segurança, criptografia (para não permitir cópia e extração de conteúdo, permitindo apenas impressão fraca) usando o padrão do Acrobat.

1 Nes Verônica T.T. de Sousa 10 por até um ano a partir da data de defesa. A extensão deste prazo suscita justificativa junto à coordenação do curso. Os dados do documento não serão disponibilizados durante o período de embargo.

Data: 20 / 04 / 2016

Assinatura do (a) autor (a)

UNIVERSIDADE FEDERAL DE GOIÁS
INSTITUTO DE CIÊNCIAS BIOLÓGICAS
PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA E EVOLUÇÃO

VERÔNICA THIEMI TSUTAE DE SOUSA

Tese de Doutorado

BIOMECÂNICA DO COMPORTAMENTO ALIMENTAR DE
GIRINOS DE ANFÍBIOS ANUROS

Orientador: Prof. Dr. Fausto Nomura

Co-orientador: Prof. Dr. Matthew Venesky

Goiânia – GO

Março, 2016

Ficha catalográfica elaborada automaticamente
com os dados fornecidos pelo(a) autor(a), sob orientação do Sibi/UFG.

Data da defesa: 22/03/2016

BIOMECÂNICA DO COMPORTAMENTO ALIMENTAR DE GIRINOS DE ANFÍBIOS ANUROS

A tese apresentada ao Instituto de Ciências Biológicas, Universidade Federal de Goiás - UFG, para a obtenção do título de Doutora em Ecologia e Evolução foi aprovada em 22/03/2016 pela banca examinadora composta por: **Fausto Nomura** (orientador, Universidade Federal de Goiás), **Marcelo Menin** (Universidade Federal do Amazonas), **Fabício Barreto Teresa** (Universidade Estadual de Goiás), **Natan Medeiros Maciel** (Universidade Federal de Goiás), e **Luis Mauricio Bini** (Universidade Federal de Goiás).

AGRADECIMENTOS

Ao Prof. Dr. Fausto Nomura e ao Prof. Dr. Matthew Venesky, pela orientação, amizade, pelos ensinamentos, pelas discussões, pela oportunidade, pela confiança na minha capacidade em desenvolver este projeto. Muito obrigada!

À Prof. Dra. Denise de Cerqueira Rossa-Feres, pela amizade e pelos conselhos. Agradeço pela orientação e pela sabedoria compartilhada ao longo de todos estes anos. Se não fosse pela confiança depositada em mim para o desenvolvimento do projeto de Iniciação Científica com os *Pseudopaludicola*, este projeto de Doutorado não teria acontecido. Serei eternamente grata!

Ao Prof. Dr. Richard Wassersug, pela confiança, pelos ensinamentos e pela colaboração neste projeto. Por ter confiado numa equipe brasileira para o desenvolvimento deste projeto de pesquisa.

À Prof. Dr. Gilda V. Andrade e ao Doutorando Tiago Pezzuti, pela colaboração e ajuda oferecida nos últimos 4 anos. Foi um imenso prazer trabalhar com vocês!

Ao Prof. Dr. John J. Wiens, por ter me recebido em seu laboratório na University of Arizona durante 1 ano para o Doutorado Sanduíche. Agradeço pela atenção e pelos ensinamentos oferecidos durante o período.

Aos amigos do IBILCE/UNESP, principalmente à Fabiane S. Annibale e ao Carlos E. de Sousa pela ajuda durante as coletas dos girinos e para a realização dos experimentos. Obrigada pela criatividade empregada na montagem dos experimentos e pelas risadas! O trabalho é sempre divertido com vocês por perto!

Aos amigos do Laboratório de Herpetologia e Comportamento Animal da UFG, pelas conversas e discussões. Um agradecimento especial vai para a Tailise Marques Dias pela amizade.

Aos Prof. Drs. Luis Maurício Bini e Tiago Rangel pela orientação durante o estágio docência.

Aos Profs. Drs. Rogério Bastos e Paulo De Marco Jr. pela conversa e pelo *feedback* durante a qualificação.

À FAPESP (2010/52321-7) e ao CNPq (563075/2010-4), pelo financiamento concedido ao projeto **Girinos de Anuros da Mata Atlântica, da Amazônia, do Pantanal, do Cerrado e de zonas de transição: caracterização morfológica, distribuição espacial e padrões de diversidade**, ao qual o presente projeto de Doutorado foi vinculado.

À Pró-Reitoria de Pesquisa e Pós-Graduação da UFG, pela Bolsa REUNI de Assistência ao Ensino, nível Doutorado. E à CAPES, pela bolsa do Programa Institucional de Doutorado Sanduíche no Exterior PDSE.

Por último, mas não menos importante, agradeço à minha família que, mesmo estando tão longe, sempre ofereceu o apoio que eu precisava para continuar seguindo em frente. Obrigada!

Sumário

Resumo	1
Abstract	3
Introdução geral	5
Referências bibliográficas	9

Capítulo 1 – Flexible feeding kinematics of a tropical carnivorous anuran tadpole

	12
Abstract	13
Introduction	14
Material and methods	15
Collection of tadpoles	15
Feeding trials	16
Feeding kinematics	18
Plasticity in jaw sheath shape	18
Statistical analysis	20
Results	21
Discussion	22
Acknowledgements	25
Tables	26
Figures	29
Literature cited	31

Capítulo 2 – Differential effects of temperature on the feeding kinematics of the tadpoles of two sympatric anuran species 36

Abstract	37
Introduction	38
Material and methods	40
Study area	40
Study species	41
Collection and maintenance of tadpoles	41
Feeding trials	42
Feeding kinematics	44
Data analysis	45
Results	46
Discussion	48
Acknowledgements	52
Tables	54
Figures	59
Literature cited	60

Capítulo 3 – Are the effect of oral morphology and ecology on tadpoles feeding behavior influenced by evolutionary history? 66

Abstract	67
----------	----

Introduction	68
Material and methods	70
Study species	70
Collection and maintenance of tadpoles	71
Feeding trials	71
Feeding kinematics	73
Phylogeny	73
Statistical analysis	76
Results	76
Discussion	78
Tables	84
Figures	93
Literature cited	99
Conclusões finais	105
Anexos	106

Resumo

Indivíduos de uma mesma espécie possuem características morfológicas, fisiológicas e comportamentais que determinam quando e onde eles poderão existir e como interagirão com indivíduos de outras espécies. A diversidade ecomorfológica de girinos de anfíbios anuros os tornam ótimos organismos de estudo, já que os padrões de uso de recursos (ecologia) e as relações filogenéticas entre as espécies podem ser inferidas a partir das propriedades fenotípicas. A influência de fatores contemporâneos sobre a estrutura das comunidades de girinos tem sido intensamente estudada há décadas, mas os padrões de uso e partilha de recursos alimentares não têm recebido a mesma atenção dos pesquisadores. Pouco se sabe sobre a ecologia e o comportamento alimentar dos girinos, incluindo a biomecânica do comportamento alimentar, a influência de fatores abióticos sobre o comportamento alimentar, além da plasticidade comportamental exibida pelos girinos na exploração dos recursos alimentares. Estas questões foram investigadas durante o Doutorado e os resultados do trabalho durante ao longo deste período são apresentados na presente tese, que compreende três capítulos. No primeiro, verificamos se o girino carnívoro de *Leptodactylus labyrinthicus* ajusta seu comportamento alimentar para maximizar o consumo de alimento obtido a partir de fontes alimentares alternativas. Os resultados indicam que, dependendo da fonte alimentar a ser explorada, os girinos podem adotar comportamentos alimentares distintos: para consumir alimentos disponíveis em suspensão na água, os girinos exibem o comportamento de filtração; já para consumir alimentos aderidos em superfícies submersas, os girinos utilizam o comportamento de raspagem. Tais comportamentos alimentares são discerníveis com relação às suas cinemáticas e aos formatos que o disco oral assume durante a alimentação por filtração e por raspagem. No segundo, testamos a

hipótese de que mudanças na temperatura média da água teriam efeitos diferenciais sobre o comportamento alimentar de girinos de duas espécies de anuros: *Rhinella schneideri*, que possui girinos bentônicos, e *Trachycephalus typhoni*, cujos girinos são nectônicos. Os resultados indicam que a temperatura afeta a cinemática do comportamento alimentar de raspagem. No entanto, devido à interação entre espécie e temperatura, o efeito da temperatura sobre as variáveis cinemáticas pode não produzir uma variação no mesmo sentido. Finalmente, no terceiro capítulo, nosso objetivo foi o de verificar se a influência dos fatores ecológicos e morfológicos sobre o comportamento alimentar dos girinos reflete as relações filogenéticas entre as espécies.

Abstract

Individuals of the same species have morphological, physiological and behavioral characteristics that determine when and where they will exist and how they will interact with individuals of other species. The ecomorphological diversity of anuran tadpoles make them great study subjects, as the resources use patterns (ecology) and the phylogenetic relationships among species can be inferred from the phenotypic properties. The influence of contemporary factors on the structure of tadpoles communities has been intensively studied for decades, but the use patterns and partitioning of food resources, have not received the same attention from researchers. Little is known about the ecology and feeding behavior of tadpoles, including its biomechanics, the influence of abiotic factors on feeding behavior, as well as the behavioral plasticity exhibited by tadpoles in the exploitation of food resources. These issues were investigated during the PhD and the results of the work developed during the course of this period are presented in this Dissertation. In the first chapter, we verified whether the carnivorous tadpole of *Leptodactylus labyrinthicus* would adjust their feeding kinematics to maximize food consumption when feeding on alternative food sources. Our results indicate that, depending on the food source to be explored, the tadpoles may exhibit different feeding behavior: to consume the food particles suspended in the water surface, the tadpoles exhibit the filter-feeding behavior; in contrast, to consume food particles that are attached to submerge surfaces, tadpoles use the scrape feeding behavior. Filtering and scraping feeding behaviors are quantitative and qualitatively distincts, i.e. these feeding behaviors kinematics and the shape of the oral disc during feeding show distinguishable features. In the second chapter, we tested the hypothesis that changes in the water mean temperature would have differential

effects on the feeding kinematics of tadpoles of two anuran species: *Rhinella schneideri*, whose tadpoles are benthic, and *Trachycephalus typhonius*, whose tadpoles are nektonic. Our results indicate that the temperature affects the kinematics of the scrape feeding behavior. However, the temperature effect on kinematics variables may depend on the species, so the changes are not in the same direction (i.e. temperature may have a positive effect on some kinematic variables but negative effects on others). Finally, in the third chapter, our aim was to verify whether the ecological and morphological influences on feeding behavior reflect the phylogenetic relationship among species.

Introdução Geral

Ecólogos frequentemente buscam entender a relação entre o fenótipo de um organismo e o ambiente em que ele vive. Indivíduos de uma mesma espécie possuem características morfológicas, fisiológicas e comportamentais que determinam quando e onde eles poderão existir e como interagirão com indivíduos de outras espécies (McGill et al., 2006; Cadotte et al., 2011). Estas características frequentemente têm raízes profundas na filogenia, que transcendem as interações entre as espécies e entre estas e seus ambientes nas comunidades contemporâneas (Cadle & Greene, 1993). No caso específico de girinos de anfíbios anuros, os atributos ecológicos (fonte do alimento consumido e os tipos de hábitat e microhábitat de ocorrência), o conjunto de características da morfologia externa e o comportamento alimentar são utilizados para classificá-los em 21 guildas ecomorfológicas (Altig & Johnston, 1989; McDiarmid & Altig, 1999). Tal diversidade ecomorfológica faz com que girinos sejam ótimos organismos de estudo, já que os padrões de uso de recursos (ecologia) e as relações filogenéticas entre as espécies podem ser inferidas a partir das propriedades fenotípicas (morfologia) (sensu Altig & McDiarmid, 1999).

A maioria dos girinos exotróficos possui um aparato oral composto por estruturas queratinizadas (i.e. mandíbulas superior e inferior, e fileiras de denticulos que estão posicionadas anteriormente à mandíbula superior e posteriormente à mandíbula inferior) e não queratinizadas (i.e. papilas marginais e submarginais) (Wassersug, 1976; Altig, 2007). Há variação interespecífica na configuração do aparato oral e alguns estudos evidenciaram que a diversidade morfológica do aparato oral reflete especializações que maximizam a eficiência da alimentação (e.g. Venesky et al., 2011, 2013). Tal diversidade pode ser exemplificada por uma das características diagnósticas

das espécies: o número de fileiras de dentículos córneos, que pode variar de 0 a 37 (McDiarmid & Altig, 1999; Faivovich et al., 2013). Estas estruturas são responsáveis pela aderência do disco oral ao substrato e pela raspagem do alimento preso à esta superfície (e.g. Venesky et al., 2011; Wassersug & Yamashita, 2001). Outras características do aparato oral que diferem entre as espécies são o número de dentículos, a queratinização das mandíbulas, e o número e a distribuição das papilas (e.g. Rossa-Feres & Nomura, 2006).

A maioria dos girinos se alimenta pela raspagem de material orgânico de um substrato pelas mandíbulas. Como demonstrado por Wassersug & Yamashita (2001), os girinos abrem a boca à medida que se aproximam do substrato de onde obterão o alimento. O disco oral adere ao substrato e as mandíbulas começam a se fechar, movimento este que promove a raspagem da superfície à qual as partículas alimentares estão aderidas. Secundariamente ao fechamento das mandíbulas, ocorre o desprendimento das fileiras de dentículos posteriores do substrato. Após o desprendimento, as fileiras de dentículos começam a retornar à posição inicial, em direção às mandíbulas. Com este movimento, as fileiras de dentículos raspam novamente o substrato, criando uma suspensão de partículas alimentares que são sugadas durante o próximo ciclo de abertura e fechamento da boca. Além das espécies que se alimentam por raspagem, há aquelas que obtêm alimento somente por filtração de partículas suspensas na superfície e/ou na coluna d'água, por macrofagia ou por carnivoría (Altig & Johnston, 1989; Altig et al., 2007; McDiarmid & Altig, 1999). As espécies podem não estar restritas à utilização de um único comportamento alimentar (e.g. Rossa-Feres et al., 2004; de Sousa et al., 2014).

A influência de fatores contemporâneos sobre a estrutura das comunidades de girinos tem sido intensamente estudada há décadas: o hidroperíodo (e.g. Wellborn et al.,

1996; Both et al., 2009), a competição (e.g. Heyer, 1976; Steinwascher, 1978), a predação (e.g. Hero et al., 1998; Azevedo-Ramos et al., 1999) e a interação entre estes fatores (e.g. Morin, 1983; Woodward, 1983; Kotler & Holt, 1989) têm sido apontados como determinantes dos padrões de coexistência encontrados. No entanto, os padrões de uso e partilha de recursos, particularmente os recursos alimentares, não têm recebido a mesma atenção dos pesquisadores. Neste sentido, as pesquisas que tentam inferir a partilha de recursos entre as espécies têm sido desenvolvidas no intuito de se verificar quais itens alimentares fazem parte da dieta de cada espécie (e.g. Rossa-Feres et al., 2004). Mas pouco se sabe sobre a biomecânica do comportamento alimentar e como ela é afetado por fatores abióticos, além da questão da plasticidade comportamental exibida pelos girinos na exploração dos recursos alimentares (e.g. Wassersug & Yamashita, 2001; Larson & Reilly, 2003).

A nossa contribuição para o preenchimento desta lacuna no conhecimento ocorre por meio de estudos experimentais. Por meio de uma parceria com pesquisadores de universidades brasileiras dos Estados de São Paulo (UNESP, campus de São José do Rio Preto), Minas Gerais (UFMG) e Maranhão (UFMA), e pesquisadores de universidades americanas (Allegheny College and University of Arizona) e canadenses (Universidade Dalhousie, Halifax, e Universidade da Columbia Britânica, Vancouver) desenvolvemos três projetos de pesquisas cujos resultados são apresentados nesta tese de Doutorado. Primeiramente, avaliamos como girinos da espécie *Leptodactylus labyrinthicus* exploram fontes alimentares distintas, i.e. avaliamos como os girinos ajustam seu comportamento alimentar de modo a consumir uma variedade maior de itens alimentares (Capítulo 1). Os resultados indicam que os girinos adotam comportamentos alimentares distintos durante a alimentação: para consumir alimentos disponíveis em suspensão na água, os girinos exibem o comportamento de filtração; já para consumir

alimentos aderidos em superfícies submersas, os girinos utilizam o comportamento de raspagem. Tais comportamentos são quantitativa e qualitativamente distintos, i.e. os comportamentos alimentares são discerníveis com relação às suas cinemáticas e aos formatos que o disco oral assume durante a alimentação por filtração e por raspagem. Portanto, apesar de apresentarem morfologia oral tipicamente carnívora, os girinos de *L. labyrinthicus* não têm sua dieta restrita pela mesma. O objetivo do segundo projeto de pesquisa que desenvolvemos foi verificar como determinado fator abiótico (i.e. a temperatura da água) influencia o comportamento alimentar de girinos bentônicos de *Rhinella schneideri* e de girinos nectônicos de *Trachycephalus typhoni* (Capítulo 2). Os resultados indicam que a temperatura afeta a cinemática do comportamento alimentar de raspagem. No entanto, o efeito da temperatura sobre as variáveis de cinemática pode depender da identidade específica. Foi detectada uma interação entre temperatura e espécie, por isso, as mudanças na cinemática do comportamento podem não variar na mesma direção (ou seja, a temperatura pode ter efeito positivo sobre algumas variáveis e efeito negativo sobre outras). Além disso, avaliamos se e como a influência das relações filogenéticas sobre a morfologia oral e sobre a ecologia das espécies afetam a biomecânica do comportamento alimentar de girinos de 22 espécies pertencentes a cinco diferentes famílias de anuros. Os Capítulos 1 e 2 já se encontram publicados (Anexos 1 e 2, respectivamente). O Capítulo 3 está sendo preparado para ser submetido a um periódico científico e sua primeira versão está sendo aqui apresentada.

REFERÊNCIAS BIBLIOGRÁFICAS

- Altig, R. 2007. A primer for the morphology of anuran tadpoles. **Herpetol. Conserv. Biol.** 2:71-74.
- Altig R, Johnston GF. 1989. Guilds of anuran larvae: relationships among developmental modes, morphologies, and habitats. **Herpetol. Monogr.** 3:81-109.
- Altig, R., Whiles, M.R.; Taylor, C.L. 2007. What do tadpoles really eat? Assessing the trophic status of an understudied and imperiled group of consumers in freshwater habitats. **Freshw. Biol.** 52:386-395.
- Azevedo-Ramos, C.; Magnusson, W.E.; Bayliss P. 1999. Predation as the key factor structuring tadpole assemblages in a savanna area in central Amazonia. **Copeia** 1999:22-33.
- Both, C.; Solé, M.; Santos, T.G.; Cechin, S.Z. 2009. The role of spatial and temporal descriptors for neotropical tadpole communities in southern Brazil. **Hydrobiologia** 624:125-138.
- Cadle, J.E.; Greene, H.W. 1993. Phylogenetic patterns, biogeography, and the ecological structure of Neotropical snake assemblages. In: Ricklefs, R.E. and Schluter, D. *Species diversity in ecological communities*. Chicago: University of Chicago Press. p. 281- 293.
- Cadotte, M.W.; Carscadden, K.; Mirotnick, N. 2011. Beyond species: functional diversity and the maintenance of ecological processes and services. **J. Appl. Ecol.** 48:1079-1087.
- de Sousa, V.T.T.; Nomura, F.; Venesky, M.D.; Rossa-Feres, D.C.; Pezzuti, T.L.; Andrade, G.V.; Wassersug, R.J. 2014. Flexible feeding kinematics of a tropical carnivorous anuran tadpole. **J. Zool.** 293:204-210.

