BRILL

Amphibia-Reptilia (2020) DOI:10.1163/15685381-bja10020

brill.com/amre

Food source determines stable isotope discrimination factors ΔN and ΔC in tadpoles

Julian Glos*, Katharina Ruthsatz, Dominik Schröder, Jana C. Riemann

Abstract. Analyses of stable isotope ratios are widely applied in studies on a large variety of aspects in trophic ecology. Most studies rely on a precise estimation of the relevant discrimination factor Δ (also called the fractionation factor), that reflects the fractionation or differences in isotope ratios of a certain element (mainly nitrogen N and carbon C) between an animal's diet and its tissue and is used to identify one step in the food web. We experimentally determined ΔN and ΔC of two species of widespread amphibians in Europe, *Rana temporaria* and *Bufo bufo*, and tested for the effect of food source (cyanobacteria *Spirulina* vs. zooplanktonic *Daphnia*) on Δ and for interspecific differences. Our study shows high variation in Δ in relation to the food source, but low interspecific differences. Tadpoles that were fed with *Spirulina* did have considerably lower ΔN than tadpoles fed with *Daphnia* in both species, and lower ΔC only in *R. temporaria*. The range of Δ obtained here can be a useful baseline for future trophic studies on tadpoles of *Rana* and *Bufo*. The strong diet-dependency of Δ, however, argues strongly against the use of a fixed discrimination factor in future isotope studies.

Keywords: diet, food web, fractionation, isotope signature, larval anurans.

Introduction

Indirect measures constitute the preferential methods in trophic ecology when direct analyses are either difficult to perform or to quantify (e.g., direct observations, gut content analyses), or are not permitted by national statutes (e.g., removal experiments) or are in contrast to rules of good scientific practice (i.e., dissection). The analysis of stable isotopes is a rather recent yet well-established and easy-to-perform method that can be used to assess a variety of relevant parameters in food web ecology. It has proven to be a more effective tool than conventional methods (e.g., gut analyses) for interpreting diet relationships as the isotopic signature of a species' niche reflects temporally and spatially integrated data, whereas stomach content data alone reflect rather a temporary snap-shot of food choice (Winemiller, 1990; Post, 2002; Winemiller and Layman, 2005; Montana et al., 2019).

Stable isotope ratios are seen as "recorders" that can be used to reconstruct ecological processes in a wide variety of organisms (West et al., 2006; Montana et al., 2019). Most applications refer to specific energy flow pathways leading to one (or a few) species of interest, i.e., they are used to calculate trophic positions (Vander Zanden et al., 1997; Post, 2002; Layman et al., 2007a), relative contribution of prey items to consumers (Vander Zanden and Vadeboncoeur, 2002), niche shifts (Post, 2003; Barnum et al., 2013; Schalk et al., 2017), and intraspecific diet variability (Bolnick et al., 2003; Bearhop et al., 2004; Matthews and Mazumder, 2004). Studies on food web ecology in amphibians using stable isotope signatures are just beginning to emerge, and include the diet and trophic niches of tadpoles (Fenolio et al., 2006; Unrine et al., 2007; Schiesari et al., 2009; Schalk et al., 2017; Schmidt et al., 2017), trophic niche shifts during development (Trakimas et al., 2011; Schriever and Williams, 2013; Glos et al., 2016) or as a reaction to changes in the species' environment (e.g., predators, density, fertilizer application; Jefferson and Russell, 2008; Caut et al., 2012; Arribas et al., 2015) and the trophic structure of mainly aquatic food

Institute of Zoology, University of Hamburg, Martin-Luther-King Platz 3, 20146 Hamburg, Germany

*Corresponding author;

e-mail: julian.glos@uni-hamburg.de

DOI:10.1163/15685381-bja10020

webs (Cogalniceanu et al., 2001; Kupfer et al., 2006; Verburg et al., 2007; Vences et al., 2016; Schalk et al., 2017; Schmidt et al., 2017).

