

4-2022

## Trophic relationships between tadpoles and other aquatic organisms in sub-tropical ponds

Kira Cates

Follow this and additional works at: [https://repository.lsu.edu/honors\\_etd](https://repository.lsu.edu/honors_etd)



Part of the [Natural Resources and Conservation Commons](#), and the [Natural Resources Management and Policy Commons](#)

---

### Recommended Citation

Cates, Kira, "Trophic relationships between tadpoles and other aquatic organisms in sub-tropical ponds" (2022). *Honors Theses*. 254.

[https://repository.lsu.edu/honors\\_etd/254](https://repository.lsu.edu/honors_etd/254)

This Thesis is brought to you for free and open access by the Ogden Honors College at LSU Scholarly Repository. It has been accepted for inclusion in Honors Theses by an authorized administrator of LSU Scholarly Repository. For more information, please contact [ir@lsu.edu](mailto:ir@lsu.edu).

Trophic relationships between tadpoles and other aquatic organisms in sub-tropical ponds

By

Kira Cates

Undergraduate honors thesis under the direction of

Dr. Michael Kaller

School of Renewable Natural Resources

Submitted to the LSU Roger Hadfield Ogden Honors College in partial fulfillment of  
the Upper Division Honors Program.

April 2022

Louisiana State University  
& Agricultural and Mechanical College  
Baton Rouge, Louisiana

## **Table of Contents**

<i>Abstract</i> .....	<b>3</b>
<i>Introduction</i> .....	<b>3</b>
<i>Methods</i> .....	<b>6</b>
<i>Results</i> .....	<b>10</b>
<i>Discussion</i> .....	<b>14</b>
<i>Conclusion</i> .....	<b>16</b>
<i>Acknowledgements</i> .....	<b>17</b>
<i>Literature Cited</i> .....	<b>18</b>

## ***Abstract***

Understanding niche overlap between taxa is vital in understanding the complexities of how ecosystems function. However, few studies have been conducted to analyze the trophic position of amphibian species in relation to other taxa. Understanding these relationships and the role that amphibians play within an ecosystem is becoming increasingly urgent, as amphibian species face rapid global declines. This study sought to analyze potential niche overlap and trophic relationships between larval anurans (tadpoles) and Red Swamp Crayfish (*Procambarus clarkii*), fish of the genus *Lepomis*, odonate larvae of Aeshnidae, and adult anurans. Using standard galvanized funnel traps and a handheld D-frame net, three adjacent ponds were sampled for the target taxa. For each sample, respective levels of stable isotopes  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  were analyzed to establish trophic position relative to other samples. The initial results indicate that there is minimal overlap between the taxa aside from predation. Most tadpoles utilized a separate carbon pathway, and there was significant variation in levels of  $\delta^{15}\text{N}$ . All levels of  $\delta^{15}\text{N}$  in each tadpole indicated that animal material was being consumed, which could be evidence supporting tadpole niche plasticity, behavioral plasticity, and omnivorous tendencies.

## ***Introduction:***

Amphibians are notably important in ecosystems, functioning as both significant predators and important food sources for many species. It is known that tadpoles function as important grazers, controlling algae and detritus within streams, as well as playing a key role in maintaining nitrogen turnover and stream respiration rates (Whiles *et al.* 2012). Despite this general knowledge, little has been done to specify the trophic ecology of amphibians. Most studies addressing this question have been carried out within the past 15 years. Now, amphibians across the world are experiencing a mass extinction at an unprecedented rate, largely due to habitat degradation and destruction, a global decline in insect populations, and disease (Collins 2010; McCallum 2007; Van der Sluijs 2020). Chytrid fungus (*Batrachochytrium dendrobatidis*) is one of the biggest threats that amphibians are facing, causing around 99 species extinctions and the decline of over 500 species which are now threatened or endangered (Lips 2016). Though the global presence of the fungus is well documented, the method of exposure to the amphibians is still not established. Despite recent breakthroughs using antimicrobial properties of Two-toed Amphiuma (*Amphiuma means*) skin and exposure to salinity (Stockwell *et al.* 2015) to potentially combat the fungus, there is still no definitive cure for animals infected with the fungus, nor has a method been discovered to rid it from an ecosystem (Pereira *et al.* 2018). On the occasion that a cure or method of eradication of the fungus is discovered, it will need to be applied in the correct and most efficient ways to be effective, which can only happen by understanding amphibian interactions with the surrounding ecosystem.