- Faivovich, J.; McDiarmid, R.W.; Myers, C.W. 2013. Two new species of *Myersiophyla* (Anura: Hylidae) from Cerro de la Neblina, Venezuela, with comments on other species of the genus. **Am. Mus. Novit.** 3792:1-63.
- Hero, J.-M.; Gascon, C.; Magnusson, W.E. 1998. Direct and indirect effects of predation on tadpole community structure in the Amazon rainforest. **Aust. J. Ecol.** 23:474-482.
- Heyer, W.R. 1976. Studies in larval amphibian habitat partitioning. **Smithson. Contrib. Zool.** 242:1-27.
- Kotler, B.P.; Holt, R.D. 1989. Predation and competition: the interaction of two types of species interactions. **Oikos** 54:256-260.
- Larson, P.M.; Reilly, S.M. 2003. Functional morphology of feeding and gill irrigation in the anuran tadpole: electromyography and muscle function in larval *Rana catesbeiana*. **J. Morphol.** 255:202-214.
- McDiarmid, R.W.; Altig, R. 1999. Research: materials and techniques. In: McDiarmid R.W.; Altig R., eds. *Tadpoles: the biology of anuran larvae*, 1st edn. The University of Chicago Press, Chicago, pp. 240-278.
- McGill, B.J.; Enquist, B.J.; Weiher, E.; Westoby, M. 2006. Rebuilding community ecology from functional traits. **Trends Ecol. Evol.** 21:178-185.
- Morin, P.J. 1983. Predation, competition, and the composition of larval anuran guilds. **Ecol. Monogr.** 53: 119-138.
- Rossa-Feres, D.C.; Jim, J.; Fonseca, M.G. 2004. Diets of tadpoles from a temporary pond in southeastern Brazil (Amphibia, Anura). **Rev. Bras. Zool.** 21:745-754.
- Rossa-Feres, D.C.; Nomura, F. 2006. Characterization and taxonomic key for tadpoles (Amphibia: Anura) from the northwestern region of São Paulo State, Brazil. **Biota**

- Neotrop.** 6, <http://www.biotaneotropica.org.br/v6n1/pt/abstract?identification-key+bn00706012006>.
- Steinwascher, K. 1978. Interference and exploitation competition among tadpoles of *Rana utricularia*. **Ecology** 59:1039-1046.
- Venesky, M.D.; Rossa-Feres, D.C.; Nomura, F.; Andrade, G.V.; Pezzuti, T.L.; de Sousa, V.T.T.; Anderson, C.V.; Wassersug, R.J. 2013. Comparative feeding kinematics of tropical hyloid tadpoles. **J. Exp. Biol.** 216:1928-1937.
- Venesky, M.D.; Wassersug, R.J.; Jorgensen, M.E.; Riddle, M.; Parris, M.J. 2011. Comparative feeding kinematics of temperate pond-dwelling tadpoles (Anura, Amphibia). **Zoomorphology** 130:31-38.
- Wassersug, R.J. 1976. Oral morphology of anuran larvae: terminology and general description. **Occas. Pap. Mus. Nat. Hist. Univ. Kansas** 48:1-23.
- Wassersug, R.J.; Yamashita, M. 2001. Plasticity and constraints on feeding kinematics in anuran larvae. **Comp. Biochem. Physiol., Part A: Mol. Integr. Physiol.** 131:183-195.
- Wellborn, G.A.; Skelly, D.K.; Werner, E.E. 1996. Mechanisms creating community structure across a freshwater habitat gradient. **Annu. Rev. Ecol. Syst.** 27:337-63.
- Woodward, B.D. 1983. Predator-prey interactions and breeding-pond use of temporary-pond species in a desert anuran community. **Ecology** 64:1549-155.

Capítulo 1

Flexible feeding kinematics of a tropical carnivorous anuran tadpole

Verônica T. T. de Sousa, Fausto Nomura, Matthew D. Venesky, Denise de C. Rossa-Feres, Tiago L. Pezzuti, Gilda V. Andrade, and Richard J. Wassersug

Manuscrito publicado em Journal of Zoology 293:204-210, 2014.

ABSTRACT

Leptodactylus labyrinthicus tadpoles are known predators of anuran eggs and hatchlings, but they are also able to filter-feed in the water column and scrape food off of firm substrates. We evaluated and compared these alternative feeding behaviors in relation to feeding kinematics and the shape of the mouth with high-speed digital imaging. We tested the hypotheses that (i) *L. labyrinthicus* tadpoles use functionally different feeding kinematics when feeding on alternative food sources and (ii) that the jaw sheaths of *L. labyrinthicus* tadpoles deform less during filter-feeding and substrate grazing compared to more common tadpoles not so specialized for macrophagous carnivory. Our results show that filtering and scraping feeding behaviors differ significantly in both kinematics and shape of the mouth. During filter-feeding, tadpoles display longer gape cycles and attain a narrower maximum gape earlier in the cycle compared to substrate grazing. Jaw deformation during opening and closing phases of the gape cycle is more pronounced during grazing on firm substrates. This deformation contributes to the achievement of a wider maximum gape during feeding. These differences appear to reflect behavioral adjustments by the tadpoles to maximize food intake. Feeding in tadpoles of *L. labyrinthicus* is not restrained by their typical carnivorous morphology. On the contrary, *L. labyrinthicus* tadpoles seem to be opportunistic feeders able to obtain nutrients from a variety of food sources by using different feeding strategies.

Key words: Anura, behavioral plasticity, biomechanics, geometric morphometrics, food source.

INTRODUCTION

Tadpole feeding is influenced by morphological, physiological and behavioral traits (e.g. Seale & Wassersug, 1979; Vera Candioti, Lavilla & Echeverría, 2004; Pryor & Bjorndal, 2005) as well as microhabitat use (e.g. Rossa-Feres, Jim & Fonseca, 2004; Sousa Filho et al., 2007) and seasonal activity (e.g. Peterson & Boulton, 1999; Echeverría, Volpedo & Mascitti et al., 2007). This has led to a variety of trophic specializations in tadpoles, although the larvae of most species are considered opportunistic omnivorous feeders (Altig, Whiles & Taylor, 2007). As such, anuran tadpoles can consume a diversity of food items, including algae, bacteria, fungi, protozoans, detritus, invertebrates, anuran eggs, and other tadpoles (reviewed by Altig et al., 2007). Depending on the quality and quantity of available food sources, many tadpoles can alternate between being omnivorous, microphagous suspension feeders, and opportunistic macrophagous predators (Petranka & Kennedy, 1999; Richter-Boix et al., 2007). To date, tadpole feeding behavior has only been described as tadpoles forage on a single type of food (e.g. Venesky et al., 2011; Venesky et al., 2013; Wassersug and Yamashita, 2001). Therefore, it is still unclear how tadpoles adjust their feeding mechanics to a diversity of food items and, consequently, to different substrates.

Herein, we explore the biomechanics of alternative feeding strategies of the macrophagous and carnivorous tadpole of *Leptodactylus labyrinthicus* (Spix, 1824) when provided with suspended and attached food sources. This species occurs in temporary aquatic habitats in Brazil and Paraguay, including Cerrado and Cerrado-like enclaves within Amazonia and the Atlantic rain forests (Heyer, 2005). Tadpoles of *L. labyrinthicus* present distinct morphological adaptations associated with macrophagy, including prominent jaw sheaths, reduced labial tooth rows (one anterior and two posterior tooth rows, Figure 1), and a slender body (Rossa-Feres & Nomura, 2006).

They prey upon con- and heterospecific tadpole eggs and hatchlings (Prado et al., 2005; Shepard & Caldwell, 2005; Silva, Giaretta & Facure, 2005). However, in captivity, tadpoles actively feed upon, and can survive through metamorphosis on, suspended particles of food as well as food attached to submerged surfaces (Rossa-Feres personal observation). Given this dietary breadth, *L. labyrinthicus* larvae can serve as a model tadpole for studying how anuran larvae adjust their feeding kinematics to different food sources.

In a laboratory experiment, we provided tadpoles of *L. labyrinthicus* with an algal-based powdered food that was either suspended on the water surface or attached to a planar glass substrate, and used high-speed digital videography to film tadpoles as they consumed the food. This methodology has allowed researchers to investigate functionally complex behaviors, such as feeding, with enough precision to visualize subtle but important differences in those behaviors (e.g. Wassersug & Yamashita, 2001; Venesky et al., 2013). Given the unpredictable hydroperiod in tropical pond systems (e.g. Rossa-Feres & Jim, 2001) and the selective pressures to reach a critical size before metamorphosis (e.g. Wilbur & Collins, 1973), we hypothesized that the carnivorous tadpoles *L. labyrinthicus* would adjust their feeding kinematics to maximize food consumption when feeding on alternative food sources. In addition, we predicted that the relatively robust jaw sheaths of *L. labyrinthicus*, which are understood to be an adaptation for macrophagous carnivory, would be less flexible and show little or no deformation (i.e., adjustment in shape) when the tadpoles were suspension feeding on finer, particulate material (Venesky et al., 2011; Venesky et al., 2013).

MATERIAL AND METHODS

Collection of tadpoles

Tadpoles of *L. labyrinthicus* were collected from marshes on the banks of ponds in Icém (20°21'47.66"S, 49°14'13.08"W), northwestern of São Paulo State, and in Buenópolis (17°54'45.50"S, 44°14'45.92"W), Minas Gerais State, Brazil. Tadpoles were transported in plastic bags filled with water from the pond to the laboratory at the Universidade Estadual Paulista – UNESP, campus of São José do Rio Preto, São Paulo State, Brazil. In the laboratory, tadpoles were maintained in polyethylene aquaria (37 x 30 x 10 cm) filled with dechlorinated and continuously aerated tap water. Tadpoles were maintained at 22°C on a natural photoperiod and were fed *ad libitum* once a day with a mixture of flocculated (Alcon BASIC®, Alcon, Brazil) and powdered (Sera Micron®, SERA, Germany) commercial fish food until the beginning of the experiments. Tadpoles were acclimated to laboratorial conditions for at least 5 days before trials. Tadpoles were collected under permit from the Brazilian Institute of Environment and Natural Resources (IBAMA) and ICMBio – Ministério do Meio Ambiente, Brazil (SISBIO no. 18163-1 to D.C.R.-F.) and maintained under approval from the Ethics Committee on the use of Animals (CEUA-IBILCE/UNESP), in accordance with the National Council for Control of Animal Experimentation (CONCEA).

Feeding Trials

In our first experiment, we filmed four tadpoles (mean \pm SD, total length: 58.45 \pm 4.37 mm; range of Gosner developmental stage: 35 - 39) at 500 frames/s as they fed on suspended particles of food on the surface of the water. Prior to filming, we placed individual tadpoles in a glass container (15 cm tall and 5 cm in diameter) that contained about 250 mL of aged tap water. We then prefocused the camera (Fastec TroubleShooter

LE 250; Fastec Imaging, California USA) on the surface of the water and sprinkled a fine particulate food (Sera Micron®) on the surface of the water until it formed a thin and uniform film. The amount of food added to the container was not standardized but appeared uniformly distributed on the surface of the water.

In our second experiment, we filmed three tadpoles (total length: 61.90 ± 4.11 mm; developmental stage: 39) as they fed on particles attached to a planar glass surface. Prior to filming, we made of a mixture of Sera Micron® and water, brushed it on one side of glass microscope slides (7.5 x 2.5 cm), and allowed it to air dry. We then mounted the clean side of one food-covered slide against the inside wall of a glass aquarium (8.5 x 8.5 x 8.5 cm), filled it with aged tap water, and filmed tadpoles in individual trials while they grazed on the food-covered surface. Because the kinematics of tadpole feeding is influenced by the resistance they encounter (Wassersug & Yamashita, 2001), all slides were made with the same concentration of Sera Micron® and we used a fresh food-covered slide for each videography trial to ensure that tadpoles had access to a substrate of similar density and food concentration.

The camera was prefocused on the food-covered surface prior to each trial following the protocol described in Venesky et al. (2011). During filming, the camera's vertical field of view was adjusted as necessary. As in the first experiment, we filmed tadpoles at 500 frames/s using the Fastec camera. In these trials, we recorded single feeding bouts with a "feeding bout" defined as beginning when a tadpole contacted the food covered surface and opened its mouth to the time when the tadpole completely closed its mouth and swam away. Each feeding bout consisted of a continuous series of "gape cycles", during which the tadpoles scraped food from the slide. As per Venesky et al. (2011), a "gape cycle" (1) starts with the jaw sheaths fully closed and the anterior and posterior tooth rows in closest proximity; (2) proceeds to the point where the mouth

is fully open and the labial tooth rows reached maximum gape; and (3) ends with full closure of the jaw sheaths and anterior and posterior tooth rows again in closest proximity to each other.

After each feeding trial, we anesthetized the tadpoles with 2% lidocaine hydrochloride and then fixed it in a 1:1 solution mixture of 70% alcohol and 15% formalin. The developmental stage was determined according to Gosner (1960). Tadpoles were deposited in the amphibian collection DZSJRP-Tadpoles of the Department of Zoology and Botany, UNESP, São José do Rio Preto, SP, Brazil.

Feeding kinematics

In order to compare the kinematics of filter-feeding at the water's surface with feeding while grazing on (i.e., scraping material off) a solid substrate, we quantified four kinematic variables that were common to feeding in both settings: (1) *Duration of the full gape cycle* – the duration of time from when the jaws begin to open until they are fully closed; (2) *Time to maximum gape* – the duration of time from when the mouth starts to open to when maximum gape is achieved; (3) *Percentage of time to maximum gape* – the duration of time, as percentage of the total time of the gape cycle, when maximum gape is achieved; and (4) *Maximum gape* – the length (in millimeters) from the internal border of upper jaw sheath to the internal border of the lower jaw sheath. We extracted data from five randomly selected gape cycles from each tadpole (n=7). All measurements were obtained using ImageJ 1.47m (Rasband, 2012).

Plasticity in jaw sheath shape

In our next set of analyses, we assessed patterns of shape variation in tadpoles' mouth between filtering and substrate grazing behavior. When tadpoles graze upon a substrate, they generate a suspension of material that is drawn into their mouths and then filtered out of the water by internal oral structures. Thus, whether a tadpole is ingesting particulate matter already in the water column or grazing on a substrate to produce a suspension, the tadpole in both situations is filter-feeding. However, to keep the wording simple here, we refer throughout to the first situation as "filter-feeding" and the second as "grazing." We obtained digital images of the tadpoles' mouth by extracting frames of high-speed video files with Midas OS (Xcitex Inc. 2012) and used the landmark-based geometric morphometrics method (Rohlf & Marcus, 1993; Zelditch et al., 2004) to evaluate shape variation in mouth. We focused our analysis to three different phases of the gape cycle: (i) opening, which is the point in the gape cycle where the anterior and posterior jaw sheaths are beginning to open but are still in contact with each other; (ii) maximum gape, which is the point in the gape cycle where the anterior and posterior jaw sheaths are furthest apart from each other; and (iii) closing, which is the point in the gape cycle where the anterior and posterior jaw sheaths are closing and regain contact with each other. For each phase, we placed five digital landmarks on each jaw sheath using TpsDIG 2.16 (Rohlf, 2010). For each jaw sheath, landmarks were placed in the left and right extremes, the center, and a point equidistant between the lateral extremes and center of jaw sheath. Selection of landmarks were based on the ability to easily and consistently identify the same position on the anatomical structure, their visibility throughout the entire gape cycle, and their ability to represent the change in shape of the structure during feeding (Venesky et al., 2013).

A Generalized Procrustes Analysis (Gower, 1975; Rohlf & Slice, 1990) was used to superimpose the specimens through the alignment of their landmarks configurations

to a Cartesian plane after accounting for differences in position, orientation, and scale. Generalized Procrustes Analysis was performed with the use of the function `gpagen` in the `geomorph` package (Adams & Otárola-Castillo, 2013) of R software (R Core Team, 2013). The resulting Procrustes-aligned coordinates represent the shape of each specimen and were used in further statistical analysis.

Statistical analysis

To determine whether the kinematics of the jaw movements differed between filter-feeding and substrate grazing behaviors, we used repeated measures multivariate analysis of covariance (MANCOVA). The kinematic variables were defined as the response variables, feeding mode (i.e., filter-feeding versus substrate grazing) was used as the independent variable, and total length of tadpoles was used as covariate to control for size differences. As measures of kinematic variables were obtained for five gape cycles of each tadpole, gape cycle was used as repeated measure. Additionally, we used repeated measures univariate analysis of covariance (ANCOVA) on each response variable to assess which variables were responsible for the significant main effects. Analysis was performed using the function `Anova` in `car` package of R software (R Core Team, 2013).

To visualize shape variation in shape space, a Principal Components Analysis based on a covariance matrix of the Procrustes-aligned coordinates was performed using `MorphoJ` (Klingenberg, 2011). Also, to verify whether the shape of tadpoles' mouth differs between filter-feeding and scraping a surface for food, we performed a Distance-based Permutational Multivariate Analysis of Variance (PERMANOVA, Anderson, 2001) based on Euclidean distance with Procrustes-aligned coordinates as dependent

variables and feeding behavior, cycle phase (opening and closing) and an interaction term between mode of feeding and cycle phase as independent variables. PERMANOVA was performed using adonis function in vegan package (Oksanen et al., 2013) of R software (R Core Team, 2013).

RESULTS

The feeding kinematics of *L. labyrinthicus* tadpoles differ in relation to the type of surface they are feeding from (repeated measures MANCOVA: $F_{1,4} = 16.55$, $p = 0.01$), except the duration of gape cycle (Table 1). Although filter-feeding tadpoles had a longer gape cycle relative to tadpoles scraping food from a firm, planar substrate (Table 2), this difference was not significant (Table 1). Filter-feeding tadpoles achieved maximum gape during the first 1/3 of the cycle, whereas tadpoles that scraped food off a firm substrate reached maximum gape during the second half of the gape cycle (Table 2). In addition, even after controlling for size differences among individuals, tadpoles that scraped the substrate for food had a larger maximum gape compared to the filter-feeding tadpoles (Table 2). Furthermore, in relation to grazing behavior, it is also noteworthy that the upper jaw does not hold to the substrate during closing phase: as the mouth starts to close, this jaw slips across the surface.

In terms of plasticity in the jaw sheaths of tadpoles, the first principal component explained 68.3% of the shape variation between feeding modes and reflects the shape of the jaw sheaths as tadpoles close their mouths. In contrast, the second principal component explained 10.6% of shape variation between the two feeding modes and is related to the opening phase of the gape cycle (Figure 2). Together the first two components explained 78.9% of the total shape variation. The only factor that influenced how *L. labyrinthicus* tadpoles move their jaw sheaths during feeding is the

behavior itself (Table 3). In other words, even after controlling for differences in the kinematics of the jaw as the mouth opened and closed, we found significant differences in the plasticity of the jaw sheaths related to feeding mode. As the tadpoles opened and closed their jaws to scrape food, the lower jaw elongated and narrowed along its transverse axis (Figure 2). In contrast, as tadpoles opened and closed their jaws while filter-feeding, the lower jaw got wider (Figure 2). Although we did not detect a significant feeding mode x gape cycle phase interaction in the jaw sheath plasticity (Table 3), changes in oral shape between feeding behaviors appeared to reflect the shape changes of the lower jaw during mouth closure.

DISCUSSION

Species that live in heterogeneous and/or temporary habitats often face unfavorable environmental conditions that can then drive the evolution of phenotypic plasticity in ecological generalists (Ghalambor et al., 2007). Ecological specialists can also persist in unstable environments, especially when the costs of generalist/plastic phenotypes outweigh those associated with specialist phenotypes (e.g. Van Tienderen, 1991; Agrawal, 2001). Behavioral flexibility might provide an intermediate strategy (Futuyma & Moreno, 1988), whereby specialists can use alternative tactics to increase fitness during unfavorable conditions. Prior to the present study, tadpoles of *L. labyrinthicus* were considered strictly carnivorous and dietary specialists (Shepard & Caldwell, 2005; Silva, Giaretta & Facure, 2005). Contrary to that perspective, our results demonstrate that tadpoles of *L. labyrinthicus* can perform as a dietary generalist and can use at least two alternative, functionally different, feeding strategies. As well as being macrophagous carnivores, they can be suspension feeders and scrape food off a firm substrate to generate a suspension of material subsequently extracted from the

water once drawn into their mouths. The feeding diversity we observed in *L. labyrinthicus* reveals how tadpoles with specialized feeding anatomy can use flexible behavioral strategies for feeding on different substrates.

Our videographic data show clear differences in the oral kinematics between filter-feeding and substrate grazing by *L. labyrinthicus* tadpoles. For instance, when the tadpoles solely filter-feed, they have relatively long gape cycles and they reach maximum gape proportionally sooner within the gape cycle than when they scrape upon a substrate. In addition, filter-feeding tadpoles of *L. labyrinthicus* achieve maximum gape proportionally earlier in the gape cycle and widen their jaws along the horizontal axis more so than when they scrape food off a solid substrate. Although we did not quantify food consumption between these two feeding strategies, these results suggest that behavioral adjustments used by the tadpoles may help maximize food intake in heterogeneous environments. Future studies could compare buccal pumping rates and capture efficiency (e.g. Seale & Wassersug, 1979; Wassersug & Hoff, 1979) between tadpoles with mixed feeding strategies to test for differences in feeding efficiency between these, and other, feeding strategies.

In terms of substrate-scraping behavior, we found similarities and differences between how this is accomplished by common grazing tadpoles with a more generalized oral morphology and *L. labyrinthicus*. In a previous study, Wassersug & Yamashita (2001) found that the dietary generalist larvae of *Lithobates catesbeianus* increase the speed in which they open and close their mouths when they meet resistance as they scrape an algal covered surface. Our result showing that *L. labyrinthicus* larvae vary the speed of their gape cycle, depending on whether they are filter feeding or foraged on an algal-covered substrate, corroborates the Wassersug & Yamashita findings. Our results

and theirs collectively suggest that kinematic adjustment in the speed that tadpoles open and close their mouths is common across diverse taxa.

In contrast to what has been described for other grazer tadpoles (Wassersug & Yamashita, 2001; Venesky et al., 2010; Venesky et al., 2013), it does not appear that tadpoles of *L. labyrinthicus* can effectively use their keratinized teeth to anchor their oral disc to the substrate when grazing on a planar surface. Instead, as the mouth starts to close, the upper jaw slips across the surface. In the present study, we focused only on feeding kinematics that were shared by the *L. labyrinthicus* tadpoles during filter-feeding and grazing, and did not quantify the extent in which the oral structures slip on the surface during grazing. As tooth rows help anchor the oral disc to the substrate (e.g. Wassersug & Yamashita, 2001, Venesky et al., 2010, Venesky et al., 2013), it is likely that poor anchoring of the mouth to the substrate is a consequence of the reduced number of labial tooth rows of *L. labyrinthicus* tadpoles (i.e., one anterior and two posterior tooth rows) compared to the common configuration in anuran tadpoles (i.e., two anterior and three posterior rows; Altig et al., 2007).