Stable isotope ratios in animal tissue derive from all trophic pathways cumulating in that individual. Applications of stable isotope ratios (typically carbon C and nitrogen N) in food web ecology take advantage of natural variation in stable isotope ratios. Nitrogen ratios of ^{15}N to ^{14}N (expressed as $\delta^{15}N$) exhibit stepwise enrichment with trophic transfers and can be powerful tools for indicating trophic position within a food web, whereas $\delta^{13}C$ (ratio of ^{13}C to ^{12}C) can be used to identify major energy sources (DeNiro and Epstein, 1981; Peterson and Fry, 1987; Post, 2002). The combination of both isotope signatures ($\delta^{15}N$ and $\delta^{13}C$) is used to determine the trophic niche of an animal (Layman et al., 2007a, b).

For most trophic studies it is essential to know the magnitude of difference in $\delta^{15}N$ and $\delta^{13}C$ between food source and focus organism which indicates one step in the food web, e.g., the difference between a pure herbivore and a carnivore feeding only on pure herbivores. This difference is called ΔN and ΔC, the discrimination factor or fractionation (Fry, 2006; Sharp, 2007). Often a ΔN of 3-4‰ is assumed (Peterson and Fry, 1987), but there can be considerable variation even within taxa (e.g., in snails and mussels, ΔN varies between 0.5 and 5.5 and ΔC between –3 and 4; Post, 2002), and can also depend on tissue and food source (Caut et al., 2009).

Although studies on amphibians that are applying stable isotope analyses are emerging, only a few studies actually have experimentally tested for discrimination factors. The existing data result in a complicated picture. While ΔN in larval wood frogs (*Lithobates sylvaticus*) was determined as about 2‰ and ΔC as 1.7‰ (Schiesari et al., 2009), Caut et al. (2012) found only small differences between tadpole species (*Pelobates cultripes* and *Bufo calamita*), but large differences between tadpoles that were raised on different diets. Here, ΔN varied between −2.3‰ (algae diet), 4.2 to 4.4‰ (zooplankton diet), and was highest when tadpoles were fed macrophytes (3.9 to 5.4‰). ΔC varied between −1.7 and 2.3‰.

Most studies on the trophic ecology of amphibians using analyses of isotopic signatures rely on a precise estimation of the relevant discrimination factor, yet many assumptions on Δ are still lacking an appropriate experimental background. Therefore, we aimed to expand the knowledge on the enrichment of isotopic ratios with one trophic transfer (i.e., planktonic food to tadpole tissue) by experimentally determining the discrimination factors ΔN and ΔC of two species of widespread amphibians in Europe, *Rana temporaria* and *Bufo bufo*. Accordingly, we tested for the effect of food source (cyanobacteria *Spirulina* vs. zooplanktonic *Daphnia*) on ΔN and ΔC, and further tested for interspecific differences.

Material and methods

Experimental procedure

Eight egg clutches of *Rana temporaria* were collected in April 2011 from a natural pond within the city limits of Hamburg, Germany ("Volksdorfer Wald", 53°6477N, 10°1436E), and eight egg strings of *Bufo bufo* from a natural breeding pond ("Teufelsee") just north of Hamburg (Bönningstedt; 53°6549N, 9°9268E) and transported to the laboratory at the University of Hamburg. Each egg clutch and string, respectively, was kept separately in an aquarium until the embryos hatched and reached developmental stage 25 (i.e., no more external gills visible, operculum developed, start of exotrophic feeding; Gosner, 1960). From each clutch/string 20 tadpoles were randomly selected, and ten of these tadpoles were randomly assigned to either the *Spirulina* or *Daphnia* treatment and subsequently raised in sibling groups in aquaria (16.8 cm × 23.9 cm, volume 9.5 l), resulting in a total of 32 aquaria (two species × eight clutches × two treatments) with ten tadpoles each. The experiment was conducted in a climatized laboratory with an ambient temperature of 13°C, a light regime of 11 h:13 h (day:night), using de-chlorinated tap water (pH between 7.0 and 7.5) changed in intervals of four days. To check for tadpole growth and development, tadpoles were measured every four days for body length (McDiarmid and Altig, 1999) and developmental stage (Gosner, 1960). When tadpoles had reached developmental stage 36 (i.e., well developed external hind legs with five distinguishable toes; Gosner, 1960), one tadpole was randomly selected from each

Table 1. Interspecific differences (*Rt* = *Rana temporaria* vs. *Bb* = *Bufo bufo*) of discrimination factors for nitrogen (ΔN) and carbon (ΔC) isotope ratios and different food sources (*Spirulina*: low quality, *Daphnia*: high quality); n = averaged values per specimen (i.e., statistical replicates); T-tests, T = test statistic.