Additionally, it has been established that a decline in anuran populations has the potential to disrupt the structure, function, and basal resource availability in some neotropical streams, and impact both aquatic and terrestrial food webs due to the nature of anuran development (Ranvestel *et al.* 2004). Local declines of tadpoles have been shown to correlate with increased levels of chlorophyll *a*, increased inorganic sediments, and a shift in the composition of primary producers within the system (Connelly *et al.* 2008). However, the full scope of the trophic and ecological consequences of this massive and widespread decline in amphibian species can only be predicted and managed if the ecological and trophic roles of amphibians are fully understood. This means that deepening our understanding of the specifics of amphibian trophic ecology is of vital importance for the future conservation of both amphibian species and all other species that could potentially be affected by their decline.

There has been a long-standing assumption that most tadpoles are herbivores, primarily consuming periphyton. However, there has been little research done to support this. Additionally, many of the studies that have been conducted to establish tadpole trophic positioning relied on mouthpart morphology or gut analyses to indicate forage behavior and diet choice, indicators which have suggested tadpoles to be herbivores, detritivores, carnivores, or even non-selective feeders depending on the respective study (Montaña *et al.* 2019). In recent studies, it has emerged that most tadpoles may rely on animal tissue in their diet, at least to some degree. It has also been suggested that larval anurans have at least some degree of trophic plasticity depending on various environmental factors, including species density and interspecific competition (Altig *et al.* 2007). This could potentially complicate the process of establishing a trophic position for tadpoles, as it could vary based on spatial and temporal differences, even within a singular species. Diet choice in larval anurans impacts their growth and development. A diet consisting of high protein promotes development but not growth, whereas a low-protein diet promotes growth but not development. There is significant variation in diet selection by tadpoles that is also influenced by environmental factors such as competition and predation (Richter-Boix *et al.* 2006). This furthers the need to understand tadpole trophic interactions within an ecosystem to establish which factors are influencing the growth and development of various tadpole species.

In addition to acting as a predator to tadpoles (Nystrom *et al.* 1997; Wilson and Williams 2014), the presence of freely roaming crayfish has been shown to affect the diet choices of tadpoles. This is likely due to the foraging habits of crayfish, which result in direct competition for macrophytes and algae, and aggressive behaviors of crayfish toward other organisms (Gamradt *et al.* 1997). They also tend to decrease plant biomass within a system, increasing the amount of sediment consumed by many tadpoles (Arribas *et al.* 2015). It has also been suggested that crayfish could potentially be a carrier and reservoir for chytrid fungus, and levels of chytrid infections in Red Swamp Crayfish (*Procambarus clarkii*) can be used as a predictor for chytrid infections in amphibians (Oficialdegui *et al.* 2019). Another study established that crayfish are viable reservoir hosts and can transmit the fungus to tadpoles (McMahon *et al.* 2013). This is particularly important, as Red Swamp Crayfish have been introduced to non-native systems around the globe, making them a potential source of global transmission of the fungus (Brannelly *et al.* 2015). Understanding the extent to which these organisms interact in a natural system, both directly and indirectly, will be the key factor in being able to confidently identify whether this is

merely a correlation or if it is, in fact, a causal mechanism, and being able to implement disease control strategies appropriately as a response.

Fish of the genus *Lepomis* spp. are known omnivores that rely on visual cues when hunting. They primarily feed on insects such as odonate larvae, but will also consume crayfish, mollusks, fish and amphibian eggs or larvae, and other fish, as well as algae and plant material when animal material is not available (Shoup *et al.* 2011; Kitchell and Windell 1970). Similar to crayfish, these fish may also act as both a predator and competition to amphibian species, as well as a potential source of chytrid fungus infections, particularly in closed systems like ponds. In one study, Mosquitofish (*Gambusia holbrooki*) were exposed to the fungus and did not become infected (McMahon *et al.* 2013). However, chytrid fungus has been found to infect non-amphibian hosts such as the Zebra Fish (*Danio rerio*), so there is still significant potential for species of *Lepomis* spp. to become infected (Liew *et al.* 2017). Considering the uncertainty and apparent variability that still surrounds pathogen growth and transmission in non-amphibian hosts, it will be important to understand the relationship between amphibians and *Lepomis* spp. as more research is conducted to determine which organisms may act as hosts for the fungus. In the event that *Lepomis* spp. are identified as a host species, an understanding of their trophic and ecological interactions will allow swift action to be taken to minimize transmission to amphibian species.