It is well known that both protein and lipid nutrients obtained from animal matter promote rapid tadpole growth and development (e.g. Kupferberg, 1997; Richter-Boix et al., 2007). The distribution of macroscopic animal prey is sometimes patchy and an ostensibly macrophagous carnivore may need to augment caloric and/or nutritional intake by consuming alternative food sources (e.g. Petranka & Kennedy, 1999; Schiesari, Werner & Kling, 2009). Suspension-feeding and substrate grazing by *L. labyrinthicus* tadpoles in natural environments could supplement their nutritional requirements through the consumption of alternative food sources available on the water surface films (Goldacre, 1949; Wotton & Preston, 2005) and on other surfaces in their aquatic habitat (McMahon, Hunter & Russel-Hunter et al., 1974; Weitzel, 1979). Future

studies that examine *L. labyrinthicus* growth rates and development raised on different larval diets, may help determine how important alternative tadpole feeding strategies are for the overall fitness of the species.

Previous studies on the feeding mechanics of tadpoles have largely focused on the buccal pumping mechanism of filter-feeding tadpoles (e.g. Wassersug & Hoff, 1979) or scraping behavior (e.g. Venesky et al., 2011). Our study shows that tadpoles of *L. labyrinthicus* are more plastic in their feeding behaviors than their external oral morphology suggests. Such plasticity appears advantageous for tadpoles of species, like *L. labyrinthicus*, that occupy temporary habitats and have to grow fast in order to metamorphose before pond drying (e.g. Richter-Boix et al., 2007). Our results demonstrate that tadpoles can adjust their feeding kinematics in response to different food and support the idea that the range of feeding strategies cannot always be inferred from external morphological features (e.g. Vera Candiotti, 2006; Schiesari, Werner & Kling, 2009). These results highlight the need for studies that link behavioral research with functional morphology.

ACKNOWLEDGEMENTS

We thank Fabiane S. Annibale, Carlos E. Sousa, Rodolfo Pelinson and Fernando Leal for assistance during field work. R.J.W.'s research is supported by the National Science and Engineering Research Council of Canada. D.C.R.F., F.N. and G.V.A. are supported by a joint CNPq (grant 563075/2010-4) and FAPESP project (grant 2010/52321-7) on Brazilian tadpole biology. D.C.R.F. and G.V.A. thank the research fellowship of CNPq. M.D.V. was supported by a Visiting Researcher grant from FAPESP (grant 2011/51724-

3). V.T.T.S. received a fellowship from Reuni/Capes. T.L.P. received a fellowship from FAPEMIG/Vale project (Process nº 17237).

TABLES

Table 1. The results from repeated measures ANCOVA documenting the variation in feeding kinematics of tadpoles of *Leptodactylus labyrinthicus* as a function of their feeding behavior (filter-feeding versus grazing). We used the total length of each tadpole as a covariate in each statistical test. df: degrees of freedom, F: F-ratio (value of observed F statistic), P: probability value.

Kinematic variables (A-D)			
Factors	df	F	P
A. Duration of			
Gape Cycle			
Behavior	1	6.069	0.069
Total length	1	0.569	0.492
Gape Cycle	4	1.429	0.269
B. Maximum			
Gape			
Behavior	1	264.334	8.38E-05
Total length	1	0.294	0.616
Gape Cycle	4	0.171	0.949
C. Time to			
Maximum Gape			
Behavior	1	14.618	0.018
Total length	1	1.697	0.262
Gape Cycle	4	1.491	0.251
D. Percentage of			
time to			
maximum gape			
Behavior	1	101.028	5.51E-04
Total length	1	1.026	0.368
Gape Cycle	4	1.814	0.175

Table 2. Mean \pm Standard Deviation of kinematic variables measured during the display of two feeding behaviors; i.e., filter-feeding versus grazing.

Kinematic variables	Feeding Behavior	
	Filtering	Scraping
Duration of Gape Cycle (ms)	87.80 ± 9.69	73.47 ± 9.78
Maximum Gape (mm)	0.79 ± 0.04	1.30 ± 0.07
Time to maximum gape (ms)	29.20 ± 4.56	41.07 ± 9.91
Percentage of time to maximum gape (as % of gape cycle)	0.33 ± 0.05	0.55 ± 0.06

Table 3. Effects of feeding behaviors (filter-feeding versus grazing), gape cycle phase (opening and closing) and the interaction term between the two factors on the shape of the mouth *Leptodacylus labyrinthicus*.

Factors	Df	F	R ²	P
Behavior	1	80.536	0.546	0.001
Cycle Phase	1	0.394	0.003	0.729
Behavior x Cycle Phase	1	0.474	0.003	0.645
Residuals	66		0.447	
Total	69		1	

FIGURES

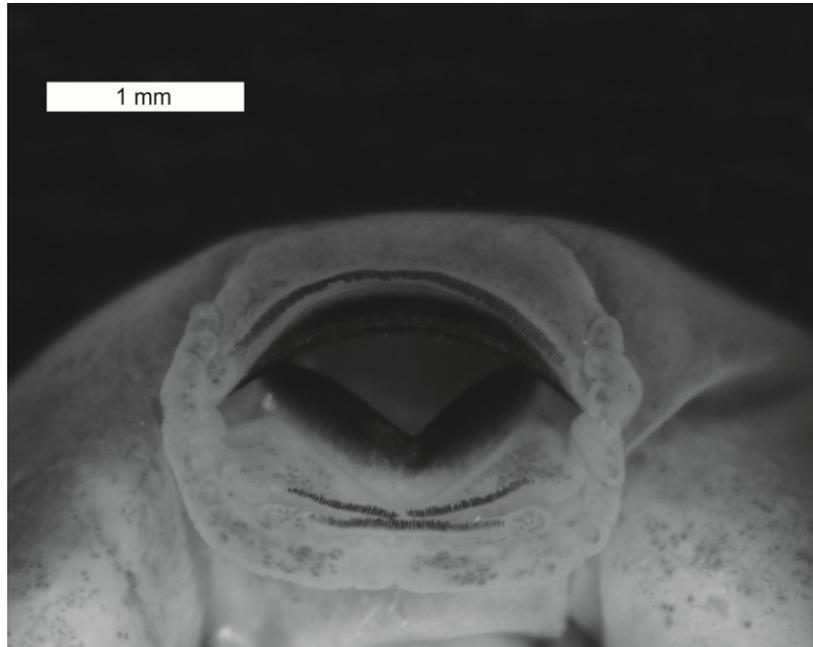


Figure 1. A representative tadpole of *Leptodactylus labyrinthicus* at Gosner developmental state 36. The tadpole was collected in northwestern region of São Paulo State, Brazil. Scale: 1 mm.

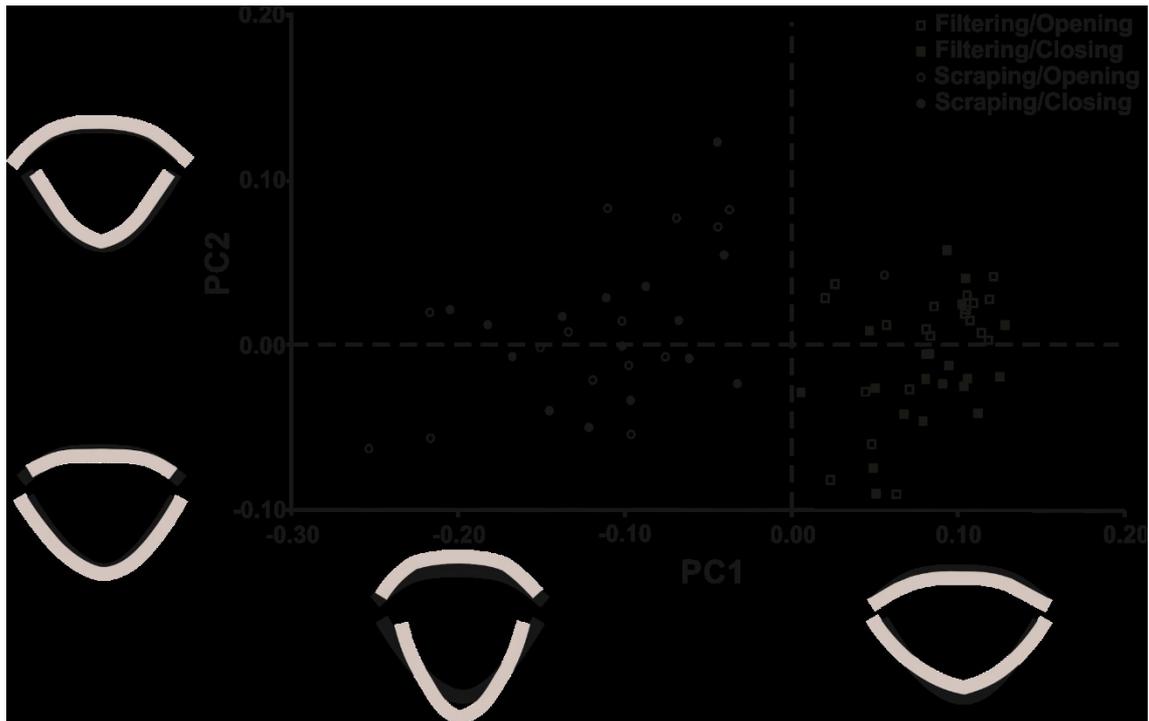


Figure 2. A plot of the Procrustes-aligned specimens of *Leptodacylus labyrinthicus* along first two dimensions of tangent space showing the plasticity of the jaw sheaths. The first axis represents closing phase, whereas the second axis represents opening phase of gape cycle. Squares represent data from filter-feeding tadpoles and circles represent data from tadpoles that fed by scraping a planar substrate. Patterns of shape variation along each component are represented by the warped outlines; black is the average shape and grey represents individuals with high scores. When filter-feeding, the posterior jaw sheath of tadpoles got wider whereas the jaw elongated and narrowed along its transverse axis when scraping for food.

LITERATURE CITED

- Adams, D. C. & Otárola-Castillo, E. (2013). geomorph: an R package for the collection and analysis of geometric morphometric shape data. *Methods Ecol. Evol.* **4**, 393-399.
- Agrawal, A. A. (2001). Phenotypic plasticity in the interactions and evolution of species. *Science* **294**, 321-326.
- Altig, R., Whiles, M. R. & Taylor, C. L. (2007). What do tadpoles really eat? Assessing the trophic status of an understudied and imperiled group of consumers in freshwater habitats. *Freshw. Biol.* **52**, 386-395.
- Anderson, M. J. (2001). A new method for non-parametric multivariate analysis of variance. *Austral Ecol.* **26**, 32-46.
- Echeverría, D. D., Volpedo, A. V. & Mascitti, V. I. (2007). Diet of tadpoles from a pond in Iguazu National Park, Argentina. *Gayana* **71**, 8-14.
- Futuyma, D. J. & Moreno, G. (1988). The evolution of ecological specialization. *Annu. Rev. Ecol. Syst.* **19**, 207-233.
- Ghalambor, C. K., McKay, J. K., Carroll, S. P., & Reznick, D. N. (2007). Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Func. Ecol.* **21**, 394-407.
- Goldacre, R. J. (1949). Surface films on natural bodies of water. *J. Anim. Ecol.* **18**, 36-39.
- Gosner, K. L. (1960). A simplified table for staging anuran embryos and larvae with note on identification. *Herpetologica* **16**, 183-190.

- Gower, J. C. (1975). Generalized Procrustes Analysis. *Psychometrika* **40**, 33-51.
- Heyer, W. R. (2005). Variation and taxonomic clarification of the large species of the *Leptodactylus pentadactylus* species group (Amphibia, Leptodactylidae) from Middle America, northern South America, and Amazonia. *Arq. Zool.* **37**, 269–348.
- Klingenberg, C. P. (2011). MORPHOJ: an integrated software package for geometric morphometrics. *Mol. Ecol. Resour.* **11**, 353-357.
- Kupferberg, S. J. (1997). The role of larval diet in anuran metamorphosis. *Amer. Zool.* **37**, 146-159.
- McMahon, R. F., Hunter, R. D. & Russel-Hunter, W. D. (1974). Variation in aufwuchs at six freshwater habitats in terms of carbon biomass and of carbon:nitrogen ratio. *Hydrobiologia* **45**, 391-404.
- Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H. & Wagner, H. (2013). *vegan: Community Ecology Package*. R package version 2.0-7. <http://CRAN.R-project.org/package=vegan>
- Peterson, C. G. & Boulton, A. J. (1999). Stream permanence influences microalgal food availability to grazing tadpoles in arid-zone springs. *Oecologia* **118**, 340-352.
- Petranka, J. W. & Kennedy, C. A. (1999). Pond tadpoles with generalized morphology: is it time to reconsider their functional roles in aquatic communities? *Oecologia* **120**, 621-631.

- Prado, C. P. A., Toledo, L. F., Zina, J. & Haddad, C. F. B. (2005). Trophic eggs in the foam nests of *Leptodactylus labyrinthicus* (Anura, Leptodactylidae): an experimental approach. *Herpetol. J.* **15**, 279-284.
- Pryor, G. S. & Bjorndal, K. A. (2005). Symbiotic fermentation, digesta passage, and gastrointestinal morphology in bullfrog tadpoles (*Rana catesbeiana*). *Physiol. Biochem. Zool.* **78**, 201-215.
- R Core Team (2013). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org/>.
- Rasband, W. S. (2012). *ImageJ*. U. S. National Institutes of Health, Bethesda, Maryland, USA, <http://imagej.nih.gov/ij/>
- Richter-Boix, A., Llorente, G. A., Montori, A. & Garcia, J. (2007). Tadpole diet selection varies with the ecological context in predictable ways. *Basic Appl. Ecol.* **8**, 464-474.
- Rohlf, F. J. (2010). *tpsDig, digitize landmarks and outlines*, version 2.16. Department of Ecology and Evolution, State University of New York at Stony Brook.
- Rohlf, F. J. & Marcus, L. F. (1993). A revolution in morphometrics. *Trends Ecol. Evol.* **8**, 129-132.
- Rohlf, F. J. & Slice, D. (1990). Extensions of the Procrustes method for the optimal superimposition of landmarks. *Syst. Zool.* **39**, 40-59.

- Rossa-Feres, D. C. & Jim, J. (2001). Similaridade no sítio de vocalização em uma comunidade de anfíbios anuros na região noroeste do estado de São Paulo, Brasil. *Rev. Bras. Zool.* **18**, 439-454.
- Rossa-Feres, D. C., Jim, J. & Fonseca, M. G. (2004). Diets of tadpoles from a temporary pond in southeastern Brazil (Amphibia, Anura). *Rev. Bras. Zool.* **21**, 745-754.
- Rossa-Feres, D. C. & Nomura, F. (2006). Characterization and taxonomic key for tadpoles (Amphibia: Anura) from the northwestern region of São Paulo State, Brazil. *Biota Neotrop.* **5**, <http://www.biotaneotropica.org.br/v6n1/pt/abstract?identification-key+bn00706012006>.
- Schiesari, L., Werner, E. E. & Kling, G. W. (2009). Carnivory and resource-based niche differentiation in anuran larvae: implications for food web and experimental ecology. *Freshw. Biol.* **54**, 572-586.
- Seale, D. & Wassersug, R. J. (1979). Suspension feeding dynamics of anuran larvae related to their functional morphology. *Oecologia* **39**, 259-272.
- Shepard, D. B. & Caldwell, J. P. (2005). From foam to free-living: ecology of larval *Leptodactylus labyrinthicus*. *Copeia* **2005**, 803-811.
- Silva, W. R., Giaretta, A. A. & Facure, K. G. (2005). On the natural history of the South American pepper frog, *Leptodactylus labyrinthicus* (Spix, 1824) (Anura, Leptodactylidae). *J. Nat. Hist.* **39**, 555-566.
- Sousa Filho, I. F., Branco, C. C., Carvalho-e-Silva, A. M. P. T., Silva G. R. & Sabagh, L. T. (2007). The diet of *Scinax angrensis* (Lutz) tadpoles in an area of the Atlantic

- Forest (Mangaratiba, Rio de Janeiro) (Amphibia, Anura, Hylidae). *Rev. Bras. Zool.* **24**, 965-970.
- Van Tienderen, P. H. (1991). Evolution of generalists and specialist in spatially heterogeneous environments. *Evolution* **45**, 1317-1331.
- Venesky, M. D., Rossa-Feres, D. C., Nomura, F., Andrade, G. V., Pezzuti, T. L., Sousa, V. T. T., Anderson, C. V. & Wassersug, R. J. (2013). Comparative feeding kinematics of tropical hylid tadpoles. *J. Exp. Biol.* **216**, 1928-1937.
- Venesky, M. D., Wassersug, R. J., Jorgensen, M. E., Riddle, M. & Parris, M. J. (2011). Comparative feeding kinematics of temperate pond-dwelling tadpoles (Anura, Amphibia). *Zoomorphology* **130**, 31-38.
- Venesky, M. D., Wassersug, R. J. & Parris, M. J. (2010). How does a change in labial tooth row number affect feeding kinematics and foraging performance of a ranid tadpole (*Lithobates sphenoccephalus*)? *Biol. Bull.* **218**, 160-168.
- Vera Candiotti, M. F. (2006). Ecomorphological guilds in anuran larvae: an application of geometric morphometric methods. *Herpetol. J.* **16**, 149-162.
- Vera Candiotti, M. F., Lavilla, E. O. & Echeverría, D. D. (2004). Feeding mechanisms in two treefrogs, *Hyla nana* and *Scinax nasicus* (Anura: Hylidae). *J. Morphol.* **261**, 206-224.
- Wassersug, R. J. & Hoff, K. (1979). A comparative study of the buccal pumping mechanism of tadpoles. *Biol. J. Linn. Soc.* **12**, 225-259.

- Wassersug, R. J. & Yamashita, M. (2001). Plasticity and constraints on feeding kinematics in anuran larvae. *Comp. Biochem. Physiol., Part A: Mol. Integr. Physiol.* **131**, 183-195.
- Weitzel, R. L. (1979). Periphyton measurements and applications. In *Methods and measurements of periphyton communities: a review*: 3-33. Weitzel, R. L. (Ed.). Baltimore: American Society for Testing and Materials.
- Wilbur, H. M. & Collins, J. P. (1973). Ecological aspects of amphibian metamorphosis. *Science* **182**, 1305-1314.
- Wotton, R. S. & Preston, T. M. (2005). Surface films: areas of water bodies that are often overlooked. *BioScience* **55**, 137-145.
- Zelditch, M. L., Swiderski, D. L., Sheets, H. D. & Fink, W. L. (2004). Geometric morphometrics for biologists: A primer. 1st edn. San Diego: Elsevier Academic Press.

Capítulo 2

Differential effects of temperature on the feeding kinematics of the tadpoles of two sympatric anuran species

Verônica T. T. de Sousa, Fausto Nomura, Denise de C. Rossa-Feres, Gilda V. Andrade,
Tiago L. Pezzuti, Richard J. Wassersug, and Matthew D. Venesky

Manuscrito publicado em Journal of Experimental Zoology 323A:456-465, 2015.

ABSTRACT

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

Key words: Biomechanics, ecomorphological guild, behavior, Bufonidae, Hylidae.

INTRODUCTION

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

Feeding is one of the most conspicuous behaviors of tadpoles and, along with other morphological and ecological features, is used for sorting tadpoles into ecomorphological guilds (Altig and Jonhston, '89). Two common guilds include benthic and nektonic tadpoles, both of which can feed by scraping food from submerged surfaces. They differ, however, in their preferred location in ponds: benthic tadpoles occur at or near the pond bottom whereas nektonic tadpoles are found within the water column. Benthic and nektonic tadpoles can be found throughout the pond, but the depth

where they can be found will vary according to the species preferences (e.g. Prado et al. 2009). As deep waters are comparatively colder than shallow waters (Newman, 1989), tadpoles occurring in different microhabitats will experience different temperatures. Tadpoles occupying shallow waters will experience drastic temperature fluctuations compared to tadpoles in deeper waters. Indeed, tadpoles' feeding rate and many fitness correlates (e.g., growth rates and size at metamorphosis) are strongly affected by variation in temperature (Marian and Pandian, '85; Warkentin, '92; Álvarez and Nicieza, 2002; Liess et al., 2013).

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

We first hypothesized that temperature would influence tadpole feeding kinematics. Irrespective of species, we predicted that tadpoles acclimated in warmer temperatures would have faster kinematics compared to tadpoles raised in colder temperatures, because muscle contractile dynamics is primarily temperature dependent

(James, 2013). Next we hypothesized that temperature would have differential effects on the feeding kinematics of these two species. Tadpoles of *R. schneideri* are lungless, negatively buoyant, and consequently benthic (Ultsch et al., '99). Because these benthic tadpoles occur primarily on the bottom of ponds in shallow depths (< 25 cm, Prado et al. 2009), and cannot regulate their position in the water column because of their negative buoyancy, they should experience relatively large diurnal shifts in environmental temperatures. In contrast, tadpoles of *T. typhoni* have functional lungs long before metamorphosis and can achieve neutral buoyancy (Zweifel, '64). They are nektonic and can swim throughout the water column. Thus they should be better buffered from extreme shifts in temperature because they can choose a microhabitat that is closest to their optimal temperature.