Isotope	Food	*Rana temporaria*	*Bufo bufo*	T	P
ΔN (‰)	*Spirulina*	3.0 ± 0.2 (n = 8)	2.8 ± 0.4 (n = 7)	1.52	0.15
	Daphnia	5.2 ± 0.2 (n = 8)	4.5 ± 0.6 (n = 4)	2.11	0.12
ΔC (‰)	*Spirulina*	2.0 ± 0.5 (n = 8)	1.9 ± 0.1 (n = 7)	0.24	0.82
	Daphnia	2.5 ± 0.3 (n = 8)	1.6 ± 0.2 (n = 4)	5.33	<0.01

aquarium, euthanized by immersion in MS222 solution and subsequently stored in 70% ethanol. BL (body length) from each specimen was measured according to the definition of McDiarmid and Altig (1999) to test whether food source affected body size. Only one tadpole per aquarium was measured to ensure independence of the data.

Experimental treatments

Tadpoles were raised either with *Spirulina* algae (cyanobacteria) (JBL Premium flakes, JBL GmbH & Co. KG, 67141 Neuhofen, Germany) *ad libitum* or with *Daphnia pulex* (Tetra Delica Daphnien, Tetra GmbH, 49304 Melle, Germany) *ad libitum* as sole food. Prior to the experiments, both food types were powdered to provide an identical mechanical texture of food, i.e., restrict the differences between food types to their chemical composition. Both food types are common planktonic food for tadpoles in their natural habitat, with *Daphnia* supposedly being of higher quality regarding protein, nitrogen and energy contents. To verify differences between food sources, nitrogen content was quantitatively analysed by the Kjeldahl method, and was converted accordingly to protein content N-factor 6.25. Energy content was analysed by bomb calorimetry (6200 Isoperobol Calorimeter, Parr Instruments, Moline, Illinois). Analyses were done at a laboratory for chemical analyses at University of Hamburg.

Isotope analyses

Prior to analysis, tail muscle tissue samples were taken from all tadpole specimens. All samples (i.e., powdered *Spirulina* and *Daphnia*, *Rana* and *Bufo* muscle tissue) were dried in an oven at 60°C for three days to eliminate any remaining moisture and to avoid any artefacts on C-isotope signatures by remaining alcohol on tadpole samples. An average of 0.6 mg material of each sample was placed in 4 × 6 mm tin cups (HEKAtech, Germany). If sufficient muscle tissue was available, two (or three, in rare cases) samples from the same tadpole specimen were analyzed. Not all isotope analyses were successful, leading to a lower than expected number of samples and replicates for the analysis. In total, 104 tadpole tissue samples were analyzed, resulting in 54 statistical replicates (i.e., means of samples from the same individual). Additionally, the food sources *Spirulina* (n = 12) and *Daphnia* (n = 11) were analyzed.

Isotope analysis was conducted in the isotope laboratory of C. Reisdorff at the Biozentrum Klein Flottbek of the University of Hamburg, Germany. Isotope ratios are given in δ notation (‰) relative to atmospheric nitrogen (AIR) for δ^{15}N, and to PeeDee Belemnite (PDB) for δ^{13}C (International reference standards; Fry, 2006). Samples were combusted in a mass spectrometer (EURO-EA 3000, Euro Vector, Italy). BBOT (2, 5-bis (5-tert-butyl-2-benzoxazol-2-yl) thiophene (6.51% N; 72.52% C; HEKAtech, Germany), KNO3, and caffeine were used as internal standards.