Odonate nymphs, particularly those of Aeshnidae, function as significant predators of tadpoles in many natural systems (Laurila *et al.* 1998). When food resources are low, tadpoles move more, leaving them at risk for predation by odonate larvae (Anholt and Werner 1995). It has been established that midges are a carrier of chytrid fungus (Toledo *et al.* 2021), as well as other aquatic insects (Gleason *et al.* 2008). Therefore, it is quite likely that odonate larvae are potential hosts for chytrid fungus in aquatic systems. Not only does this pose a risk for larval anurans, but it could also result in the infection of adult anurans, as well. It is well documented that adult anurans eat odonates (Ruppell *et al.* 2020). Should an odonate become infected and then ingested by an adult anuran, the risk of infection to the anuran is considerably high.

Determination of stable isotopic signatures has become an increasingly ubiquitous method of assessing trophic niche overlap between species, especially when dealing with amphibians (Arribas *et al.* 2015) and other aquatic organisms (Zanden and Rasmussen 1999). Specifically, assessing stable ratios of carbon and nitrogen provides insight as to the trophic relationships and flow of energy within an ecosystem. In any consumer, their  $\delta^{13}\text{C}$  ratio will resemble the  $\delta^{13}\text{C}$  ratio of the organisms that they consume.  $\delta^{15}\text{N}$  ratios indicate trophic position relative to the  $\delta^{15}\text{N}$  ratio of the lowest trophic clade within the consumer's food web, as  $\delta^{15}\text{N}$  ratios tend to become enriched by around 3-4% relative to the organism's diet (DeNiro and Epstein 1981; Minagawa and Wada 1984; Peterson and Fry 1987). A key benefit of stable isotope analysis is the insight it provides as to what an organism is assimilating from its diet rather than just what it is ingesting. Multiple studies have shown that when stable isotopes are examined, diet contents ingested differ from food sources assimilated (Cummins and Klug 1979; Evans-White *et al.* 2003; Parkyn *et al.* 2001). Assimilated food sources are more important for growth and development, which is particularly important for organisms like tadpoles with many predators. (Altig *et al.* 2017).

This study sought to analyze potential niche overlap between larval anurans (tadpoles) and adult anurans, freshwater crayfish, fish of the genus *Lepomis*, and odonate larvae using stable isotope analysis. Considering the lack of literature regarding the trophic positioning of amphibian species, I decided that analyzing these relationships was prudent due to their high levels of co-occurrence on both a local and a global scale. I hypothesized that there would be significant niche overlap between all taxonomic groups due to their proximity in the closed system from which they were to be sampled.

## ***Methods:***

### *Study system and sampling:*

Samples were collected from three adjacent sub-tropical ponds in Baton Rouge, Louisiana at the LSU Aquaculture Research Station over the course of 12 trap nights. The first sampling period took place from February 12, 2021 – April 23, 2021, while the second sampling period took place from September 22 - November 3, 2021. Two of the ponds, respectively labelled “Pond 1” and “Pond 2”, were shallow with minimal overhead forest canopy and a moderate amount of aquatic vegetation near the banks. The primary vegetation in both ponds were cattails of genus *Typha*, *Hydrilla verticillata*, and grass of genus *Juncus*. The third pond, labelled “Ditch,” was a very shallow waterbody with a moderate to intense amount of overhead forest canopy. It is an ephemeral waterbody, exhibiting a completely dry period in November 2021, and was densely covered in *Hydrilla verticillata* and other emergent aquatic vegetation. There was an abundance of algae in all three waterbodies.