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

MATERIAL AND METHODS

Study area

Tadpoles of *Rhinella schneideri* and *Trachycephalus typhoni* were obtained in ponds located in Nova Itapirema (21°04'44.83"S, 49°32'22.00"W), northwestern of São Paulo State, Brazil. The climate of this region is Aw Köppen-Geiger (Alvares et al.

2013), characterized by hot and wet summers (October to March) and dry winters (April to September). The annual rainfall varies from 1200 to 1650 mm (Carvalho and Assad, 2005) and the onset of the rainy season varies each year (Rossa-Feres and Jim, 2001). In the beginning of the rainy season (during October), the temperature in ponds may be as low as 21°C, and it may reach 37°C in the middle of the season (during January) (DC Rossa-Feres, unpublished data). The original vegetation cover, of Mesophitic Semideciduous Forest (Atlantic Forest Domain) with patches of Cerrado, was intensively deforested for agro-pastoral activities, and the remaining fragments of original vegetation are few and small (SMA/IF, 2005).

Study species

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

Collection and maintenance of tadpoles

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

Feeding Trials

To evaluate the effect of temperature on feeding kinematics, ten tadpoles of *R. schneideri* and ten tadpoles of *T. typhoni* were randomly selected and allocated to one of two treatment groups: cold (22°C) or warm temperatures (30.5–32°C). The temperatures in which tadpoles were tested fell within the range of the water temperature of the ponds in which they naturally occurred. Tadpoles of different groups

were kept in two separate rooms and acclimated at their respective temperatures for five days before the start of the feeding trials. Ideally, we would have placed all of the tadpoles in the same room and controlled each container individually. However, we had technical difficulties regulating the appropriate temperature in the small chamber that we used during our feeding trials because tadpoles did not always feed immediately when placed in the filming chamber. Thus, we elected to film in two separate rooms so that we would minimize temperature variation between each replicate. Water temperature was measured with a thermometer immediately before the start of each feeding trial.

Our feeding experiment followed the protocol described in Venesky et al. (2011). Briefly, prior to each feeding trial, we made a mixture of Sera Micron® and water, brushed it on one side of a glass microscope slide, and allowed it to air dry. As tadpole feeding kinematics is influenced by the resistance they encounter (Wassersug and Yamashita, 2001), all slides were made with the same concentration of Sera Micron®. We then mounted the clean side of one food-covered slide against the inside wall of a glass container (8.5 x 8.5 x 8.5 cm) and filled it with aged dechlorinated tap water. We prefocused a Fastec TroubleShooter LE 250 camera (Fastec Imaging, San Diego, California, USA) on the food-covered surface prior to each trial and video-recorded the tadpoles in individual trials while they grazed on this surface. We used a fresh food-covered slide for each trial to ensure that tadpoles had access to a substrate of similar density and food concentration. Video images were captured at 500 frames per second and the camera's vertical field of view was adjusted as necessary during recording.

In these trials, we recorded single feeding bouts, with a "feeding bout" defined as beginning when a tadpole contacted the food covered surface and opened its mouth to the time when the tadpole completely closed its mouth and swam away. Each feeding

bout consisted of a continuous series of “gape cycles”, during which the tadpoles scraped food from the slide. As per Venesky et al. (2011), a “gape cycle” (1) starts with the jaw sheaths fully closed and the anterior and posterior tooth rows in closest proximity; (2) proceeds to the point where the mouth is fully open and the labial tooth rows reached maximum gape; and (3) ends with full closure of the jaw sheaths and anterior and posterior tooth rows again in closest proximity to each other.

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

Feeding kinematics

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

percentage of the total time of the gape cycle, from when the mouth starts to open to when the lower jaw starts to narrow; (v) *Opening phase velocity*—the velocity at which upper and lower jaws move from the initial position of full contact between jaws to the maximum gape position; and (vi) *Closing phase velocity*— the velocity at which upper and lower jaws move from the initial position of maximum gape to the final position of jaws fully closed. Velocity was calculated as the displacement (in millimeters) of the jaws during opening and closing phases of a gape cycle divided by the time elapsed during the considered phase of the gape cycle. We randomly extracted data from 4-5 gape cycles from each tadpole. Thus, 48 gape cycles were analyzed for *R. schneideri* and 50 for *T. typhoni*. All measurements were obtained using ImageJ 1.47m (Rasband, 2012).

Data analyses

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

increasing temperature, the Q_{10} will be greater than 1. If the reaction rate decreases with increasing temperature, the Q_{10} will be less than 1.

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

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

RESULTS

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

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

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

Although Q_{10} values indicate that tadpoles do not adjust the velocity at which they open their jaws according to the temperature they experience (i.e., Q_{10} values were not different from 1; Table 2), both species tended to increase the velocity of opening

phase when in warmer water (Table 1). For the velocity of closing phase, Q_{10} values were significantly different from a value of one (Table 2) indicating that changes in the mean temperature affected this component of feeding. Tadpoles of *T. typhoni* slightly decreased the velocity at which they close their jaws in warmer water, while *R. schneideri* increased it (Tables 1 and 2).

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

DISCUSSION

Temperature has the potential to significantly impact ectotherm performance by influencing many of their physiological processes. In the present study, we show how temperature alters tadpoles feeding kinematics. Irrespective of species, tadpoles that feed in warm water have shorter gape cycles and faster opening phase. The fact that we

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

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

previously thought. For example, Venesky et al. (2013) found that the total number of labial tooth rows that a tadpole had was a better predictor of the duration of the gape cycle than whether a particular species was categorized as a benthic or nektonic tadpole. When tadpoles feed, one of the functions of their labial tooth rows is to anchor the oral disc to the substrate so that the jaws can scrape food from the surface (Wassersug and Yamashita, 2001). During the closing phase of the gape cycle, the posterior tooth rows are generally released in a serial fashion, with the inner row being the first to disengage from the substrate and the outer row, the last (Wassersug and Yamashita, 2001; Venesky et al., 2010). Because of this, the duration of the gape cycle should be positively related to the number of tooth rows (Venesky et al., 2010). Thus, the inconsistency between the results of our present study and those of Venesky et al. (2013) is likely explained by the differences in the oral morphology of the study species in each guild, or an interaction between feeding guild and the number of tooth rows for the species in either guild.

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

During feeding, six hyoid and mandibular muscles are activated, with different phases of a gape cycle being controlled by different muscles (Larson and Reilly, 2003).

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

One of our most intriguing results was that temperature does not necessarily shift the feeding kinematics of both species in the same direction. In cold water, the benthic feeding tadpoles of *R. schneideri* (and also the species with fewer tooth rows) achieved the maximum gape and started to close their mouths proportionally earlier in the gape cycle compared to the nektonic feeding tadpoles of *T. typhoni* (the species with more tooth rows). In warm water, this pattern is inverted. In relation to the closing phase velocity, tadpoles close their jaws in a similar rate in cold temperatures; but in warm water, tadpoles of *R. schneideri* increases the velocity at which they close their jaws, while *T. typhoni* slightly decreases it. The interaction between temperature and species is logical, if one considers the fact that tadpoles of *R. schneideri* should be well adapted to larger thermal variation given their ecology and behavior. As tadpoles of *R. schneideri* occur in the bottom of ponds but in shallow waters (Prado et al., 2009), they experience both colder and warmer temperatures. This can be seen in the results found for their feeding at the colder temperature. It is known that tadpoles with larger maximum gapes open and close their jaws slower than the ones that exhibit smaller

maximum gapes (Venesky et al., 2013), but that is not how *R. schneideri* behaves. In colder water, tadpoles of *R. schneideri* achieve larger maximum gapes than both tadpoles of the same species subjected to the warmer temperatures, and tadpoles of *T. typhoni* in colder temperature. Yet, tadpoles of *R. schneideri* achieved a larger maximum gape sooner in cold water than in warm water, indicating that their jaw movements are not restrained by the cold temperature and, possibly that they function better in colder temperatures.

Although this paper focused primarily on the functional morphology of tadpole feeding, the interaction between species and temperature on tadpole feeding kinematics could have broader ecological implications. Our data show that temperature changes can affect feeding kinematics of sympatric tadpoles in different ways. It is thus plausible that climate related changes in pond temperature could indirectly drive changes in the structure of the community within ponds upon which tadpoles feed. Given that tadpoles can act as "ecosystem engineers" by modifying habitat structure and biomass availability in waterbodies (e.g. Flecker et al. 1999; Ranvestel et al., 2004; Wood and Richardson, 2010), such changes in feeding behavior for different species could lead to cascading effects on the full food webs (e.g., Wilbur, '97). These effects in pond communities could become more pronounced because of the projected increase in extreme temperature events (Easterling et al. 2000).

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

ecomorphology, behavioral plasticity and muscle physiology interact to affect feeding kinematics are needed to fully understand tadpole feeding at the individual and community level.

ACKNOWLEDGEMENTS

We thank Fabiane S. Annibale and Carlos E. Sousa for assistance during field work, and Prof. Dr. Luiz Henrique Florindo for support. We also thank the two anonymous reviewers and the associate editor for their insightful comments that helped to significantly improve the final version. This research was supported by a joint CNPq (grant 563075/2010-4) and FAPESP project (grant 2010/52321-7) on Brazilian tadpoles biology. R.J.W.'s research is supported by the National Science and Engineering Research Council of Canada. D.C.R.F. and G.V.A. thank the research fellowships provided by CNPq (303522/2013-5 and 309479/2012-6). V.T.T.S. received a fellowship from Bolsa REUNI de Assistência ao Ensino. T.L.P. received a fellowship from FAPEMIG/Vale project (Process nº 17237).

TABLES

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

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

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

Kinematic variables	Q₁₀	95% IC	t	P
<i>Trachycephalus typhoni</i>				
Maximum gape	0.932	0.895 - 0.967	-4.023	< 0.001
Duration of gape cycle	1.177 ¹	1.092 - 1.261	4.379	< 0.001
% Time to maximum gape	1.120 ¹	1.031 - 1.209	2.817	0.011
% Time to lower jaw starts to narrow	1.169 ¹	1.099 - 1.238	5.105	< 0.001
Opening phase velocity	1.119	0.912 - 1.325	1.206	0.242
Closing phase velocity	0.884	0.825 - 0.942	-4.167	< 0.001
<i>Rhinella schneideri</i>				
Maximum gape	0.904	0.823 - 0.987	-2.407	0.025
Duration of gape cycle	1.318 ¹	1.228 - 1.410	7.265	< 0.001
% Time to maximum gape	0.899 ¹	0.804 - 0.994	-2.211	0.038
% Time to lower jaw starts to narrow	0.914 ¹	0.868 - 0.959	-3.961	< 0.001
Opening phase velocity	1.188	0.984 - 1.390	1.914	0.069
Closing phase velocity	1.504	1.281 - 1.727	4.697	< 0.001

¹The Q₁₀ temperature coefficient was calculated as Q₁₀ inverse (i.e., 1/Q₁₀).

Table 3. Results of the two-sample t-test for equal means applied to verify whether tadpoles of *Rhinella schneideri* and *Trachycephalus typhoni* exhibited comparatively different temperature sensitivity for each kinematics variable measured. Sensitivity was evaluated through the Q₁₀ or 1/Q₁₀ temperature coefficients.

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

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

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

Closing phase velocity

Species	19.191	< 0.001
Temperature	12.883	0.002
Total length	2.173	0.141
Species x Temperature	7.482	0.006

FIGURES

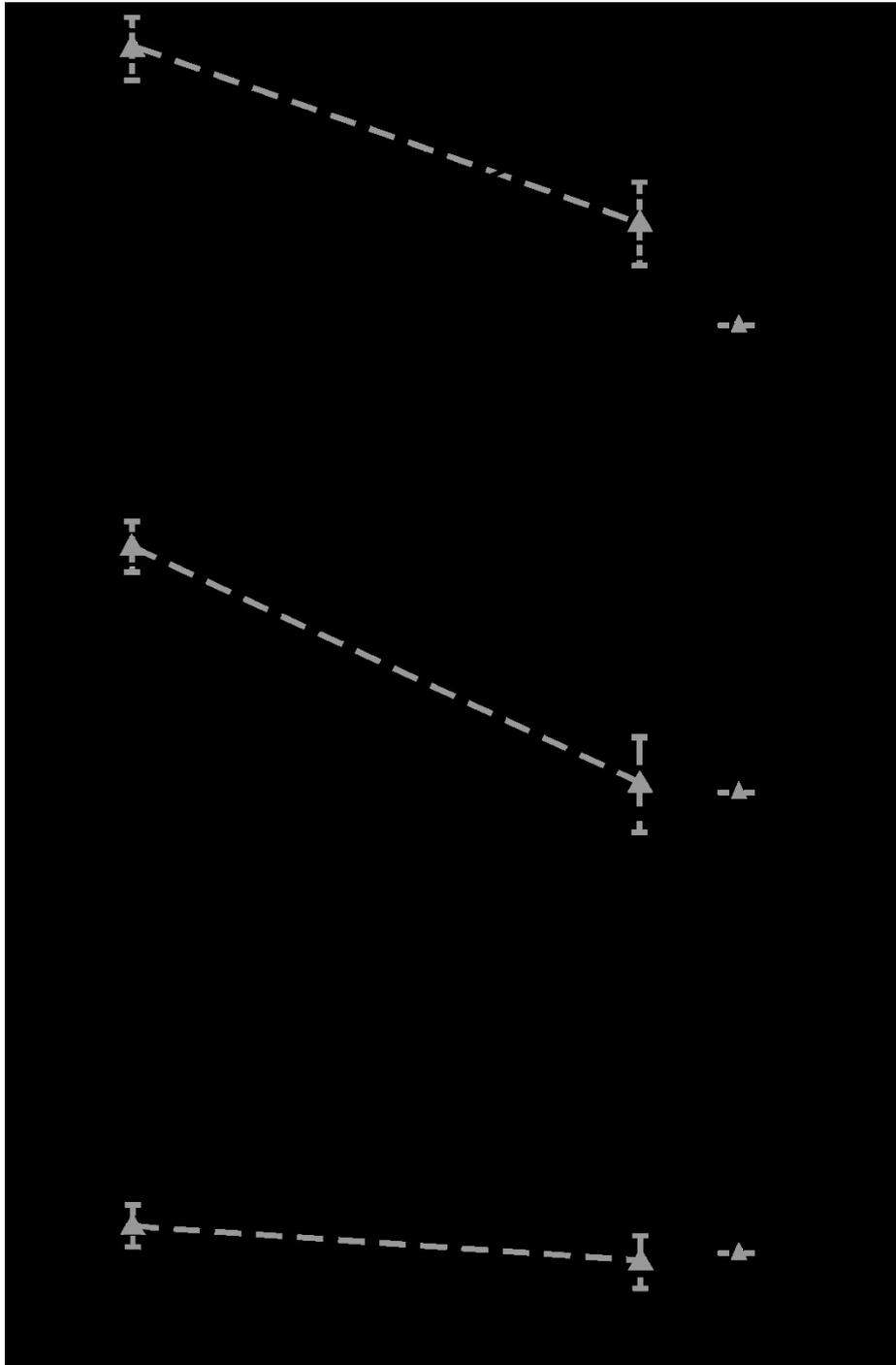


Figure 1. The species x temperature interaction effect on (A) the percentage of time tadpoles take to achieve maximum gape (MG), (B) the percentage of time tadpoles take to narrow the lower jaw (LJ) as mouth start closing, and (C) the velocity at which

tadpoles close their mouth. Solid triangles and circles represent mean values and bars indicate standard error.

LITERATURE CITED

- Altig R, Johnston GF. 1989. Guilds of anuran larvae: relationships among developmental modes, morphologies, and habitats. *Herpetol Monogr* 3:81-109.
- Alvares CA, Stape JL, Sentelhas PC, de Moraes Gonçalves JL, Sparovek G. Köppen's climate classification map for Brazil. *Meteorol. Z.* 22:711-728.
- Álvarez D, Nicieza G. 2002. Effects of temperature and food quality on anuran larval growth and metamorphosis. *Funct Ecol* 16:640–648.
- Berven KA. 1990. Factors affecting population fluctuations in larval and adult stages of the wood frog (*Rana sylvatica*). *Ecology* 71:1599-1608.
- Bates D, Maechler M, Bolker B, Walker S. 2014. lme4: Linear mixed-effects models using Eigen and S4. R package version 1.1-7, <URL: <http://CRAN.R-project.org/package=lme4>>.
- Borges Júnior VNT, Rocha CFD. 2013. Tropical tadpole assemblages: which factors affect their structure and distribution? *Oecol. Aust.* 17:27-38.
- Cannatella D. 1999. Architecture: cranial and axial musculoskeleton. In: McDiarmid RW, Altig R, editors. *Tadpoles: the Biology of Anuran Larvae*. Chicago: The University of Chicago Press. p. 52-91.
- Carvalho JRP, Assad ED. 2005. Análise espacial da precipitação pluviométrica no Estado de São Paulo: comparação de métodos de interpolação. *Eng Agr - Jaboticabal* 25:377-384.
- de Sousa VTT, Nomura F, Venesky MD, Rossa-Feres DC, Pezzuti TL, Andrade GV, Wassersug RJ. 2014. Flexible feeding kinematics of a tropical carnivorous anuran tadpole. *J Zool* 293:204-210.

- Duarte H, Tejedo M, Katzenberger M, Marangoni F, Baldo D, Beltrán JF, Martí DA, Richter-Boix A, Gonzalez-Voyer A. 2012. Can amphibians take the heat? Vulnerability to climate warming in subtropical and temperate larval amphibian communities. *Global Change Biol* 18:412–421.
- Easterling DR, Meehl GA, Parmesan C, Changnon SA, Karl TR, Mearns LO. 2000. Climate extremes: observations, modeling, and impacts. *Science* 289:2068-2074.
- Flecker AS, Feifarek BP, Taylor BW. 1999. Ecosystem engineering by a tropical tadpole: density-dependent effects on habitat structure and larval growth rates. *Copeia* 1999: 495-500.
- Frost DR. 2014. Amphibian Species of the World: an Online Reference. Version 6.0. Electronic Database accessible at <http://research.amnh.org/herpetology/amphibia/index.html>. American Museum of Natural History, New York, USA.
- Gosner KL. 1960 A simplified table for staging anuran embryos and larvae with note on identification. *Herpetologica* 16:183-190.
- Gradwell N. 1972. Gill irrigation in *Rana catesbeiana*. Part II. On the musculoskeletal mechanism. *Can J Zool* 50:501-521.
- Hernández LP. 2000. Intraspecific scaling of feeding mechanics in an ontogenetic series of zebrafish, *Danio rerio*. *J Exp Biol* 203:3033-3043.
- Herrel A, Van Wassenbergh S, Wouters S, Adriaens D, Aerts P. 2005. A functional morphological approach to the scaling of the feeding system in the African catfish, *Clarias gariepinus*. *J Exp Biol* 208:2091-2102.
- Hill AV. 1950. The dimensions of animals and their muscular dynamics. *Sci Prog* 38:209-230.

- Hoff KS, Blaustein AR, McDiarmid RW, Altig R. 1999. Behaviour – Interactions and their consequences. In: McDiarmid RW, Altig R, editors. Tadpoles: the biology of anuran larvae. Chicago: The University of Chicago Press. p. 215-239.
- Huey RB, Stevenson RD. 1979. Integrating thermal physiology and ecology of ectotherms: a discussion of approaches. *Am Zool* 19:357-366.
- [James](#) RS. 2013. A review of the thermal sensitivity of the mechanics of vertebrate skeletal muscle. *J Comp Physiol B* 183:723-733.
- Kupferberg S. 1997. Facilitation of peryphiton production by tadpole grazing: functional differences between species. *Freshwater Biol* 37:427-439.
- Larson PM, Reilly SM. 2003. Functional morphology of feeding and gill irrigation in the anuran tadpole: electromyography and muscle function in larval *Rana catesbeiana*. *J Morphol* 255:202-214.
- Liess A, Rowe O, Guo J, Thomsson G, Lind MI. 2013. Hot tadpoles from cold environments need more nutrients – Life history and stoichiometry reflects latitudinal adaptation. *J Anim Ecol* 82:1316–1325.
- Marian MP, Pandian TJ. 1985. Effect growth of temperature on development, and bioenergetics of the bullfrog tadpole *Rana tigrina*. *J Therm Biol* 10:157-161.
- Newman RA. 1989. Developmental plasticity of *Scaphiopus couchii* tadpoles in an unpredictable environment. *Ecology* 70:1775-1787.
- Niehaus AC, Angilletta MJ, Sears MW, Franklin CE, Wilson RS. 2011. Predicting the physiological performance of ectotherms in fluctuating thermal environments. *J Exp Biol* 215:694-701.
- Prado VHM, Fonseca MG, Almeida FVR, Necchi Jr O, Rossa-Feres DC. 2009. Niche occupancy and the relative role of micro-habitat and diet in resource partitioning among pond dwelling tadpoles. *South Am J Herpetol* 4:275-285.