Statistical analysis

All statistical analyses were run with the average of multiple samples per specimen as one replicate (n_{total} = 54 for tadpole tissues; table 1). The discrimination factor ΔN for each tadpole individual was calculated as $\Delta N = \delta^{15}N_{food\ source}$ (average of replicates) − $\delta^{15}N_{individual}$ (average of replicates per specimen), and correspondingly for ΔC. We checked a priori for homogeneity of variances among the two treatment groups (*Spirulina*, *Daphnia*) and performed relevant parametric statistics (T-tests) using SPSS software (IBM® SPPS® Statistics Version 23).

Results

The protein content of *Spirulina* food was 35%, and of *Daphnia* 54%. Nitrogen content of *Spirulina* was 5.6% vs. 8.6% of *Daphnia*. Energy content was 1256 kJ/100 g (*Spirulina*) and 1503 kJ/100 g (*Daphnia*). For both species, tadpoles that were fed *Spirulina* food did not significantly differ in body length to tadpoles fed *Daphnia* (*R. temporaria*: Mann-Whitney U-test: Z = −0.52, p = 0.64, n = 16; *B. bufo* Z = −1.55, p = 0.33, n = 16).

Isotopic signatures and discrimination factors

The isotopic ratios of the *Spirulina* food source were 7.5 ± 0.2‰ for δ^{15}N and −23.1 ± 0.3‰ for δ^{13}C (n = 12), and 12.9 ± 0.5‰ for δ^{15}N and −21.5 ± 0.3‰ for δ^{13}C (n = 11) for *Daphnia*. In tadpole muscle tissues, variation in both δ^{15}N and δ^{13}C and for both species was very

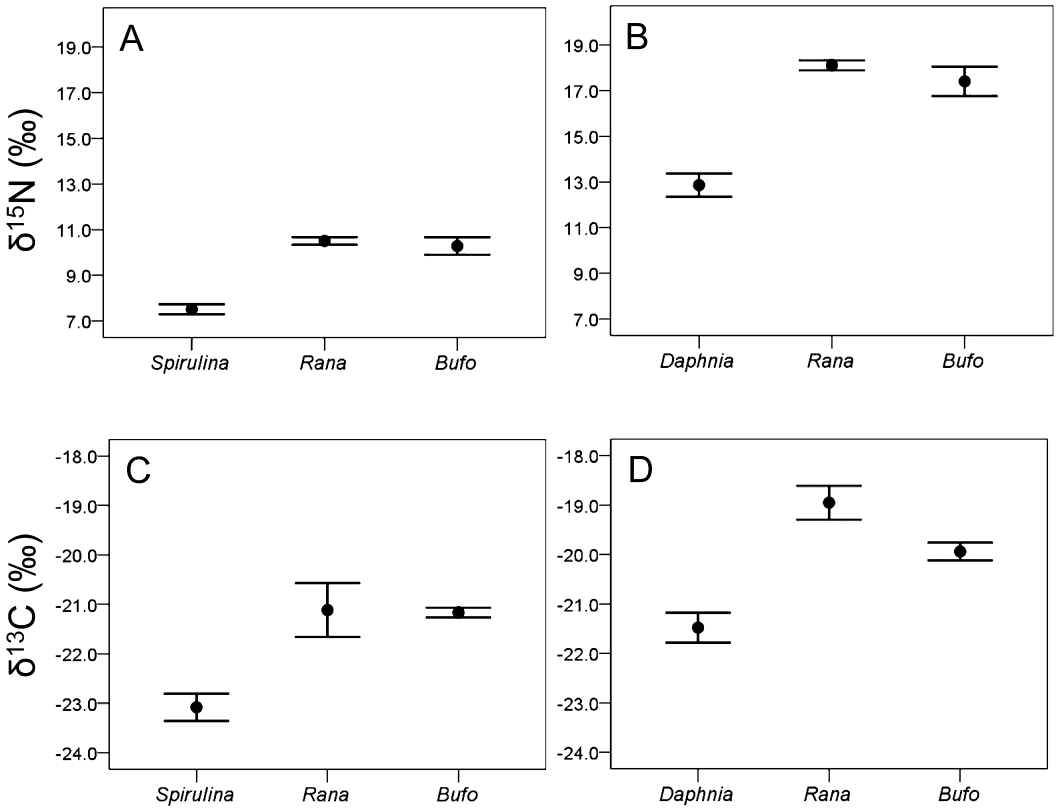

Figure 1. Stable isotope ratios δ^{15}N (A, B) and δ^{13}C (C, D) (in‰) for tadpole food sources *Spirulina* and *Daphnia* and muscle tissues of *Rana temporaria* and *Bufo bufo* tadpoles fed on *Spirulina* (A, C) and *Daphnia* (B, D). Dots indicate means, whiskers are ± 1 SD, N = nitrogen, C = carbon.