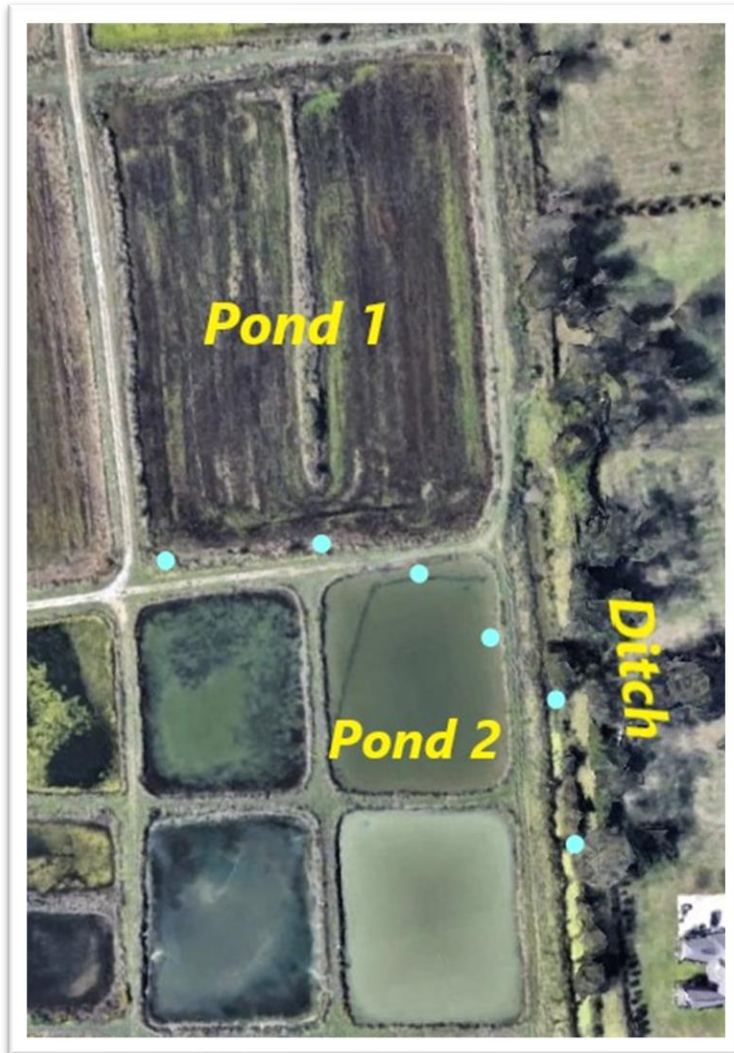


Figure 1: A map of the three sampled waterbodies and trap sites indicated by blue points

Passive sampling has commonly been used as an effective method of sampling for aquatic organisms. As such, two standard galvanized funnel traps were set in each pond at the same site on every trap night (Figure 1). Each trap was baited with an industrial strength glow stick to function as a light trap, (Bennett *et al.* 2012). To minimize trap mortality, trap nights were limited to <30 hours and traps were set half sub-merged (Figure 3; Willson and Gibbons 2010). After traps were retrieved, a hand-held D-frame net was passed through aquatic vegetation in the littoral zone of each pond at three sites no deeper than 1m with the intention of collecting odonate larvae (Bright 1999).

Collected specimens were recorded and placed in bags labeled with the date, site, and capture method used. Specimens were then euthanized on ice in a standard 4.5L ice chest, following standard protocols approved by the Institutional Animal Care and Use Committee (IACUC – A2021-15). At the end of each trap night, water quality data consisting of pH, temperature, and



dissolved oxygen (mg/L) for each pond was taken at the same site using a YSI multiprobe, as water quality plays a key role in habitat suitability for amphibian species (Glooschenko et al. 1992; Sparling 2010). The captured specimens were then taken back to the lab and whole samples were preserved in a deep freezer.



Figure 2: An image of trap retrieval



Figure 3: An image depicting the galvanized minnow trap being set

### *Sample processing:*

All collected specimens were identified to lowest practical unit, producing 4 taxonomic groups: odonate larvae of Aeshnidae, crayfish of *Procambarus clarkii*, anurans (larval and adult) of *Lithobates* spp. and *Acris* spp., and fish of *Lepomis* spp. Adult anurans were later identified to species, producing a Southern Leopard Frog (*Lithobates sphenoccephalus*) and two Eastern Cricket Frogs (*Acris crepitans*). Specimens were sorted into groups of like-taxa by sampling date. They were then placed on trays of aluminum foil to dry in an oven at 60 degrees °C for 48 hours, followed by a period of desiccation of 24 hours (Kaller *et al.* in press). Following this, specimens were ground into powder using a mortar and pestle. White muscle tissue was ground for *Lepomis* spp., tail muscle for crayfish, leg muscle tissue from the thigh of the Southern Leopard Frog, and whole samples were ground for odonate larvae, tadpoles, and the Eastern Cricket Frogs. Three

vials of ground tissue were collected per taxa for each sampling date. Individuals of the same taxa were composited through random selection.