- R Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org/>.
- Ranvestel AW, Lips KR, Pringle CM, Whiles MR, Bixby RJ. 2004. Neotropical tadpoles influence stream benthos: evidence for the ecological consequences of decline in amphibian populations. *Freshwater Biol* 49:274-285.
- Rasband WS. 2012. ImageJ. U. S. National Institutes of Health, Bethesda, Maryland, USA, <http://imagej.nih.gov/ij/>
- Reilly SM. 1995. The ontogeny of aquatic feeding behavior in *Salamandra salamandra*: stereotypy and isometry in feeding kinematics. *J Exp Biol* 198:701-708.
- Richard BA, Wainwright PC. 1995. Scaling the feeding mechanism of largemouth bass (*Micropterus salmoides*): kinematics of prey capture. *J Exp Biol* 198:419-433.
- Rossa-Feres DC, Jim J. 2001. Similaridade no sítio de vocalização em uma comunidade de anfíbios anuros na região noroeste do Estado de São Paulo, Brasil. *Rev Bras Zool* 18:439-454.
- Rossa-Feres DC, Jim J, Fonseca MG. 2004. Diets of tadpoles from a temporary pond in southeastern Brazil (Amphibia, Anura). *Rev Bras Zool* 21:745-754.
- Rossa-Feres DC, Nomura F. 2006. Characterization and taxonomic key for tadpoles (Amphibia: Anura) from the northwestern region of São Paulo State, Brazil. *Biota Neotrop* 6, <http://www.biotaneotropica.org.br/v6n1/pt/abstract?identification-key+bn00706012006>.
- Ryerson WG, Deban SM. 2010. Buccal pumping mechanics of *Xenopus laevis* tadpoles: effects of biotic and abiotic factors. *J Exp Biol* 213:2444-2452.
- SMA/IF (Secretaria do Meio Ambiente/Instituto Florestal). 2005. Inventário florestal da vegetação natural do Estado de São Paulo. São Paulo: Imprensa Oficial. 200 p.

- Ultsch GR, Bradford DF, Freda J. 1999. Physiology: coping with the environment. In: McDiarmid RW, Altig R, editors. Tadpoles: the biology of anuran larvae. Chicago: The University of Chicago Press. p. 189-214.
- Van Wassenbergh S, Herrel A, James RS, Aerts P. 2007. Scaling of the contractile properties of catfish feeding muscles. *J Exp Biol* 210:1183-1193.
- Venesky MD, Rossa-Feres DC, Nomura F, Andrade GV, Pezzuti TL, Sousa VTT, Anderson CV, Wassersug RJ. 2013. Comparative feeding kinematics of tropical hylid tadpoles. *J Exp Biol* 216:1928-1937.
- Venesky MD, Wassersug RJ, Jorgensen ME, Riddle M, Parris MJ. 2011. Comparative feeding kinematics of temperate pond-dwelling tadpoles (Anura, Amphibia). *Zoomorphology* 130:31-38.
- Venesky MD, Wassersug RJ, Parris MJ. 2010. How does a change in labial tooth row number affect feeding kinematics and foraging performance of a ranid tadpole (*Lithobates sphenoccephalus*)? *Biol Bull* 218: 160-168.
- Warkentin KM. 1992. Effects of temperature and illumination on feeding rates of Green Frog tadpoles (*Rana clamitans*). *Copeia* 1992:725-730.
- Wassersug RJ, Yamashita M. 2001. Plasticity and constraints on feeding kinematics in anuran larvae. *Comp Biochem Physiol A Mol Integr Physiol* 131:183-195.
- Wilbur HM. 1997. Experimental ecology of food webs: complex systems in temporary ponds. *Ecology* 78:2279-2302.
- Willmer P, Stone G, Johnston I. 2005. Environmental physiology of animals. Oxford: Blackwell Publishing. 768 p.
- Wilson RS, Franklin CE. 1999. Thermal acclimation of locomotor performance in tadpoles of the frog *Limnodynastes peronii*. *J Comp Physiol B* 169:445-451.

- Wood SLR, Richardson JS. 2010. Evidence for ecosystem engineering in a lentic habitat by tadpoles of the western toad. *Aquat Sci* 72:499-508.
- Wu H-J, Yen C-F, Kam Y-C. 2007. Metabolic compensation and behavioral thermoregulation of subtropical rhacophorid (*Polypedates megacephalus*) tadpoles in container habitats. *Comp Biochem Physiol B Biochem Mol Biol* 146:101–106.
- Zeileis A, Hothorn T. 2002. Diagnostic checking in regression relationships. *R News* 2:7-10. Available in <http://CRAN.R-project.org/doc/Rnews/>.
- Zweifel RG. 1964. Life history of *Phrynohyas venulosa* (Salientia: Hylidae) in Panamá. *Copeia* 1964:201-208.

Capítulo 3

Are the effect of oral morphology and ecology on tadpoles feeding behavior influenced by evolutionary history?

Verônica T. T. de Sousa, Tiago L. Pezzuti, Fabiane S. Annibale, Denise de C. Rossa-Feres, Gilda V. Andrade, Richard J. Wassersug, Matthew D. Venesky, John J. Wiens, and Fausto Nomura

ABSTRACT

The oral morphological variation in anuran tadpoles can be associated to species ecology, especially to the characteristics of the habitat and microhabitat where they occur, to the foraging position in the water column and to the type of food they feed on. Although correlations between tadpoles' oral morphology and ecology have been found, there also are studies showing that the morphological variation can also be attributed to evolutionary history. Our aim was to verify whether the ecological and morphological influences on feeding behavior are affected by the phylogenetic relationship among species. We assessed the feeding behavior of tadpoles of 22 species assigned to five anuran families while we conducted laboratory experiments in which tadpoles were filmed as they grazed on a food-covered planar substrate. From the video images, a series of standard feeding kinematic parameters were extracted. We confirm that ecology and morphology influence the way tadpoles feed, but we found they don't act on the same feeding kinematics variables. Ecological factors do not impact the same feeding kinematics that varies with morphological factors. Regarding the effect of oral morphology on kinematics, the evolutionary history only influences the relationship between one morphological variable (the labial tooth row formulae) and feeding kinematics. So it seems that the feeding behavior of tadpoles is very complex. The feeding behavior seems to be plastic enough to be adjusted accordingly to species ecology, but the variation in behavior that is linked to tadpoles morphology seems to be determined by evolutionary history.

Key words: tadpoles, feeding behavior, ecology, oral morphology, phylogeny.

INTRODUCTION

Feeding is one of the most conspicuous behaviors of tadpoles, as they need to gather a lot of energy in a short amount of time. Tadpoles that can grow to the largest size fast will avoid being captured by gape-limited predators (e.g. Richards & Bull, 1990). And, in the case they inhabit temporary habitats, they will likely avoid death by habitat desiccation if they reach the body size threshold they need to metamorphose earlier (e.g. Lawler, 1989). Tadpoles optimize growth by consuming a great variety of food items (e.g. algae, plant fragments, particulate organic debris, invertebrates, anuran eggs and tadpoles) (Vera Candioti, 2005; Giaretta & Facure, 2006; Echeverría et al., 2007; Sousa Filho et al., 2007; Wickramasinghe et al., 2007; Schiesari et al., 2009). They are considered opportunistic omnivorous (Altig, 2007; Schiesari et al., 2009) and the varied diet is possible mainly because many generalized tadpoles exhibit feeding behavior plasticity. Tadpoles can alternate between filter-feeding on suspended particles and scraping food off submerged surfaces, and can even exhibit some degree of macrophagy (Altig & Johnston, 1989; McDiarmid & Altig, 1999; Schiesari et al., 2009; de Sousa et al., 2014). Moreover, ecological factors such as microhabitat use (e.g. Rossa-Feres et al., 2004; Sousa Filho et al., 2007), time of occurrence (e.g. Peterson & Boulton, 1999; Echeverría et al., 2007) and intra- and interspecific interactions (e.g. Richter-Boix et al., 2004, 2007; Ledón-Rettig & Pfennig, 2012) also influence feeding and allow tadpoles to acquire food from a variety of sources.

Most exotrophic tadpoles have an oral apparatus comprised of keratinized structures (i.e. the anterior and posterior jaw sheaths, and the rows of labial teeth) surrounded by a soft tissue with a free, fringed margin (Wassersug, 1976; Altig, 2007). However, the variation in the oral apparatus configuration among species is noteworthy. A quick morphological examination of tadpoles of different species will reveal

differences in the width of the jaw sheaths keratinization, in the number of the anterior and posterior tooth rows, and in the arrangement of the papillae rows, among others. Therefore, the oral apparatus configuration is useful in the identification of species (e.g. Rossa-Feres & Nomura, 2006) and together with other morphological features (such as the eye position, and the shapes of the body, the tail and its fins), ecology and behavior of tadpoles allow researchers to assort tadpoles into ecomorphological guilds (Altig & Johnston, 1989).

Many herpetological studies showed that the oral morphological variation in anuran tadpoles can be associated to species ecology, especially to the characteristics of the habitat and microhabitat where they occur, to the foraging position in the water column and to the type of food they feed on (e.g. Orton, 1953; Altig & Johnston, 1989; Vera Candiotti, 2007; Van Buskirk, 2009). Although correlations between tadpoles' oral morphology and ecology have been found, there also are studies showing that the amount of variation associated to evolutionary history is actually higher than the one associated to ecology. Van Buskirk (2009) results suggest that morphology reflects mainly historical constraints, and that the phenotypes of species occurring along an environmental gradient may not be optimal for the habitat where they occur. Vera Candiotti (2007) suggested that oral morphological designs may be flexible enough not to require modifications linked to specific feeding habits. When it comes to feeding behavior, the correspondence between morphological and ecological variation does not always hold as species similar in their ecomorphology and/or similar in their oral configurations may exhibit different feeding kinematics (Venesky et al., 2011, 2013).

Besides the correlations between morphology and ecology and between morphology and phylogenetic relationships, a third scenario in which oral morphology cannot be fully explained by neither ecology nor evolutionary history is also plausible.

Studies pointing towards this direction have been developed considering an extensive set of phenotypic traits (instead of just oral morphology) such as the one developed by Richardson (2001), who found a weak phylogenetic signal in tadpoles' phenotypic traits, and suggested that multiple adaptive peaks within an environment may be occurring.

It is clear that, despite all the research on tadpoles' biology, we have a very limited understanding of how tadpoles' morphology, ecology, behavior and phylogeny relates explicitly to one another. Therefore, our aim was to verify whether the ecological and morphological influences on feeding behavior are affected by the phylogenetic relationship among species. We conducted laboratory experiments in which tadpoles were filmed as they grazed on a food-covered planar substrate. From the video images, a series of standard kinematic parameters were extracted. If phylogeny has a large influence on the way tadpoles feed, we would expect that species from the same family would be more similar in oral morphology and ecology than species from different lineages. This way, closely related species would exhibit similar feeding kinematics and oral shape. If the evolutionary history is not important, then distantly related species would exhibit similarity in feeding behavior due to a convergent adaptive process on morphology and, consequently, ecology.

MATERIAL AND METHODS

Study species

In the present study, we assessed the feeding behavior of tadpoles of 22 species assigned to five anuran families (Bufonidae, Hylidae, Hylodidae, Leptodactylidae and Odontophrynidae; Table 1). Species can be found in Brazilian Atlantic Forest and Cerrado Domains, with the exception of *Agalychnis lemur*, which occurs in humid

lowlands and montane primary forests of Costa Rica, Panama and Colombia. Species differ in their ecological and external oral morphological features (Table 1).

Collection and maintenance of tadpoles

Tadpoles of *A. lemur* were captive-born in 2010 from a colony at The Atlanta Botanical Garden, Atlanta, GA, USA, and were maintained at The University of Memphis, Memphis, TN, USA. Brazilian tadpoles were field collected from temporary and permanent ponds and streams located in São Paulo and Minas Gerais States, southeastern Brazil, from 2012 to 2015, during the rainy seasons, and were transported to a laboratory at the Universidade Estadual Paulista (IBILCE/UNESP, campus of São José do Rio Preto, São Paulo State, Brazil). Brazilian and *A. lemur* tadpoles were maintained in similar conditions: at 22°C on a natural photoperiod and acclimated to laboratorial conditions for at least 5 days before trials. Species were maintained separated from each other, i.e. each species was kept in a singular polyethylene aquarium (37 x 30 x 10 cm) filled with dechlorinated tap water, which was continuously aerated. They were fed *ad libitum* once a day with a mixture of flocculated (Alcon BASIC®, Alcon, Camboriú, Santa Catarina, Brazil) and powdered (Sera Micron®, SERA, Heinsberg, Germany) commercial fish food until the beginning of the experiments. Tadpoles were collected under a permit from the Brazilian Institute for Environment and Natural Renewable Resources (IBAMA) and Chico Mendes Institute for Biodiversity Conservation (ICMBio), Brazil (SISBIO no. 18163-1 to D.C.R.F.) and maintained with approval from the Ethics Committee on the use of Animals (CEUA-IBILCE/UNESP) in accordance with the National Council for Control of Animal Experimentation (CONCEA).

Feeding trials

Our feeding experiment followed the standard protocol developed by Wassersug & Yamashita (2001) and further refined by Venesky et al. (2011, 2013). Prior to each feeding trial, a mixture of Sera Micron® and water was brushed on one side of a glass microscope slide, which was allowed to air dry. The clean side of one food-covered slide was mounted against the inside wall of a glass container (8.5 x 8.5 x 8.5 cm). Containers were filled with aged dechlorinated tap water. Then, we prefocused a Fastec TroubleShooter LE 250 camera (Fastec Imaging, San Diego, California, USA) on the food-covered surface. The feeding behavior of tadpoles was video-recorded in individual trials while they grazed on this surface. As tadpoles feeding kinematics is influenced by the resistance they encounter (Wassersug and Yamashita 2001), all slides were made with the same concentration of Sera Micron® and a fresh food-covered slide was used in each trial to ensure that tadpoles had access to a substrate of similar density and food concentration. Video images were capture at 500 frames per second and the camera's vertical field of view was adjusted as necessary during recording.

In each trial, we recorded a continuous series of gape cycles (i.e. a single feeding bout), during which tadpoles scraped food from the slide. One gape cycle (1) starts with the jaw sheaths fully closed and the anterior and posterior tooth rows in closest proximity; (2) proceeds to the point where the mouth is fully open and the labial tooth rows reached maximum gape; and (3) ends with full closure of the jaw sheaths and anterior and posterior tooth rows again in closest proximity to each other (Venesky et al. 2011).

During the experiment, we filmed 3 to 6 tadpoles of each species (N = 97). After each feeding trial, we euthanized the tadpoles with an overdose of an anesthetic (2% lidocaine hydrochloride) and fixed them in a 1:1 solution mixture of 70% alcohol and

15% formalin. The developmental stage of the tadpoles was determined according to Gosner (1960) and the total length of each larvae was measured (species means and standard deviation are given in Table 1). All specimens were subsequently deposited in the amphibian collection of the Department of Zoology and Botany of IBILCE/UNESP (DZSJRP-Amphibia-Tadpoles).

Feeding kinematics

For each tadpole, we extracted data from 2 to 6 gape cycles (N = 446). We used ImageJ 1.47m (Rasband 2012) to obtain the following measurements: (i) Duration of the full gape cycle: the duration of time (in milliseconds) from when the jaws begin to open until they are fully closed; (ii) Percentage of time to maximum gape: the duration of time, as a percentage of the total time of the gape cycle, from when the mouth starts to open to when maximum gape is achieved; (iii) Maximum gape: the length (in millimeters) from the internal border of the upper jaw sheath to the internal border of the lower jaw sheath as a percentage of the upper jaw sheath width; (iv) Smallest width of the lower jaw sheath: the length (in millimeters) measured during closing phase at the smallest width of the lower jaw sheath while we can see the left and right extremes (right before the extremes start to hidden under the upper jaw); (v) Opening phase velocity: the displacement (in millimeters) of the jaws, from the initial position of full contact between them to the maximum gape position, divided by the time elapsed during opening phase; and (vi) Closing phase velocity: the displacement (in millimeters) of the jaws, from the initial position of maximum gape to the final position in which jaws were fully closed, divided by the time elapsed during closing phase. For statistical analyses, we calculated the species mean value for each one of the measured kinematics variable after averaged it for each tadpole.

Phylogeny

Although an extensive time-calibrated phylogeny is available for anuran species (Pyron & Wiens, 2013), it does not include all the 22 anuran species comprised by the present study. In order to obtain such a phylogeny, we combined the phylogenies from Pyron & Wiens (2013) and Veiga-Menoncello et al. (2014) studies after reduce them to include only the species that were common to our study.

We generated a time-calibrated tree containing the 22 anuran species included in the present study. We started by using the functions `extract.clade` and `drop.tip` of R software's `ape` package (Paradis et al., 2004) to reduce the phylogeny of Pyron & Wiens (2013) to 19 anuran species of which 16 were common to our study. We kept the branches of *Bokermannohyla martinsi*, *Scinax catharinae*, and *S. nasicus* and followed Wiens et al. (2010) to adjust their length to match those of *B. alvarengai*, *S. machadoi* and *S. similis*, respectively.

Because the *Pseudopaludicola* species (*P. atragula*, *P. mystacalis* and *P. ternetzi*) are lacking from Pyron & Wiens (2013) phylogeny and we couldn't find a time-calibrated tree that included these species, we estimated a time-calibrated phylogeny based on the phylogenetic analysis of the genera *Pseudopaludicola* performed by Veiga-Menoncello et al. (2014). We reduced the mtDNA sequences dataset compiled by the authors to include only the samples obtained from Leiuperinae species. The reduced data matrix included only one sample of each species. For the three *Pseudopaludicola* species included in our study, we kept the samples obtained from individuals collected in Icém, northwestern São Paulo State, as the tadpoles we collected for our experiments were obtained from this location. We used the Multiple Sequence Comparison by Log-Expectation (MUSCLE) algorithm (Edgar, 2004) to align the sequences.

We used the Bayesian phylogenetic analysis implemented in the BEAST 2.1.3 software (Bouckaert et al., 2014) to estimate the time-calibrated phylogeny of Leiuperinae species. We assumed a general time-reversible (GTR) model of nucleotide substitution (Rodríguez et al., 1990) with the among-site rate variation following a gamma (Γ) distribution with 4 rate categories for Γ (Yang, 1994), a proportion of invariant sites, and estimated base frequencies. Phylogeny was inferred under the uncorrelated relaxed clock model for lineage-specific substitution rate variation (Drummond et al., 2006). In order to estimate absolute node ages, we based on the time-calibrated tree provided by Pyron & Wiens (2013) to define three calibration points: (i) 60.2208 Myr as the age of the root of the tree, (ii) 49.2672 Myr as the divergence time between *Pleurodema brachyops* and the clade consisting of the species of the genera *Edalorhina*, *Engystomops*, and *Physalaemus*, and (iii) 43.2294 Myr as the divergence time between *Edalorhina perezii* and the clade consisting of *Engystomops*, and *Physalaemus* species. For each calibration point, we used a normal prior distribution and standard deviation of 1. The starting tree was generated under a Yule speciation prior. We performed four independent Markov chain Monte Carlo (MCMC) analysis (Drummond et al., 2002), each run for 10,000,000 generations (sampled every 1,000 generations). The first 10% of sampled generations of each MCMC analysis were discarded as burn-in based on the maximum clade credibility trees and mean node heights using TreeAnnotator 2.1.2 (Rambaut & Drummond, 2014). We verified the adequacy of sampling from the posterior probability distribution using Tracer 1.6.0 (Rambaut et al., 2014). Three of the MCMC analysis resulted in acceptable mixing and adequate effective sample sizes (ESS > 200) and were combined using LogCombiner 2.1.3 (Rambaut & Drummond, 2014). We used FigTree 1.4.2. (Rambaut, 2014) to view the results.

We used the `drop.tip` function of R software's `ape` package (Paradis et al., 2004) to delete tips from the generated Leiuperinae time-calibrated tree so it only included the three *Pseudopaludicola* species. Then we used the `bind.tree` function of R software's `ape` package (Paradis et al., 2004) to bind together the reduced trees and generate the final phylogeny (Figure 1).

Statistical analysis

We applied the phylogenetic Analysis of Variance (phylogenetic ANOVA) to verify whether species that differ in their ecology and external oral morphology exhibit different feeding kinematics. Ecological and morphological data were obtained from literature sources or unpublished data (Table 2). As ecological predictors of feeding kinematics, we used (i) tadpoles position in the water column, (ii) type of habitat where tadpoles occur, and (iii) hydroperiod of habitats where tadpoles occur. To check for the influence of external oral morphological features on kinematics, we used (i) labial tooth row formulae (LTRF), and (ii) oral disc position (anteroventral or ventral) as morphological predictors of feeding kinematics. We only used predictors for which data are available for all the species included in this study. We used the `aov.phylo` function of R software's `geiger` package (Harmon et al., 2008) to run the phylogenetic ANOVA. This function calculates the test statistic for the conventional ANOVA; then, for the phylogenetic ANOVA, it generates a null distribution of the test statistic by simulating new sets of the response variables on the phylogeny under a Brownian-motion model (Garland et al., 1993). Additionally, we applied Principal Components Analysis (PCA) to better visualize how feeding kinematics, species, and predictors are related.