low (fig. 1). The isotopic ratios with *Spirulina* as food source were 10.5 ± 0.2‰ for δ^{15}N and −21.1 ± 0.5‰ for δ^{13}C for *Rana*, and 10.3 ± 0.4‰ for δ^{15}N and −21.2 ± 0.1‰ for δ^{13}C for *Bufo*. The isotopic ratios with *Daphnia* as food source were 18.1 ± 0.2‰ for δ^{15}N and −18.9 ± 0.3 ‰ for δ^{13}C for *Rana*, and 17.4 ± 0.6 ‰ for δ^{15}N and −19.9 ± 0.2‰ for δ^{13}C for *Bufo*. The discrimination factors of all treatments varied between 2.8 and 5.3 in ΔN, and 1.6 and 2.5 in ΔC (means). In *R. temporaria*, ΔN and ΔC differed significantly between tadpoles that were raised on *Spirulina* (i.e., low quality food) and *Daphnia* (i.e., high quality) food (table 1; fig. 1; T-tests: for δ^{15}N, $T = -22.7$, $p < 0.001$, for δ^{13}C, $T = -2.5$, $p < 0.05$). Likewise, both ΔN and ΔC differed significantly between the *Spirulina* and *Daphnia* feeding treatment in *B. bufo* (table 1; fig. 1; T-tests: for δ^{15}N, $T = -5.6$, $p < 0.001$, for δ^{13}C, $T = 4.5$, $p = 0.001$).

Interspecific differences in ΔN and ΔC

The discrimination factor ΔN did not differ between *R. temporaria* (3.0 ± 0.2 for *Spirulina*, 5.2 ± 0.2 for *Daphnia*) and *B. bufo* (2.8 ± 0.4 for *Spirulina*, 4.5 ± 0.6 for *Daphnia*). Also, ΔC of tadpoles fed on *Spirulina* food did not differ between species, however, ΔC of tadpoles fed on *Daphnia* food was significantly higher in *R. temporaria* than in *B. bufo* (table 1, fig. 1).

Discussion

Analyses of stable isotope ratios are widely applied in studies on a large variety of aspects in trophic ecology. Either as a standing alone

analysis, or in combination with direct studies such as direct observations of feeding or gut analyses, isotope ratios indicate trophic position within a food web (δ^{15}N) or identify major energy sources (δ^{13}C) (DeNiro and Epstein, 1981; Peterson and Fry, 1987; Post, 2002; Montana et al., 2019). One essential factor in these studies is the discrimination factor Δ (also called the fractionation factor), that reflects the fractionation or differences in isotope ratios of a certain element between an animal's diet and its tissue. In early trophic isotope studies often a fixed Δ was used across taxa and food sources. More recent studies, however, revealed considerable differences in Δ among taxonomic groups (e.g., in ΔC; Caut et al., 2009), animal tissue (Caut et al., 2008, of rats; Caut et al., 2009, of different vertebrate taxa), and food source (e.g., Caut et al., 2012 for tadpoles). These differences between food sources can be caused by differences in protein quality, lipid content, type of food and diet isotopic ratio (see Post et al., 2007; Caut et al., 2008, 2009).