*Stable isotope analysis:*

To prepare for isotopic analysis, 0.55± 0.025 SE mg of tissue was weighed for all respective tadpole, frog, crayfish, and fish tissue samples, while 0.60± 0.025mg of each odonate larvae tissue sample was measured, placed into tin capsules, and given to the LSU Wetland Biogeochemistry Analytical Services Unit for analysis. Samples were then flash-combusted using a Costech ECS4010 elemental analyzer coupled to a Thermo-Fisher Delta Plus XP continuous-flow stable isotope ratio mass spectrometer for carbon and nitrogen stable isotope analyses, respectively. Stable isotope values were normalized using a two-point system with glutamic acid reference material (USGS-40 and USGS-41). Sample precision was based on the standard deviation of repeated reference materials (USGS-40 and USGS-41) and an internal laboratory standard (red drum; see QAQC below). Stable isotope values were calculated with the following equation and are expressed in standard delta ( $\delta$ ) notation in per mil units (‰):

$$\delta X = \left[ \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} \right) - 1 \right] \times 1000$$

Where X is  $^{13}\text{C}$  or  $^{15}\text{N}$  and R is the corresponding ratio  $^{13}\text{C}/^{12}\text{C}$  or  $^{15}\text{N}/^{14}\text{N}$ . The  $R_{\text{standard}}$  values were based on Vienna Pee Dee Belemnite (VPDB) for  $\delta^{13}\text{C}$  and atmospheric  $\text{N}_2$  (AIR) for  $\delta^{15}\text{N}$  values.

*Statistical analysis:*

Multivariate analysis was used to determine whether tadpoles exhibited niche overlap with the other aquatic organisms. Typical stable isotope analysis methods, such as mixture models, were not used because basal food resource samples were not taken. Therefore,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values were compared simultaneously by perMANOVA with taxonomic groups as fixed effects (package vegan, Program R vers. 4.1.0; Oksanen et al. 2020; R Core Team 2021). Prior to performing perMANOVA, data were tested for homogeneity of dispersion.

Intraspecific differences in  $\delta^{15}\text{N}$  can sometimes be explained by the size of sampled individuals, a factor which affects prey availability (Akin and Winemiller 2008). I examined the relationship between  $\delta^{15}\text{N}$  with dry mass of respective taxa using a simple linear regression. For each taxa, the  $\delta^{15}\text{N}$  was estimated as a function of dry weight using the data analysis tool in Microsoft Excel. Using program R, a normality test was conducted to confirm the appropriateness of each dataset for linear regression. To establish significance, I conducted a likelihood ratio test against a null model on each function (package tidyverse, package broom, Program R vers. 4.1.0; ; R Core Team 2021; Robinson et al. 2022; Wickham et al. 2019).

## Results

Although in close proximity, each pond and the ditch differed somewhat in water quality. Pond 1 had an average temperature of 25.4 °C, ranging from 21.2 °C to 29.9 °C throughout the sampling periods. Pond 2 was the warmest pond, with an average temperature of 26.55 °C and ranging from 22.0 °C to 32.3 °C. The Ditch was the coldest pond, but also the pond with the greatest variability (likely due to its shallow depth and significant shade), with an average temperature of 22.96 °C and ranging from 17.1 °C to 28.4 °C. Pond 1 had an average pH level of 8.45, ranging from 7.39-9.5 throughout the sampling periods. Pond 2 had the highest average pH level at 9.09, and was the pond with the most variability, ranging from 6.72-10.13. The Ditch had the lowest average pH level at 7.15, as well as the least variability, ranging from 6.72-7.71. Pond 1 had an average DO level of 8.13 (mg/L), ranging from 3.4 (mg/L) to 12.34 (mg/L) throughout the sampling periods. Pond 2 had the highest average DO level at 12.45 (mg/L), as well as the highest variability, ranging from 6.05 (mg/L) to 16.99 (mg/L). The Ditch had the lowest average DO level at 2.00 (mg/L), ranging from 0.5 (mg/L) to 3.53 (mg/L).