RESULTS

The type of habitat where tadpoles occur also influence how tadpoles use their jaws during feeding (Table 4). Tadpoles that occur exclusively in swamps (i.e. *Pseudopaludicola* species) do not narrow their lower jaw, while tadpoles occurring exclusively in streams narrow their lower jaw sheaths only by 11% of their maximum widths (Table 3). Tadpoles occurring exclusively in ponds (i.e. *Trachycephalus typhoni*) can narrow their lower jaw sheaths by 25% of their maximum widths (Table 3). Tadpoles that can occur in more than one type of habitat (i.e. ponds and streams, or ponds and swamps) can narrow their jaw sheaths but only by 9% of the maximum width, whereas tadpoles that can occur in all habitats (i.e. ponds, streams, and swamps) can narrow their lower jaw sheath in 22% (Table 3). Hydroperiod did not influence feeding kinematics (Table 4). Species occupying different portions of the water column (i.e. mid-water vs. bottom) differed in the maximum gape they can attain and in the opening velocity of their mouths (Tables 3 and 4). Tadpoles that occur in mid-water displayed smaller maximum gapes and attained the maximum gape faster than tadpoles living near the bottom of the water bodies (Table 3). The results indicate that ecology do not exert a selective influence on feeding kinematics, i.e. evolutionary history does not explain differences in feeding kinematics due to species ecology (Table 4, Figures 2-4).

Concerning the morphological features, tadpoles having different number of tooth rows (i.e. different LTRFs) showed different feeding kinematics (Table 3). LTRF influenced both the duration of the gape cycle and the percentage of time tadpoles take to attain maximum gape (Table 5). While tadpoles with the LTRF of 2/2 showed shorter gape cycles, the duration of the gape cycle shown by tadpoles with the most common LTRF (2/3) varied largely, from less than 28ms up to almost 100ms (Table 3). Tadpoles with specialized oral morphology (i.e. with LTRF of 0/1, 1/2, 2/5, and 3/5) performed comparatively longer gape cycles, but tadpoles with a higher number of tooth rows did

not showed longer gape cycle (e.g. the duration of the gape cycle exhibited by *Dendropsophus minutus* (LTRF of 0/1 is similar to the one exhibited by *Bokermannohyla alvarengai* (LTRF of 2/5)) (Table 3). Results for the phylogenetic ANOVA were significant (Table 5, Figure 5). In general, non-hylids species showed shorter gape cycles (except for tadpoles of *Leptodactylus labyrinthicus* and *Odontophrynus carvalhoi*), while Hylidae species showed longer cycles. Oral disc position did not have a significant effect on feeding kinematics (Table 3, Figure 6).

DISCUSSION

We confirm that ecology and morphology influence the way tadpoles feed, but we found they don't act on the same feeding kinematics variables. Two ecological factors don't impact the same kinematics variable. The same way, two different morphological variables don't impact the same feeding kinematics variables. The evolutionary history only influences the relationship between one morphological variable (the LTRF) and both duration of the gape cycle and the time (relative to the total duration of the cycle) tadpoles take to achieve maximum gape.

It is known that the amplitude of tadpoles' maximum gapes and the velocity of the opening phase of the gape cycle vary with the feeding behavior (de Sousa et al., 2014), the substrate tadpoles graze on (Wassersug & Yamashita, 2001), and the habitat conditions tadpoles experience (de Sousa et al., 2015). We found that tadpoles occurring in mid-water (i.e. nektonic tadpoles) display smaller maximum gapes and attain the maximum gapes faster than tadpoles occurring at the bottom of the water bodies (i.e. benthic tadpoles). Because, in the present study, data were collected while tadpoles were scrape-feeding on a standard algal-covered surface, the results found cannot be attributed to the use of different feeding strategies (i.e. filter-feeding versus scrape-

feeding), but results may indicate that nektonic tadpoles may scrape food off soft substrates. Generally, tadpoles grazing on high resistant substrates are able to achieve larger maximum gapes but later in the gape cycle (e.g. Wassersug & Yamashita, 2001; Venesky et al., 2013), and this might be the case of tadpoles that feed at the bottom of water bodies. Alternatively, because tadpoles occupying different positions in the water column may experience different ranges of temperature, it's plausible that such differences reflect temperature differences instead of grazing on substrates of different resistance.

Johnston (1982) and Taylor et al. (1996) have already speculated about the flexibility of tadpoles jaws and this was later confirmed by Wassersug & Yamashita (2001), who demonstrated that tadpoles were able to protract and retract, and also narrow and widen, their jaws during a gape cycle. They suggested that the narrowing allow tadpoles to better focus their bite force over a smaller area when grazing on high frictional surfaces. Furthermore, Johnston (1982) suggested that species would differ in the magnitude the tadpoles can narrow their lower jaws. Indeed, among species differences were detected in previous studies (e.g. Venesky et al., 2011), but our results indicate that the phylogenetic relationships among species do not explain these differences. We found that species occupying different types of habitat differ in the way they move their lower jaw sheath during the closing phase of the gape cycle. In one extreme, there are the tadpoles that occur exclusively in swamps and do not narrow their lower jaws. In the other, tadpoles living in ponds that are able to narrow their lower jaws up to 25% of the maximum gape. The magnitude to which tadpoles narrow their lower jaw sheaths depend on the habitat probably due to structural differences among habitats and, consequently, to the availability of microhabitats in each habitat (Eterovick & Barata, 2006), but these results might also reflect differences in the species

preferences for scraping food from a specific substrate as many of the studied species can be found in more than one type of habitat. To our knowledge, data on which type of substrate tadpoles prefer to forage is not available. So we cannot state that the differences we found reflect adaptations to scrape soft versus resistant substrates. Tadpoles may graze on different substrates as they occur in distinct microhabitats (e.g. Altig & Jonhston, 1989; Prado et al., 2009; Marques & Nomura, 2015). For instance, nektonic tadpoles occur at the mid-water and scrape food from macrophytes and other plants (e.g. *Scinax fuscovarius*), while benthic tadpoles occur in the bottom of the water bodies and scrape food from surfaces at the bottom (e.g. *S. machadoi*). However, tadpoles of some species alternate their position between microhabitats. Tadpoles of *Rhinella schneideri* ingest mainly planktonic algae; so, even though they show primarily benthic habits, they are able to move along the water column and use the resources available in it (Rossa-Feres et al., 2004).

The effect of the LTRF on feeding kinematics was demonstrated in previous studies (e.g. Venesky et al., 2010, 2013; de Sousa et al., 2015): tadpoles with more labial tooth rows consistently displayed longer gape cycles. However, our results do not corroborate the positive relationship between the LTRF and the total duration of the gape cycle. The duration of the gape cycles performed by *D. minutus* (LTRF of 0/1) and *B. alvarengai* (LTRF of 2/5), for instance, was very similar (107 and 109 ms, respectively). The same pattern was found for the percentage of time tadpoles take to achieve the maximum gape: *D. minutus*, *Leptodactylus labyrinthicus* (LTRF of 1/2), and *Phyllomedusa ayeaye* (LTRF of 2/3) spent more than half of the total duration of their gape cycles opening their mouths, while *B. alvarengai* spent just 25% of the duration of their gape cycle doing the same. Besides, tadpoles with the same number of tooth rows frequently display different feeding kinematics (e.g. Venesky et al., 2013; present

study). So, we found no evidence that tadpoles that have more tooth rows would require more time to complete a gape cycle and to achieve the maximum gape when grazing on substrates. This may be explained by the way tadpoles disengage the tooth rows from the substrate. Some species released their lower tooth rows from the substrate in a serial fashion (Wassersug and Yamashita, 2001; Venesky et al., 2010), while others may disengage the rows at the same time (Venesky et al., 2013). We did not measure the time tadpoles released each tooth rows but, if species with more posterior tooth rows disengage them concurrently, they may display gape cycles of the same duration as tadpoles with less lower tooth rows.

Although we could not confirm that the relationship between feeding kinematics and the dental formulae is positive, the LRTF explained great part of the variation found in the feeding kinematics variables. This may be due to species ecology. Differences in the duration of the gape cycle may occur because of differences in the resistance of the substrates from where tadpoles obtain food through scrape feeding. Wassersug & Yamashita (2001) showed that the gape cycle last longer when the jaws meet little resistance. In our study, tadpoles that displayed shorter gape cycles have benthic habits, and graze on the algal-covered substrates at the bottom of the water bodies. The same results were found by Venesky et al. (2011, 2013). The variation in the distribution of tadpoles among habitats and microhabitats may influence the feeding kinematics (e.g. Venesky et al., 2013; de Sousa et al., 2015), but it does not explain all our findings. For instance, the similarity between *D. minutus* and *B. alvarengai* regarding the duration of their gape cycle cannot be explained by the use of habitats and microhabitats by species: tadpoles of *D. minutus* inhabit ponds and swamps and are nektonic, while *B. alvarengai* tadpoles are found only in lotic environments and are benthic. *Dendropsophus minutus* and *B. alvarengai* are two species that belong to the Hylidae anuran family and our

analysis showed that the relationship between LTRF and both the duration of the gape cycle and the percentage of time to reach the maximum gape are significant in a phylogenetic context.

The number of labial tooth rows varies greatly among species, ranging from 0 to 37 rows (Altig and McDiarmid, 1999; Faivovich et al., 2013), but the LTRF is consistent at the generic level and, therefore, phylogenetically informative (Altig and McDiarmid, 1999; Haas, 2003). Our results indicate that the differences in the duration of the gape cycle and the time to maximum gape are due to differences occurring between two major clades: one composed by hylids and the other by non-hylids (tadpoles of the Bufonidae, Leptodactylidae, Hylodidae, and Odontophrynidae families). Hylids showed gape cycles of longer duration than tadpoles belonging to other families. Regarding the percentage of time tadpoles take to attain the maximum gape (relative to the total duration of the gape cycle), the Hylidae clade can be divided into species that attained the maximum gape by 25 to 35% of the total duration of the gape cycle, and species that achieved the maximum gape after the cycle has reached half of its total duration. Non-hylids values fell between these two hylid groups. However, although evolutionary history may have constrained the feeding behavior of tadpoles of these two groups, close related species not always show the same feeding kinematics. For instance, the duration of the gape cycle displayed by *Odontophrynus americanus* is more similar to the duration of *P. ayeaye* gape cycles than to *O. carvalhoi*. It's possible that limiting factors, such as the resources available at a habitat and the species niche similarity (MacArthur & Levins, 1967; Pianka, 1974), are leading to differences in feeding behavior, which would allow tadpoles to explore distinct parts of habitat or different food resources.

The feeding behavior seems to be plastic enough to be adjusted accordingly to species ecology, but the variation in behavior that is linked to tadpoles morphology seems to be determined by evolutionary history. Correlations between tadpoles' oral morphology and the kind of habitat they live have long been noted (e.g. Orton, 1953; Altig & Johnston, 1989; Vera Candiotti, 2007). But few studies demonstrated intra- or interspecific variation in microhabitat use (e.g. Warkentin, 1992; Eterovick & Barata, 2003, 2006; Prado et al., 2009). This kind of research will produce data that will allow researchers to better categorize the available microhabitats into retreat, resting, and feeding areas. To know where tadpoles prefer to feed, which kind of substrate they scrape food from, and whether they show behavioral plasticity (i.e. they are able to alternate between microhabitats) is fundamental for understanding the biomechanics of their oral apparatus. Additionally, we only included a small proportion of anuran species in the present study, and only included one species for some of the clades. Regarding the oral morphological, the variation found in nature is greater than the one present here. So, in order for us to understand better the results found, a more comprehensive study is needed.

ACKNOWLEDGEMENTS

We thank Carlos E. Sousa for assistance during field work. This research was supported by a joint CNPq (grant 563075/2010-4) and FAPESP project (grant 2010/52321-7) on Brazilian tadpoles biology. R.J.W.'s research is supported by the National Science and Engineering Research Council of Canada. D.C.R.F. and G.V.A. thank the research fellowships provided by CNPq (303522/2013-5 and 309479/2012-6). V.T.T.S. received fellowships from Bolsa REUNI de Assistência ao Ensino, and from CAPES that

supported her visit to Arizona to work on this project with JJW. T.L.P. received a fellowship from FAPEMIG/Vale project (Process nº 17237).

TABLES

Table 1. Mean \pm standard deviation (SD) of the total length (mm) and Gosner developmental stage of each of the 22 anuran species included in the present study.

Species	Total Length (mm)	Gosner Stage
<i>Hypsiboas raniceps</i>	59.25 \pm 5.28	36 \pm 1
<i>Hypsiboas albopunctatus</i>	38.96 \pm 2.22	31 \pm 0
<i>Bokermannohyla alvarengai</i>	46.97 \pm 2.51	36 \pm 2
<i>Scinax machadoi</i>	28.88 \pm 2.85	31 \pm 1
<i>Scinax fuscovarius</i>	44.66 \pm 1.42	38 \pm 1
<i>Scinax similis</i>	32.66 \pm 3.25	35 \pm 2
<i>Dendropsophus minutus</i>	28.20 \pm 1.44	32 \pm 1
<i>Trachycephalus typhonius</i>	37.16 \pm 2.17	36 \pm 1
<i>Agalychnis lemur</i>	32.25 \pm 1.96	34 \pm 1
<i>Phyllomedusa ayeaye</i>	25.95 \pm 3.76	26 \pm 1
<i>Odontophrynus carvalhoi</i>	46.91 \pm 2.31	47 \pm 2
<i>Odontophrynus americanus</i>	30.69 \pm 2.28	31 \pm 1
<i>Crossodactylus caramaschii</i>	33.44 \pm 3.38	28 \pm 1
<i>Rhinella schneideri</i>	23.19 \pm 3.17	35 \pm 1
<i>Leptodactylus labyrinthicus</i>	61.90 \pm 4.11	39 \pm 0
<i>Leptodactylus fuscus</i>	25.21 \pm 0.80	34 \pm 1
<i>Leptodactylus podicipinus</i>	30.83 \pm 1.43	37 \pm 0
<i>Physalaemus nattereri</i>	18.19 \pm 1.22	31 \pm 3
<i>Physalaemus cuvieri</i>	19.86 \pm 2.33	32 \pm 2
<i>Pseudopaludicola ternetzi</i>	23.40 \pm 2.76	33 \pm 2
<i>Pseudopaludicola atragula</i>	19.34 \pm 2.79	32 \pm 3
<i>Pseudopaludicola mystacalis</i>	18.03 \pm 2.68	32 \pm 4

Table 2. Anuran species included in the present study and their ecological and external oral morphological features. PWC: Position in the Water Column; LTRF: Labial Tooth Row Formulae; ODP: Oral Disc Position.

Species	Family	Habitat	Hydroperiod	PWC	LTRF	ODP
<i>Hypsiboas raniceps</i>	Hylidae	Lentic/Swamps Lotic/Lentic/Swamp	Permanent/Temporary	Bottom	2/3	Ventral
<i>Hypsiboas albopunctatus</i>	Hylidae	s	Permanent/Temporary	Bottom	2/3	Ventral
<i>Bokermannohyla alvarengai</i>	Hylidae	Lotic	Permanent/Temporary	Bottom	2/5	Anteroventral
<i>Scinax machadoi</i>	Hylidae	Lotic	Permanent	Bottom	2/3	Ventral
<i>Scinax fuscovarius</i>	Hylidae	Lentic/Swamps	Permanent/Temporary	Mid-water	2/3	Anteroventral
<i>Scinax similis</i>	Hylidae	Lentic/Swamps	Permanent/Temporary	Mid-water	2/3	Anteroventral
<i>Dendropsophus minutus</i>	Hylidae	Lentic/Swamps	Permanent/Temporary	Mid-water	0/1	Anteroventral
<i>Trachycephalus typhonius</i>	Hylidae	Lentic	Temporary	Mid-water	3/5	Anteroventral
<i>Agalychnis lemur</i>	Hylidae	Lentic/Lotic	Permanent/Temporary	Mid-water	2/3	Anteroventral
<i>Phyllomedusa ayeaye</i>	Hylidae	Lentic/Lotic	Permanent/Temporary	Mid-water	2/3	Anteroventral
<i>Odontophrynus carvalhoi</i>	Odontophrynidae	Lentic/Lotic	Temporary	Bottom	2/3	Ventral
<i>Odontophrynus americanus</i>	Odontophrynidae	Lotic/Lentic/Swamp	Temporary	Bottom	2/3	Ventral
<i>Crossodactylus caramaschii</i>	Hylodidae	s Lotic	Permanent	Bottom	2/3	Ventral
<i>Rhinella schneideri</i>	Bufonidae	Lentic/Swamps	Permanent/Temporary	Bottom	2/3	Anteroventral
<i>Leptodactylus labyrinthicus</i>	Leptodactylidae	Lentic/Swamps	Permanent/Temporary	Bottom	1/2	Anteroventral
<i>Leptodactylus fuscus</i>	Leptodactylidae	Lentic/Swamps	Permanent/Temporary	Bottom	2/3	Anteroventral
<i>Leptodactylus podicipinus</i>	Leptodactylidae	Lentic/Swamps	Permanent/Temporary	Bottom	2/3	Anteroventral
<i>Physalaemus nattereri</i>	Leptodactylidae	Lentic/Swamps	Permanent/Temporary	Bottom	2/3	Ventral
<i>Physalaemus cuvieri</i>	Leptodactylidae	Lentic/Swamps	Permanent/Temporary	Bottom	2/3	Ventral
<i>Pseudopaludicola ternetzi</i>	Leptodactylidae	Swamps	Temporary	Bottom	2/2	Ventral

<i>Pseudopaludicola atragula</i>	Leptodactylidae	Swamps	Temporary	Bottom	2/3	Ventral
<i>Pseudopaludicola mystacalis</i>	Leptodactylidae	Swamps	Permanent/Temporary	Bottom	2/2	Ventral

Table 3. Mean of the feeding kinematics measured for tadpoles of 22 anuran species. Maximum gape was measured as a percentage of the upper jaw sheath width (UJW). The smallest lower jaw width (LJW) was measured as a percentage of the lower jaw sheath width at maximum gape. GC: Gape Cycle, MG: Maximum Gape, LJW: Lower jaw width.

Species	Duration of GC (ms)	% Time to MG	Maximum Gape (mm)	Smallest LJW (mm)	Opening velocity (mm/s)	Closing velocity (mm/s)
<i>Hypsiboas</i>	90.18	34.04	92.76	98.37	46.27	23.70
<i>raniceps</i>						
<i>Hypsiboas</i>	75.00	30.34	136.10	71.48	56.07	24.34
<i>albopunctatus</i>						
<i>Bokermannohyla</i>	109.83	25.77	125.61	84.81	69.81	23.36
<i>alvarengai</i>						
<i>Scinax machadoi</i>	60.82	29.73	110.64	81.64	89.74	37.53
<i>Scinax fuscovarius</i>	54.20	34.80	135.96	98.81	85.89	47.02
<i>Scinax similis</i>	53.30	30.65	104.83	97.01	66.38	29.18
<i>Dendropsophus</i>	107.16	55.59	76.21	100.00	9.77	12.55
<i>minutus</i>						
<i>Trachycephalus</i>	60.80	49.30	119.31	74.52	69.91	67.70
<i>typhonius</i>						

<i>Agalychnis lemur</i>	95.33	44.98	104.59	100.00	63.99	53.99
<i>Phyllomedusa</i>	50.43	54.03	129.07	83.68	47.19	52.91
<i>ayeaye</i>						
<i>Odontophrynus</i>	70.00	41.57	142.96	89.41	108.80	68.38
<i>carvalhoi</i>						
<i>Odontophrynus</i>	50.29	37.67	161.23	83.96	119.44	70.47
<i>americanos</i>						
<i>Crossodactylus</i>	31.11	40.46	184.69	100.00	150.57	101.66
<i>caramaschii</i>						
<i>Rhinella</i>	53.25	42.80	135.67	80.94	88.11	65.10
<i>schneideri</i>						
<i>Leptodactylus</i>	72.50	54.55	128.01	87.68	66.81	78.71
<i>labyrinthicus</i>						
<i>Leptodactylus</i>	38.00	37.85	153.98	86.18	97.73	58.06
<i>fuscus</i>						
<i>Leptodactylus</i>	40.50	36.74	169.37	83.75	112.87	61.26
<i>podicipinus</i>						
<i>Physalaemus</i>	38.48	38.86	137.67	84.25	69.96	41.55
<i>nattereri</i>						
<i>Physalaemus</i>	27.60	35.68	123.68	91.37	77.89	41.42

<i>cuvieri</i> <i>Pseudopaludicola</i>	30.38	45.30	121.03	100.00	58.48	47.59
<i>ternetzi</i> <i>Pseudopaludicola</i>	35.42	49.05	166.03	100.00	58.97	56.34
<i>atragula</i> <i>Pseudopaludicola</i>	26.17	39.08	116.37	100.00	85.88	54.52
<i>mystacalis</i>						

Table 4. Results of the Analysis of variance (ANOVA) and phylogenetic ANOVA in which the species ecology influence on feeding kinematics was evaluated. Significant effects are in bold.

Feeding kinematics variables	R²	Adj R²	Pr (>F)	Pr (phy)
Water Column Position				
Duration of the gape cycle	0.095	0.050	0.162	0.277
% Time to maximum gape	0.113	0.069	0.126	0.263
Maximum gape	0.213	0.173	0.031	0.090
Smallest lower jaw width	0.028	-0.020	0.454	0.583
Opening phase velocity	0.181	0.141	0.048	0.120
Closing phase velocity	0.042	-0.005	0.258	0.486
Habitat				
Duration of the gape cycle	0.023	-0.005	0.461	0.656
% Time to maximum gape	0.373	0.180	0.150	0.310
Maximum gape	0.100	-0.181	0.870	0.936
Smallest lower jaw width	0.495	0.337	0.037	0.140
Opening phase velocity	0.157	-0.106	0.702	0.847
Closing phase velocity	0.084	-0.203	0.910	0.954
Hydroperiod				
Duration of the gape cycle	0.068	-0.030	0.510	0.595
% Time to maximum gape	0.102	0.008	0.359	0.464
Maximum gape	0.126	0.034	0.278	0.394
Smallest lower jaw width	0.001	-0.104	0.988	0.994
Opening phase velocity	0.257	0.189	0.060	0.124
Closing phase velocity	0.209	0.126	0.108	0.166

Table 5. Results of the Analysis of variance (ANOVA) and phylogenetic ANOVA in which the species morphology influence on feeding kinematics was evaluated. Significant effects are in bold.