Our study showed an overall high variation in discrimination factors in larvae of frogs *R. temporaria* and toads *B. bufo*, and that diet-dependent variation in Δ within amphibian species can be greater than differences among species. The discrimination factors for our two study species are within the range of discrimination factors found for other tadpole species, *Lithobathes sylvaticus* (Schiesari et al., 2009) and *Pelobates cultripes* and *Bufo calamita* (Caut et al., 2012), considering mean values and the diet-dependent high variability of ΔN and ΔC. Intra- and interspecific differences in ΔN and ΔC that were revealed in our study, however, showed important factors that have to be considered in stable isotope studies. Tadpoles that were fed with *Spirulina* cyanobacteria did have considerably lower ΔN than tadpoles fed with *Daphnia* in both species. A reverse pattern was found in ΔC. *Rana temporaria* tadpoles fed on *Spirulina* had lower ΔC values than those fed on *Daphnia*, and in contrast, *B. bufo* tadpoles fed on *Spirulina* had higher ΔC values than those fed on *Daphnia*. Furthermore, we found no differences in ΔN among species, but diet-dependent interspecific differences in ΔC (only for *Daphnia* food). Generally, Δ is known to vary among major taxonomic groups (mammals, birds, fish, invertebrates; Caut et al., 2009). This study on two (within the order Anura) only very distantly related species (*Rana* and *Bufo*), together with a study by Caut et al. (2012) that found no differences between *Pelobates cultripes* (Pelobatidae) and *Bufo calamita* (Bufonidae), indicates that there is no strong difference of ΔN between species or families within larval amphibians. On the contrary, variation in ΔC may be greater between different amphibian taxa and depends greatly on the animal's diet.

To deal with the overall strong diet-dependency of Δ, (Caut et al., 2008, 2009) proposed a calibration of Δ for different food sources (i.e., using a "diet-dependent discrimination factor"). In a multi-taxon review (on mammals, birds, fishes, invertebrates) they found a significant negative relationship between the discrimination factors ΔN and ΔC and the isotopic ratios δ^{15}N and δ^{13}C of the food sources. Accordingly, Δ could be calculated from this relationship when δ of the respective food source is known. In *R. temporaria* and *B. bufo*, we also found that ΔN and ΔC differed with the respective δ. However, for nitrogen isotopes in both species Δ was greater when δ of the food source was higher and thus, the relationship was rather positive, while carbon isotopes showed an opposing Δ-δ-relationship in our two study species. Our results suggest therefore that Δ-δ-dependencies might be different in larval amphibians and the use of a calibration factor needs further studies.

Beside the need for practicable solutions to deal with the diet-dependency of Δ in applied studies, it is important to investigate and understand its cause. The biochemical mechanism leading to considerable higher ΔN values of tadpoles that were fed with *Daphnia* compared to those fed with *Spirulina* cyanobacteria in

both species remains speculative, but might be caused by the higher protein content of *Daphnia*, higher protein quality, lipid content, or other chemical properties of the food (see Caut et al., 2008, 2009; Post et al., 2007).

Conclusions

The range of discrimination factors obtained here provides a useful baseline for future trophic studies on tadpoles of the two most-widespread amphibian species in Europe, *R. temporaria* and *B. bufo*. Also, in accordance with Caut et al. (2012), our data indicates that the transfer of nitrogen discrimination factors to other larval amphibian species (using the same food source) might be generally possible, although this needs further validation by studies on a wider spectrum of amphibian taxa. The strong diet-dependency of Δ, however, argues strongly against the use of a fixed discrimination factor for a variety of applications (e.g., diet reconstruction, identification of trophic level and niche) when different food sources are consumed. Therefore, we recommend to experimentally assess Δ for any potential food source when reconstructing diets using isotope ratios. Also, isotopic analyses in trophic studies on larval amphibian communities should be interpreted cautiously. Differences in food sources between species can translate into differences in δ^{15}N and δ^{13}C between species within one community. Due to the strong diet-dependency of Δ, however, these interspecific differences do not necessarily indicate differences in the relative trophic position within this community, e.g., that a species with higher δ^{15}N is a consumer of higher order than a species with lower δ^{15}N. Finally, for the development of future approaches of stable isotope analyses and applications it will be essential to understand the mechanisms causing the strong diet-dependency of Δ.

Acknowledgements. We are grateful to C. Reisdorff for his aid in the isotope analyses. Collecting permits were provided by the Behörde für Stadtentwicklung und Umwelt, Hamburg, and the permit for conducting the experiments was provided by the Behörde für Soziales, Familie, Gesundheit und Verbraucherschutz (Permit Number 8/11 from 9th Feb 2011).