Feeding kinematics variables	R²	Adj R²	Pr (>F)	Pr (phy)
Labial Tooth Row Formulae				
Duration of the gape cycle	0.549	0.407	0.017	0.030
% Time to maximum gape	0.527	0.380	0.023	0.039
Maximum gape	0.285	0.062	0.321	0.427
Smallest lower jaw width	0.334	0.125	0.216	0.289
Opening phase velocity	0.299	0.080	0.288	0.365
Closing phase velocity	0.357	0.156	0.175	0.230
Oral disc position				
Duration of the gape cycle	0.136	0.092	0.092	0.203
% Time to maximum gape	0.063	0.016	0.259	0.381
Maximum gape	0.039	-0.008	0.376	0.484
Smallest lower jaw width	0.014	-0.035	0.600	0.069
Opening phase velocity	0.051	0.003	0.312	0.415
Closing phase velocity	0.001	-0.048	0.863	0.887

FIGURES

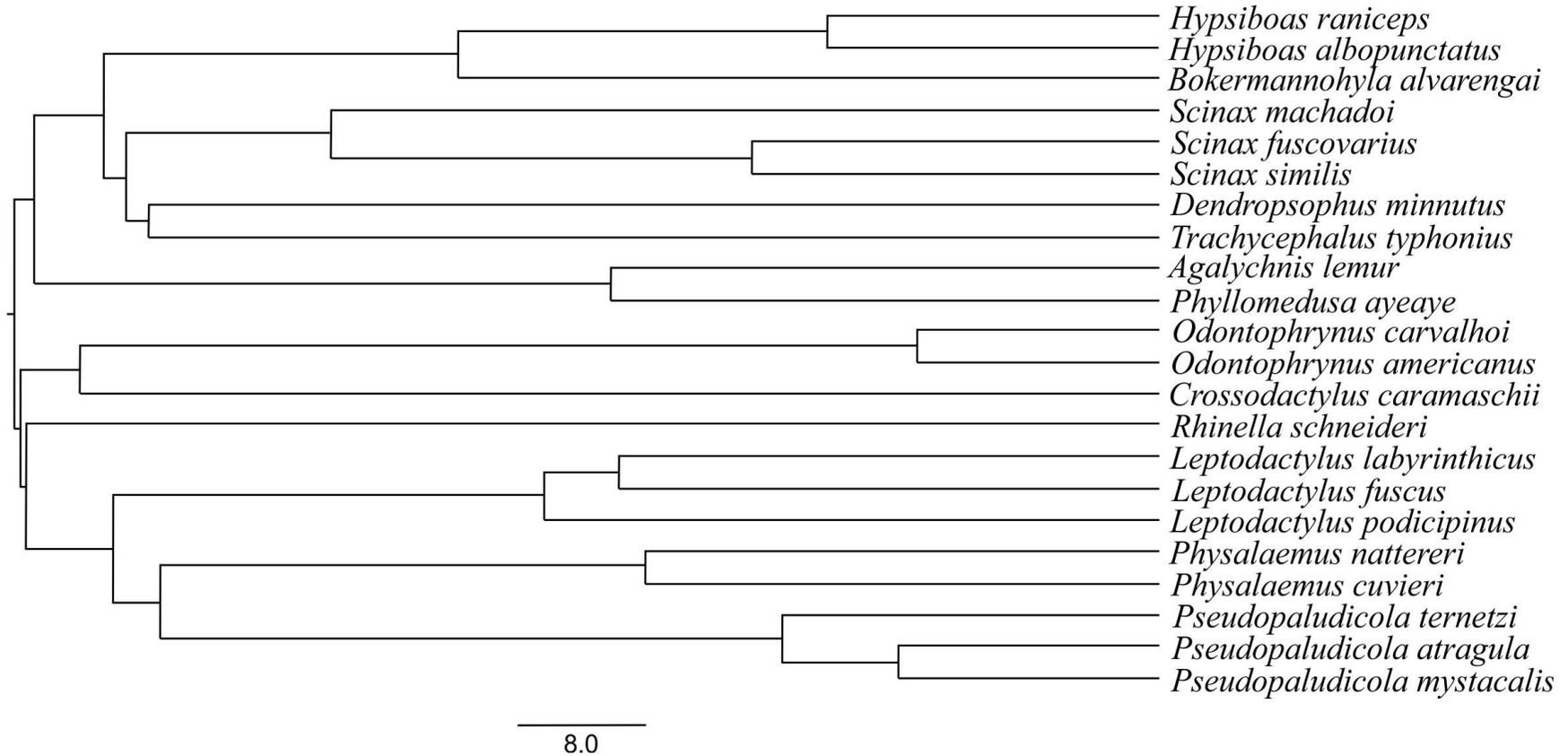


Figure 1. Phylogeny including the 22 anuran species whose tadpoles feeding behavior was evaluated in the present study.

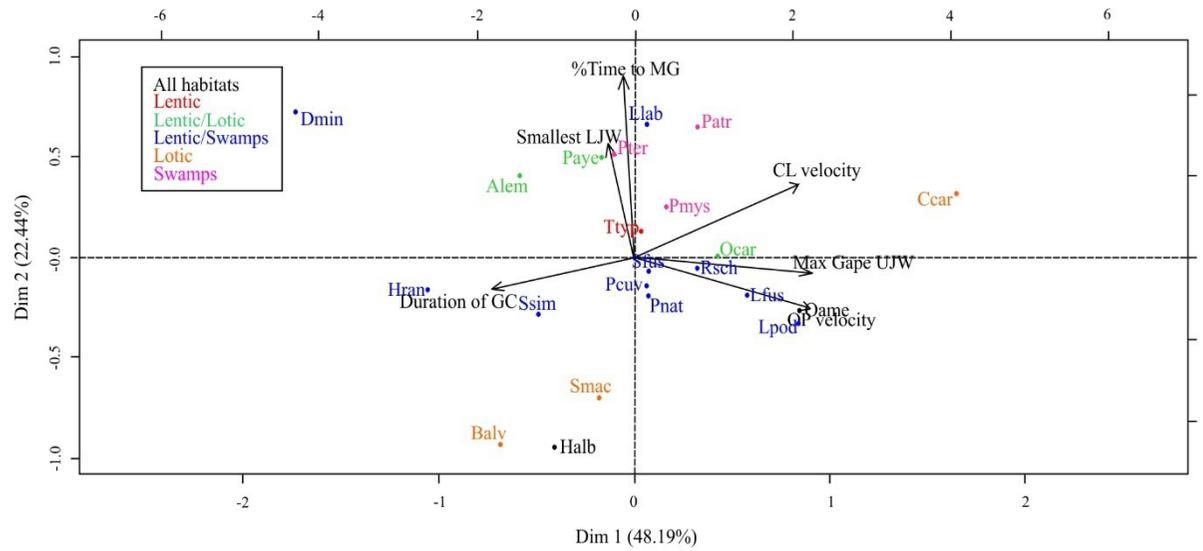
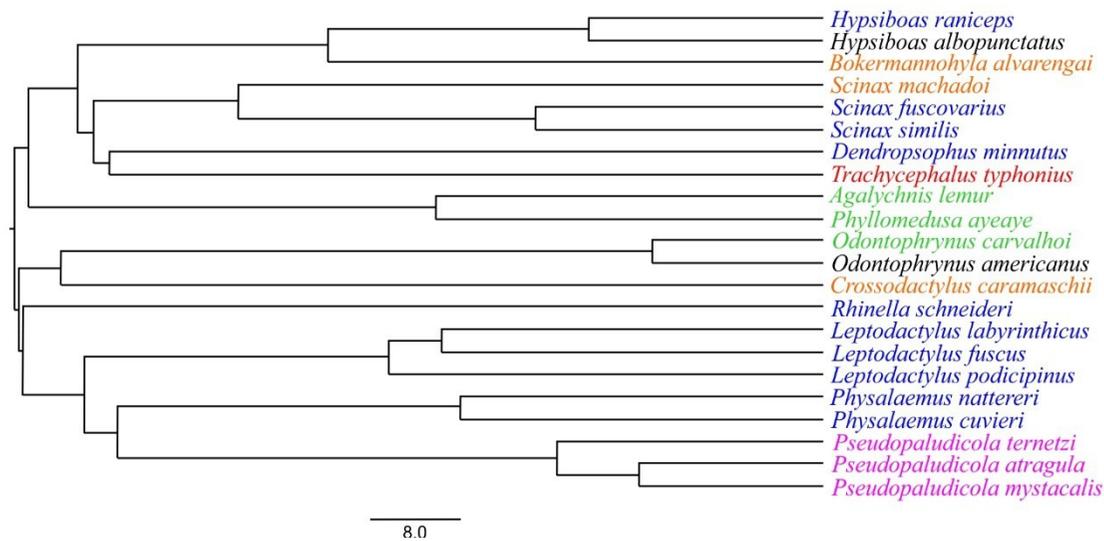


Figure 2. Principal component analysis result for feeding kinematics variables plotted against the first two principal components axes, and the phylogenetic tree. Colors indicate in which type of habitat species occur. Hran: *H. raniceps*, Halb: *H. albopunctatus*, Balv: *B. alvarengai*, Smac: *S. machadoi*, Sfus: *S. fuscovarius*, Ssim: *S. similis*, Dmin: *D. minutus*, Ttyp: *T. typhonius*, Alem: *A. lemur*, Paye: *P. ayeaye*, Ocar: *O. carvalhoi*, Oame: *O. americanus*, Ccar: *C. caramaschii*, Rsch: *R. schneideri*, Llab: *L. labyrinthicus*, Lfus: *L. fuscus*, Lpod: *L. podicipinus*, Pnat: *P. nattereri*, Pcuv: *P. cuvieri*, Pter: *P. ternetzi*, Patr: *P. atragula*, Pmys: *P. mystacalis*.



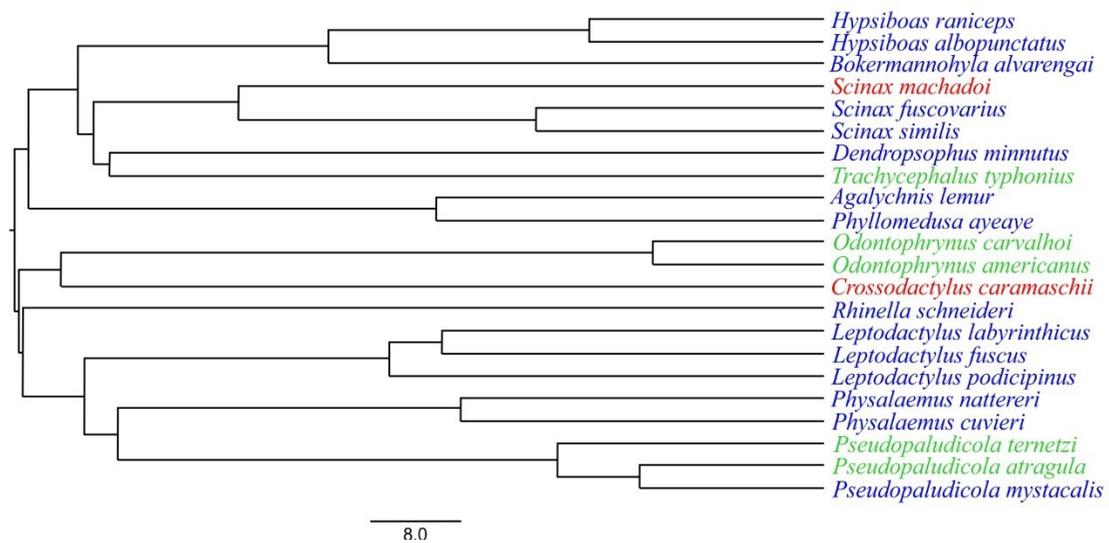
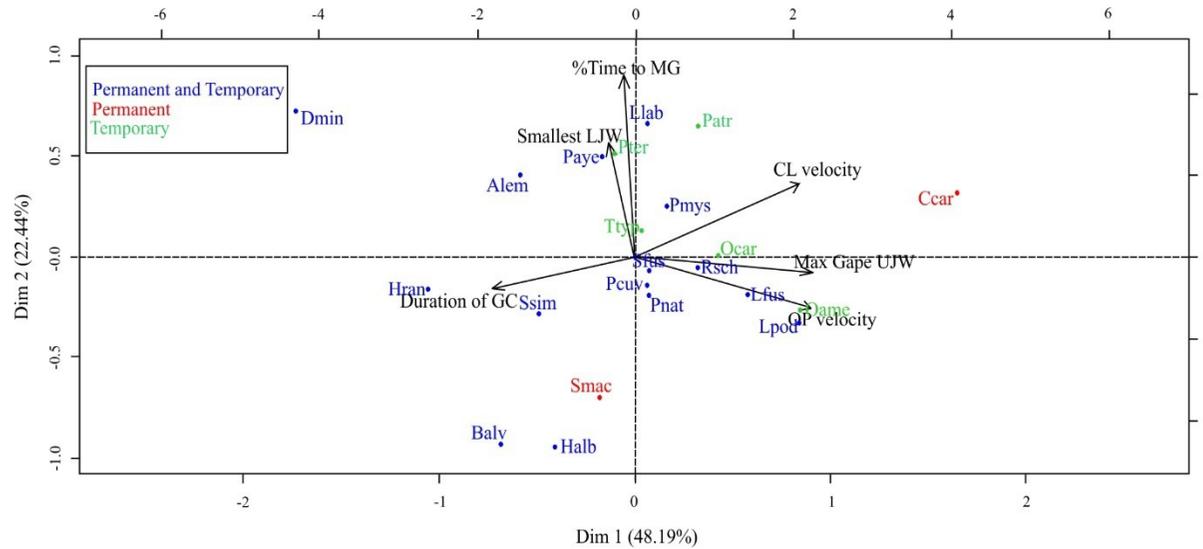


Figure 3. Principal component analysis result for feeding kinematics variables plotted against the first two principal components axes, and the phylogenetic tree. Colors indicate the hydroperiod of the habitats where species occur. Hran: *H. raniceps*, Halb: *H. albopunctatus*, Balv: *B. alvarengai*, Smac: *S. machadoi*, Sfus: *S. fuscovarius*, Ssim: *S. similis*, Dmin: *D. minutus*, Ttyp: *T. typhonius*, Alem: *A. lemur*, Paye: *P. ayeaye*, Ocar: *O. carvalhoi*, Oame: *O. americanus*, Ccar: *C. caramaschii*, Rsch: *R. schneideri*, Llab: *L. labyrinthicus*, Lfus: *L. fuscus*, Lpod: *L. podicipinus*, Pnat: *P. nattereri*, Pcu: *P. cuvieri*, Pter: *P. ternetzi*, Patr: *P. atragula*, Pmys: *P. mystacalis*.

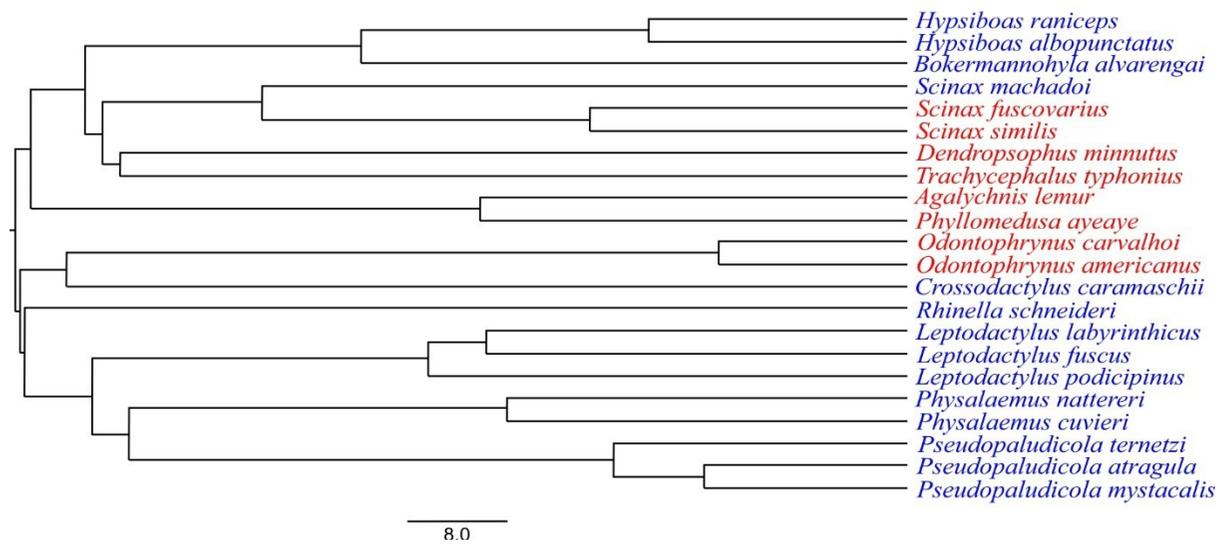
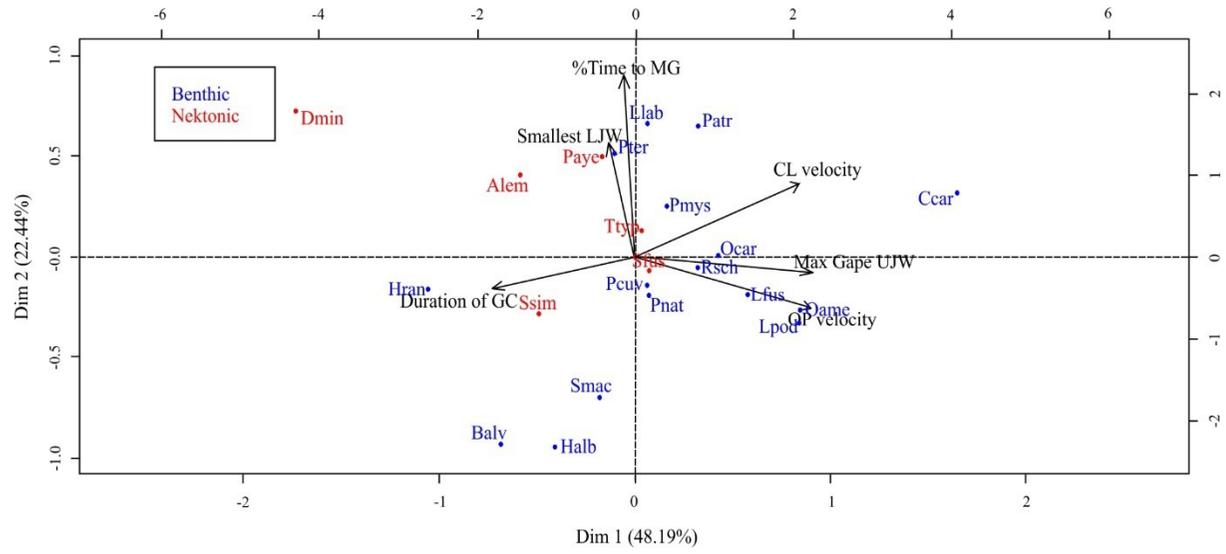


Figure 4. Principal component analysis result for feeding kinematics variables plotted against the first two principal components axes, and the phylogenetic tree. Colors indicate tadpole position at the water column. Hran: *H. raniceps*, Halb: *H. albopunctatus*, Balv: *B. alvarengai*, Smac: *S. machadoi*, Sfus: *S. fuscovarius*, Ssim: *S. similis*, Dmin: *D. minutus*, Ttyp: *T. typhonius*, Alem: *A. lemur*, Paye: *P. ayeaye*, Ocar: *O. carvalhoi*, Oame: *O. americanus*, Ccar: *C. caramaschii*, Rsch: *R. schneideri*, Llab: *L. labyrinthicus*, Lfus: *L. fuscus*, Lpod: *L. podicipinus*, Pnat: *P. nattereri*, Pcuv: *P. cuvieri*, Pter: *P. ternetzi*, Patr: *P. atragula*, Pmys: *P. mystacalis*.