References

Arribas, R., Díaz-Paniagua, C., Caut, S., Gomez-Mestre, I. (2015): Stable isotopes reveal trophic partitioning and trophic plasticity of a larval amphibian guild. PLoS One **10**: e0130897.

Barnum, T.R., Verburg, P., Kilham, S.S., Whiles, M.R., Lips, K., Colón-Gaud, C., Pringle, C.M. (2013): Use of stable isotope ratios to characterize potential shifts in the isotopic niches of grazing insects following an amphibian decline in a Neotropical stream. J. Trop. Ecol. **29**: 291-299.

Bearhop, S., Adams, C.E., Waldron, S., Fuller, R.A., Macleod, H. (2004): Determining trophic niche width: a novel approach using stable isotope analysis. J. Anim. Ecol. **73**: 1007-1012.

Bolnick, D.I., Svanback, R., Fordyce, J.A., Yang, L.H., Davis, J.M., Hulsey, C.D., Forrister, M.A. (2003): Comparative approaches to intra-population niche variation. Integr. Comp. Biol. **43**: 1078.

Caut, S., Angulo, E., Courchamp, F. (2008): Discrimination factors (Δ15N and Δ13C) in an omnivorous consumer: effect of diet isotopic ratio. Funct. Ecol. **22**: 255-263.

Caut, S., Angulo, E., Courchamp, F. (2009): Variation in discrimination factors (Δ15N and Δ13C): the effect of diet isotopic values and applications for diet reconstruction. J. Appl. Ecol. **46**: 443-453.

Caut, S., Angulo, E., Díaz-Paniagua, C., Gomez-Mestre, I. (2012): Plastic changes in tadpole trophic ecology revealed by stable isotope analysis. Oecologia **173**: 95-105.

Cogalniceanu, D., Palmer, M.W., Ciubuc, C. (2001): Feeding in anuran communities on islands in the Danube floodplain. Amphibia-Reptilia **22**: 1-19.

DeNiro, M.J., Epstein, S. (1981): Influence of diet on the distribution of nitrogen isotopes in animals. Geochim. Cosmochim. Acta **45**: 341-351.

Fenolio, D.B., Graening, G.O., Collier, B.A., Stout, J.F. (2006): Coprophagy in a cave-adapted salamander; the importance of bat guano examined through nutritional and stable isotope analyses. Proc. R. Soc. Lond. (Biol.) **273**: 439-443.

Fry, B.G. (2006): Stable Isotope Ecology. Springer, New York.

Glos, J., Wolter, J., Struck, U., Rödel, M.-O. (2016): Ecological shifts during larval development in two West African savanna frogs. J. Trop. Ecol. **33**: 50-59.

Gosner, K.L. (1960): A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica **16**: 183-190.

Jefferson, D.M., Russell, R.W. (2008): Ontogenetic and fertilizer effects on stable isotopes in the green frog (*Rana clamitans*). Appl. Herpetol. **5**: 189-196.

Kupfer, A., Langel, R., Scheu, S., Himstedt, W., Maraun, M. (2006): Trophic ecology of a tropical aquatic and ter-

restrial food web: insights from stable isotopes (N-15). J. Trop. Ecol. **22**: 469-476.

Layman, C.A., Arrington, D.A., Montana, C.G., Post, D.M. (2007a): Can stable isotope ratios provide for community-wide measures of trophic structure? Ecology **88**: 42-48.

Layman, C.A., Quattrochi, J.P., Peyer, C.M., Allgeier, J.E. (2007b): Niche width collapse in a resilient top predator following ecosystem fragmentation. Ecol. Letters **10**: 937-944.

Matthews, B., Mazumder, A. (2004): A critical evaluation of intrapopulation variation of delta C-13 and isotopic evidence of individual specialization. Oecologia **140**: 361-371.

McDiarmid, R.W., Altig, R. (1999): Tadpoles: the Biology of Anuran Larvae. University of Chicago Press, Chicago, IL.