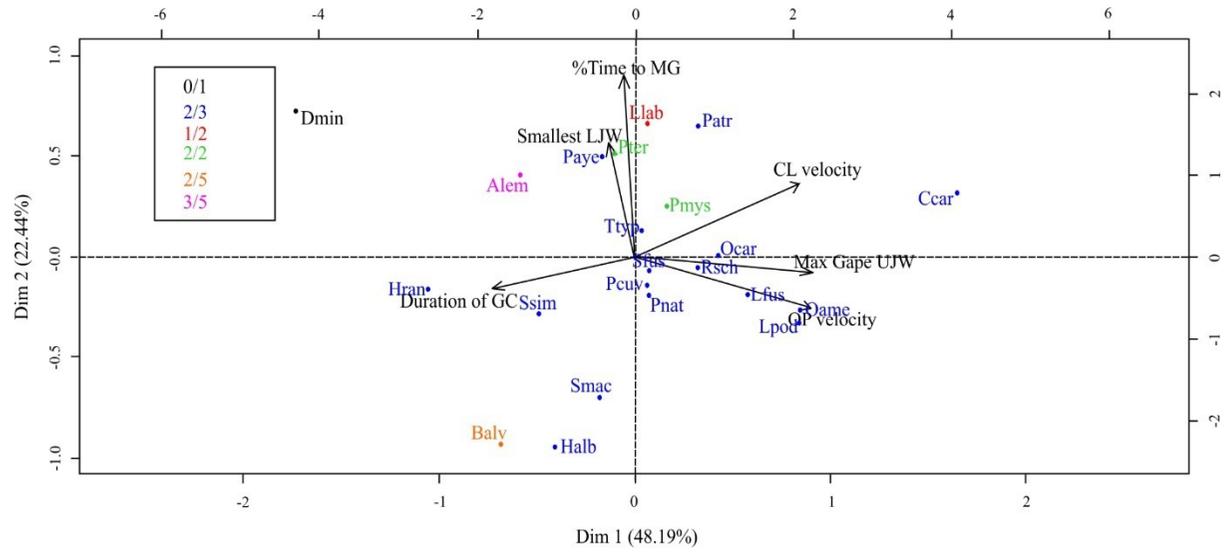
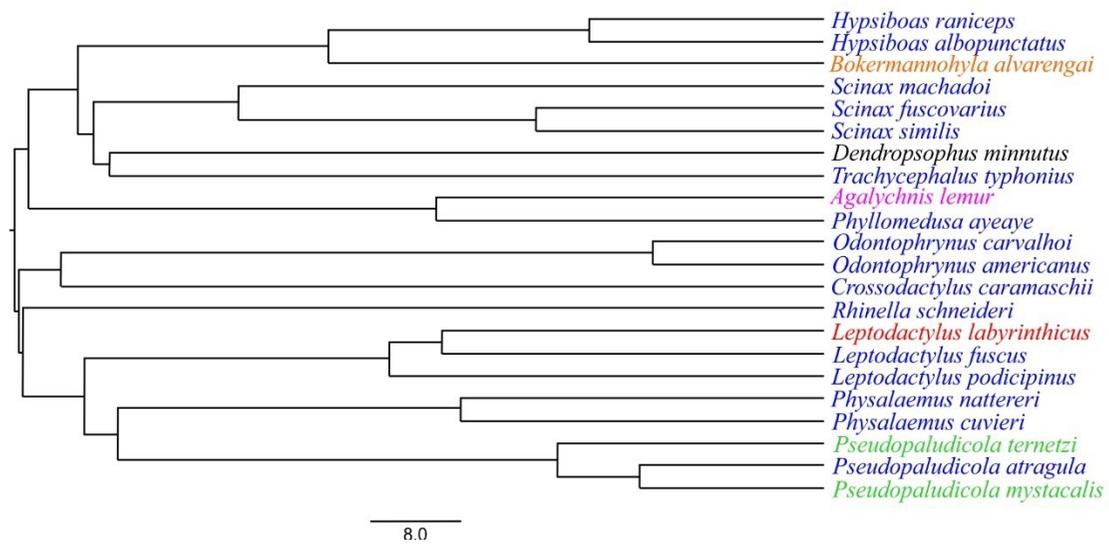


Figure 5. Principal component analysis result for feeding kinematics variables plotted against the first two principal components axes, and the phylogenetic tree. Colors indicate tadpoles labial tooth row formulae (LTRF). Hran: *H. raniceps*, Halb: *H. albopunctatus*, Balv: *B. alvarengai*, Smac: *S. machadoi*, Sfus: *S. fuscovarius*, Ssim: *S. similis*, Dmin: *D. minutus*, Ttyp: *T. typhonius*, Alem: *A. lemur*, Paye: *P. ayeaye*, Ocar: *O. carvalhoi*, Oame: *O. americanus*, Ccar: *C. caramaschii*, Rsch: *R. schneideri*, Llab: *L. labyrinthicus*, Lfus: *L. fuscus*, Lpod: *L. podicipinus*, Pnat: *P. nattereri*, Pcuv: *P. cuvieri*, Pter: *P. ternetzi*, Patr: *P. atragula*, Pmys: *P. mystacalis*.



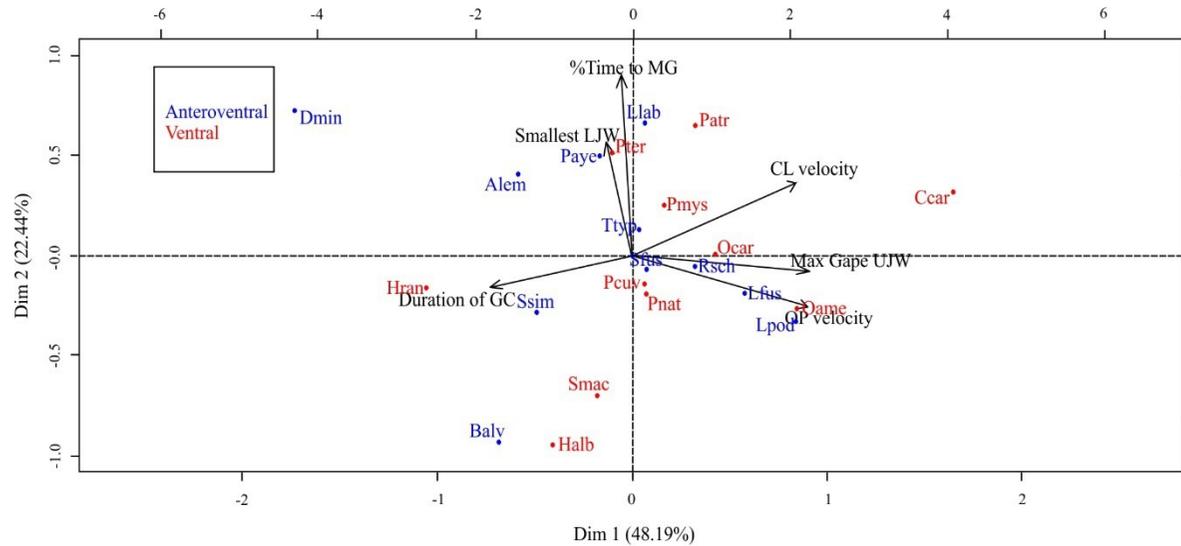
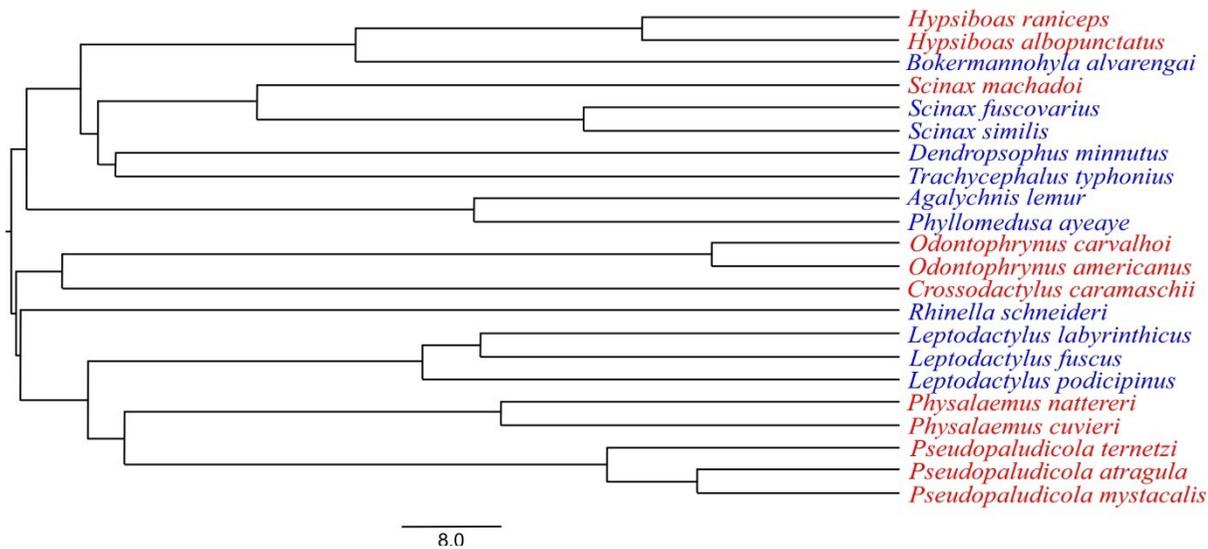


Figure 6. Principal component analysis result for feeding kinematics variables plotted against the first two principal components axes, and the phylogenetic tree. Colors indicate tadpoles oral disc position. Hran: *H. raniceps*, Halb: *H. albopunctatus*, Balv: *B. alvarengai*, Smac: *S. machadoi*, Sfus: *S. fuscovarius*, Ssim: *S. similis*, Dmin: *D. minutus*, Ttyp: *T. typhonius*, Alem: *A. lemur*, Paye: *P. ayeaye*, Ocar: *O. carvalhoi*, Oame: *O. americanus*, Ccar: *C. caramaschii*, Rsch: *R. schneideri*, Llab: *L. labyrinthicus*, Lfus: *L. fuscus*, Lpod: *L. podicipinus*, Pnat: *P. nattereri*, Pcuv: *P. cuvieri*, Pter: *P. ternetzi*, Patr: *P. atragula*, Pmys: *P. mystacalis*.



LITERATURE CITED

- Altig, R. 2007. A primer for the morphology of anuran tadpoles. **Herpetol. Conserv. Biol.** 2:71-74.
- Altig, R.; Johnston, G.F. 1989. Guilds of anuran larvae: relationships among developmental modes, morphologies, and habitats. **Herpetol. Monogr.** 3:81-109.
- Bouckaert, R.; Heled, J.; Kühnert, D.; Vaughan, T.; Wu, C.-H.; Xie, D.; Suchard, M.A.; Rambaut, A.; Drummond, A.J. 2014. BEAST 2: A Software Platform for Bayesian Evolutionary Analysis. **PLoS Comput. Biol.** 10:e1003537. doi:10.1371/journal.pcbi.1003537.
- de Sousa, V.T.T.; Nomura, F.; Venesky, M.D.; Rossa-Feres, D.C.; Pezzuti, T.L.; Andrade, G.V.; Wassersug, R.J. 2014. Flexible feeding kinematics of a tropical carnivorous anuran tadpole. **J. Zool.** 293:204-210.
- de Sousa, V.T.T.; Nomura, F.; Rossa-Feres, D.C.; Andrade, G.V.; Pezzuti, T.L.; Wassersug, R.J.; Venesky, M.D. 2015. Differential effects of temperature on the feeding kinematics of the tadpoles of two sympatric anuran species. **J. Exp. Zool.** 323A:456-465.
- Drummond, A.J.; Nicholls, G.K.; Rodrigo, A.G.; Solomon, W. 2002. Estimating mutation parameters, population history and genealogy simultaneously from temporally spaced sequence data. **Genetics** 161:1307-1320.
- Drummond, A.J.; Ho, S.Y.W.; Phillips, M.J.; Rambaut, A. 2006. Relaxed phylogenetics and dating with confidence. **PLoS Biol.** 4: e88. doi:10.1371/journal.pbio.0040088
- Edgar, R.C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. **Nucleic Acids Res.** 32:1792-1797.

- Eterovick, P.C.; Barata, I.M. 2006. Distribution of tadpoles within and among Brazilian streams: the influence of predators, habitat size and heterogeneity. **Herpetologica**, 62:367-379.
- Eterovick, P.C.; Barros, I.S. 2003. Niche occupancy in South-eastern Brazilian tadpole communities in mountain-meadow streams. **J. Trop. Ecol.** 19:439-448.
- Faivovich, J.; McDiarmid, R.W.; Myers, C.W. 2013. Two new species of *Myersiophyla* (Anura: Hylidae) from Cerro de la Neblina, Venezuela, with comments on other species of the genus. **Am. Mus. Novit.** 3792, 1-63.
- Garland, T.Jr.; Dickerman, A.W.; Janis, C.M.; Jones, J.A. 1993. Phylogenetic analysis of covariance by computer simulation. **Syst. Biol.** 42, 265-292.
- Haas, A. 2003. Phylogeny of frogs as inferred from primarily larval characters (Amphibia: Anura). **Cladistics** 19:23-89.
- Harmon, L.J.; Weir, J.T.; Brock, C.D.; Glor, R.E.; Challenger, W. 2008. GEIGER: investigating evolutionary radiations. **Bioinformatics** 24,129-131.
- Johnston, G.F., 1982. Functions of the keratinized oral features in anuran tadpoles with an analysis of ecomorphological tadpole types. Master's thesis, Mississippi State University, Mississippi.
- Lawler, S.P. 1989. Behavioural responses to predators and predation risk in four species of larval anurans. **Anim. Behav.** 38:1039-1047.
- Ledón-Rettig, C.C.; Pfennig, D.W. 2012 Antipredator behavior promotes diversification of feeding strategies. **Integr. Comp. Biol.** 52:53-63. doi:10.1093/icb/ics074
- MacArthur, R; Levins, R. 1967. The limiting similarity, convergence and divergence of coexisting species. **The Am. Nat.**, 101:377–385.

- Marques, N.S.; Nomura, F. 2015. Where to live? How morphology and evolutionary history predict microhabitat choice by Tropical tadpoles. **Biotropica** 47:227-235. doi: 10.1111/btp.12199
- McDiarmid, R.W.; Altig, R. 1999. Research: materials and techniques. In: *Tadpoles: the biology of anuran larvae*, 1st edn (McDiarmid R.W. & Altig R., eds). The University of Chicago Press, Chicago, pp. 240-278.
- Orton, G.L. 1953. The systematics of vertebrate larvae. **Syst. Zool.** 2:63-75.
- Paradis, E.; Claude, J.; Strimmer, K. 2004. APE: analyses of phylogenetics and evolution in R language. **Bioinformatics** 20, 289-290.
- Peterson, C.G.; Boulton, A.J. 1999. Stream permanence influences microalgal food availability to grazing tadpoles in arid-zone springs. **Oecologia** 118:340-352.
- Pianka, E.R. 1974. Niche overlap and diffuse competition. **Proc. Nat. Acad. Sci. USA**, 71:2141-2145.
- Prado, V.H.M.; Fonseca, M.G.; Almeida, F.V.R., Necchi Jr., O.; Rossa-Feres, D.C. 2009. Niche occupancy and the relative role of micro-habitat and diet in resource partitioning among pond dwelling tadpoles. **South Am. J. Herpetol.** 4:275-285.
- Pyron, R.A.; Wiens, J.J. 2013. Large-scale phylogenetic analyses reveal the causes of high tropical amphibian diversity. **Proc. R. Soc. B** 280: 20131622. <http://dx.doi.org/10.1098/rspb.2013.1622>
- Rambaut, A. 2014. FigTree v1.4.2. Available from: <http://beast.bio.ed.ac.uk/>
- Rambaut, A.; Drummond, A.J. 2014. TreeAnnotator v2.1.2. Available from: <http://beast.bio.ed.ac.uk/TreeAnnotator>.
- Rambaut, A.; Suchard, M.A.; Xie, D.; Drummond, A.J. 2014. Tracer v1.6. Available from: <http://beast.bio.ed.ac.uk/Tracer>.

- Richards, S.J.; Bull, C.M. 1990. Size-limited predation on tadpoles of three Australian frogs. **Copeia** 1990: 1041-1046.
- Richardson, J.M.L. 2001. The relative roles of adaptation and phylogeny in determination of larval traits in diversifying anuran lineages. **The Am. Nat.** 157, 282-299.
- Richter-Boix, A.; Llorente, G.A.; Montori, A. 2004. Responses to competition effects of two anuran tadpoles according to life-history traits. **Oikos** 106:38-50.
- Richter-Boix, A.; Llorente, G.A.; Montori, A.; Garcia, J. 2007. Tadpole diet selection varies with the ecological context in predictable ways. **Basic Appl. Ecol.** 8:464-474.
- Rodríguez, F.; Oliver, J.L., Marín, A.; Medina, J.R. 1990. The general stochastic model of nucleotide substitution. **J. Theor. Biol.** 142:485-501.
- Rossa-Feres, D.C.; Jim, J.; Fonseca, M.G. 2004. Diets of tadpoles from a temporary pond in southern Brazil (Amphibia, Anura). **Rev. Brasil. Biol.** 21(4):745-754.
- Rossa-Feres, D.C.; Nomura, F. 2006. Characterization and taxonomic key for tadpoles (Amphibia: Anura) from the northwestern region of São Paulo State, Brazil. **Biota Neotrop.** 6:<http://www.biotaneotropica.org.br/v6n1/pt/abstract?identification-key+bn00706012006>.
- Schiesari, L.; Werner, E.E.; Kling, G.W. 2009. Carnivory and resource-based niche differentiation in anuran larvae: implications for food web and experimental ecology. **Freshw. Biol.** 54:572-586.
- Sousa Filho, I.F.; Branco, C.C.; Carvalho-e-Silva, A.M.P.T.; Silva, G.R.; Sabagh, L.T. 2007. The diet of *Scinax angrensis* (Lutz) tadpoles in an area of the Atlantic Forest (Mangaratiba, Rio de Janeiro) (Amphibia, Anura, Hylidae). **Rev. Bras. Zool.** 24, 965-970.

- Taylor, C.L., Altig, R., Boyle, C.R., 1996. Oral disc kinematics of four lentic anuran tadpoles. **Herpetol. Nat. Hist.** 4:49-56.
- Van Buskirk, J. 2009. Getting in shape: adaptation and phylogenetic inertia in morphology of Australian anuran larvae. **J. Evol. Biol.** 22, 1326-1337.
- Veiga-Menoncello, A.C.P.; Lourenço, L.B.; Strüsmann, C.; Rossa-Feres, D.C.; Andrade, G.V.; Giaretta, A.A.; Recco-Pimentel, S.M. 2014. A phylogenetic analysis of *Pseudopaludicola* (Anura) providing evidence of progressive chromosome reduction. **Zool. Scr.** 43, 261-272.
- Venesky, M.D., Rossa-Feres, D.C., Nomura, F., Andrade, G.V., Pezzuti, T.L., de Sousa, V.T.T., Anderson, C.V. & Wassersug, R.J. 2013. Comparative feeding kinematics of tropical hyloid tadpoles. **J. Exp. Biol.** 216, 1928-1937.
- Venesky, M.D., Wassersug, R.J., Jorgensen, M.E., Riddle, M. & Parris, M.J. 2011. Comparative feeding kinematics of temperate pond-dwelling tadpoles (Anura, Amphibia). **Zoomorphology** 130, 31-38.
- Venesky, M.D., Wassersug, R.J. & Parris, M.J. 2010. How does a change in labial tooth row number affect feeding kinematics and foraging performance of a ranid tadpole (*Lithobates sphenoccephalus*)? **Biol. Bull.** 218:160–168.
- Vera Candiotti, M.F. 2005. Morphology and feeding in tadpoles of *Ceratophrys cranwelli* (Anura: Leptodactylidae). **Acta Zoologica** (Stockholm) 86:1-11.
- Vera Candiotti, M.F. 2007. Anatomy of anuran tadpoles from lentic water bodies: systematic relevance and correlation with feeding habits. **Zootaxa** 1600, 1-175.
- Warkentin 1992. Microhabitat use and feeding rate variation in green frog tadpoles (*Rana clamitans*). **Copeia** 1992:731-740.
- Wassersug, R.J. 1976. Oral morphology of anuran larvae: terminology and general description. **Occas. Pap. Mus. Nat. Hist. Univ. Kansas** 48, 1-23.

- Wassersug, R.J.; Yamashita, M. 2001. Plasticity and constraints on feeding kinematics in anuran larvae. **Comp. Biochem. Physiol., Part A: Mol. Integr. Physiol** 131, 183-195.
- Wickramasinghe, D.D.; Oseen, K.L.; Wassersug, R.J. 2007. Ontogenetic changes in diet and intestinal morphology in semi-terrestrial tadpoles of *Nannophrys ceylonensis* (Dicroglossidae). **Copeia** 2007:1012-1018.
- Wiens, J.J.; Kuczynski, C.A.; Hua, X.; Moen, D.S. 2010. An expanded phylogeny of treefrogs (Hylidae) based on nuclear and mitochondrial sequence data. **Mol. Phylogenet. Evol.** 55:871-882.
- Yang, Z. 1994. Maximum likelihood phylogenetic estimation from DNA sequences with variable rates over sites: approximate methods. **J. Mol. Evol.** 39:306-314.

Conclusões Finais

- Os girinos exibem plasticidade em seus comportamentos alimentares, sendo capazes de ajustá-lo de acordo com os diferentes tipos alimentares a serem consumidos.
- A ecologia das espécies, principalmente o tipo de hábitat que ocupam e a posição assumida na coluna d'água, influenciam o comportamento alimentar dos girinos.
- Os girinos podem apresentar comportamentos que não necessariamente se correlacionam com as adaptações morfológicas utilizadas para categorizá-los em guildas ecomorfológicas.
- A variação interespecífica no comportamento alimentar que, à princípio, é atribuída às diferenças morfológicas entre as espécies, também pode ser associada às relações filogenéticas entre as espécies.

ANEXOS