Montana, C.G., Silva, S., Hagyari, D., Wager, J., Tiegs, L., Sadeghian, C., Schriever, T.A., Schalk, C.M. (2019): Revisiting "what do tadpoles really eat?" A 10-year perspective. Freshwater Biol. **64**: 2269-2282. DOI:10.1111/fwb.13397.

Peterson, B.J., Fry, B. (1987): Stable isotopes in ecosystem studies. Ann. Rev. Ecol. Syst. **18**: 293-320.

Post, D.M. (2002): Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology **83**: 703-718.

Post, D.M. (2003): Individual variation in the timing of ontogenetic niche shifts in largemouth bass. Ecology **84**: 1298-1310.

Post, D.M., Layman, C.A., Arrington, D.A., Takimoto, G., Quattrochi, J., Montana, C.G. (2007): Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. Oecologia **152**: 179-189.

Schalk, C.M., Montana, C.G., Winemiller, K.O., Fitzgerald, L.A. (2017): Trophic plasticity, environmental gradients and food-web structure of tropical pond communities. Freshwater Biol. **54**: 519-529.

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. Freshwater Biol. **54**: 572-586.

Schmidt, K., Blanchette, M., Pearson, R.G., Alford, R.A., Davis, A.M. (2017): Trophic roles of tadpoles in tropical Australian streams. Freshwater Biol. **62**: 1929-1941.

Schriever, T.A., Williams, D.D. (2013): Ontogenetic and individual diet variation in amphibian larvae across an environmental gradient. Freshwater Biol. **58**: 223-236.

Sharp, Z.D. (2007): Principles of Isotope Geochemistry. Pearson Prentice Hall, Upper Saddle River, NJ.

Trakimas, G., Jardine, T.D., Bariseviciu, R., Garbaras, A., Skipityte, R., Remeikis, V. (2011): Ontogenetic dietary shifts in European common frog (*Rana temporaria*) revealed by stable isotopes. Hydrobiologia **675**: 87-95.

Unrine, J.M., Hopkins, W.A., Romanek, C.S., Jackson, B.P. (2007): Bioaccumulation of trace elements in omnivorous amphibian larvae: implications for amphibian health and contaminant transport. Environ. Pollut. **149**: 182-192.

Vander Zanden, M.J., Cabana, G., Rasmussen, J.B. (1997): Comparing trophic position of freshwater fish calculated using stable nitrogen isotope ratios (delta N-15) and literature dietary data. Can. J. Fish. Aquat. Sci. **54**: 1142-1158.

Vander Zanden, M.J., Vadeboncoeur, Y. (2002): Fishes as integrators of benthic and pelagic food webs in lakes. Ecology **83**: 2152-2161.

Vences, M., Lyra, M.L., Kueneman, J.G., Bletz, M., Archer, H.M., Canitz, J., Handreck, S., Randrianiaina, R.D., Struck, U., Bhuju, S., Jarek, M., Geffers, R., McKenzie, V.J., Tebbe, C.C., Haddad, C.F.B., Glos, J. (2016): Gut bacterial communities across tadpole ecomorphs in two diverse tropical anuran faunas. Naturwissenschaften **103**: 25.

Verburg, P., Kilham, S.S., Pringle, C.M., Lips, K.R., Drake, D.L. (2007): A stable isotope study of a Neotropical stream food web prior to the extirpation of its large amphibian community. J. Trop. Ecol. **23**: 643-651.

West, J.B., Bowen, G.J., Cerling, T.E., Ehleringer, J.R. (2006): Stable isotopes as one of nature's ecological recorders. Trends Ecol. Evolut. **21**: 408-414.

Winemiller, K.O. (1990): Spatial and temporal variation in tropical fish trophic networks. Ecol. Monograph. **60**: 331-367.

Winemiller, K.O., Layman, C.A. (2005): Pattern, process and scale in the food web paradigm: moving on the path from abstraction to prediction. In: Dynamic Food Webs: Multispecies Assemblages, Ecosystem Development, and Environmental Change, p. 10-23. Elsevier, Amsterdam.

Submitted: October 1, 2019. Final revision received: May 10, 2020. Accepted: May 13, 2020.
Associate Editor: Roberto Sacchi.