Table 1. Temperature(°C) in each waterbody on each sampling date

Date	Pond 1 Temp(°C)	Pond 2 Temp(°C)	Ditch Temp(°C)
12-Feb	NA	NA	NA
26-Feb	21.7	22.4	19.4
12-Mar	22.9	23.7	22.6
9-Apr	25.5	26.5	21.6
23-Apr	21.6	22	17.1
22-Sep	29.9	31.2	28.4
29-Sep	26	26.8	24.1
6-Oct	29.2	30.2	24.8
13-Oct	29.6	32.3	26.5
20-Oct	25.4	27.1	22.3
27-Oct	25.7	26.9	23.7
3-Nov	21.2	23	22.1

Table 2. pH in each waterbody on each sampling date

Date	Pond 1 pH	Pond 2 pH	Ditch pH
12-Feb	NA	NA	NA
26-Feb	9.32	9.71	7.71
12-Mar	9.5	10.13	7.3
9-Apr	9.3	9.94	7
23-Apr	8.6	9.28	7.26
22-Sep	8.43	8.91	7.08
29-Sep	7.4	8.89	7.06
6-Oct	8.73	9.22	6.72
13-Oct	8.36	9.77	7.18

20-Oct	8.11	9.54	7.3
27-Oct	7.39	7.84	6.84
3-Nov	7.83	6.72	7.17

Table 3. Dissolved Oxygen (%/mL) in each waterbody on each sampling date

Date	Pond 1 DO (mg/L)	Pond 2 DO (mg/L)	Ditch DO (mg/L)
12-Feb	NA	NA	NA
26-Feb	12.34	13.81	2.24
12-Mar	9.8	13.33	3.24
9-Apr	6.77	10.45	0.5
23-Apr	6.5	11.9	0.48
22-Sep	8.33	12.7	3.53
29-Sep	5.6	10.86	1.94
6-Oct	9.99	16.99	2.2
13-Oct	8.4	16.72	2.81
20-Oct	8.48	11.45	2.6
27-Oct	3.4	6.05	1.01
3-Nov	9.8	12.68	1.53

Trapping and netting resulted in collecting different taxa from each pond, reflecting the differences in habitat among ponds (Table 4). In Pond 1, 366 *Lepomis* spp., one frog, three tadpoles, 13 crayfish, and 53 odonates were captured. In Pond 2, 130 *Lepomis* spp., one frog, no tadpoles, three crayfish, and 12 odonates were captured. In the Ditch, 16 *Lepomis* spp., one frog, 15 tadpoles, 81 crayfish, and six odonates were captured. Most taxa were represented for each sampling date.

Table 4. Taxa type caught by sampling date (\* indicates that at least 1 tadpole was caught)

Date	Crayfish	Amphibian	Fish	Odonate
12-Feb	Y	Y*	Y	Y
26-Feb	Y	N	Y	Y
12-Mar	Y	Y*	Y	N
9-Apr	Y	Y*	Y	Y
23-Apr	Y	Y*	Y	Y
22-Sep	N	N	Y	Y
29-Sep	Y	Y*	Y	Y
6-Oct	N	Y*	Y	Y
13-Oct	N	N	Y	N
20-Oct	N	Y	Y	Y
27-Oct	Y	Y	Y	Y
3-Nov	N	N	Y	Y

The initial results showed that each taxa occupied a separate niche, with slight overlap between odonates and crayfish, crayfish and adult frogs, and adult frogs and *Lepomis* spp. (Figure 4). Tadpoles, with the exception of two individuals, utilized a separate carbon pathway than the other taxa and had significant variability in  $\delta^{15}\text{N}$  levels. The remaining two individuals, the only two *Acris* spp., utilized the same carbon pathway as the other taxa and exhibited similar  $\delta^{15}\text{N}$  levels as crayfish and adult frogs.

The data were appropriate for multivariate analyses ( $F_{4,55} = 0.93$ ,  $p = 0.44$ ). Multivariate analyses demonstrated that significant differences in isotopic dispersion existed among the groups ( $F_{4,55} = 26.7$ ,  $p < 0.01$ ). The perMANOVA did not specify which group differed, however, by inspection of Figure 4, the tadpole samples appear to be driving the difference.

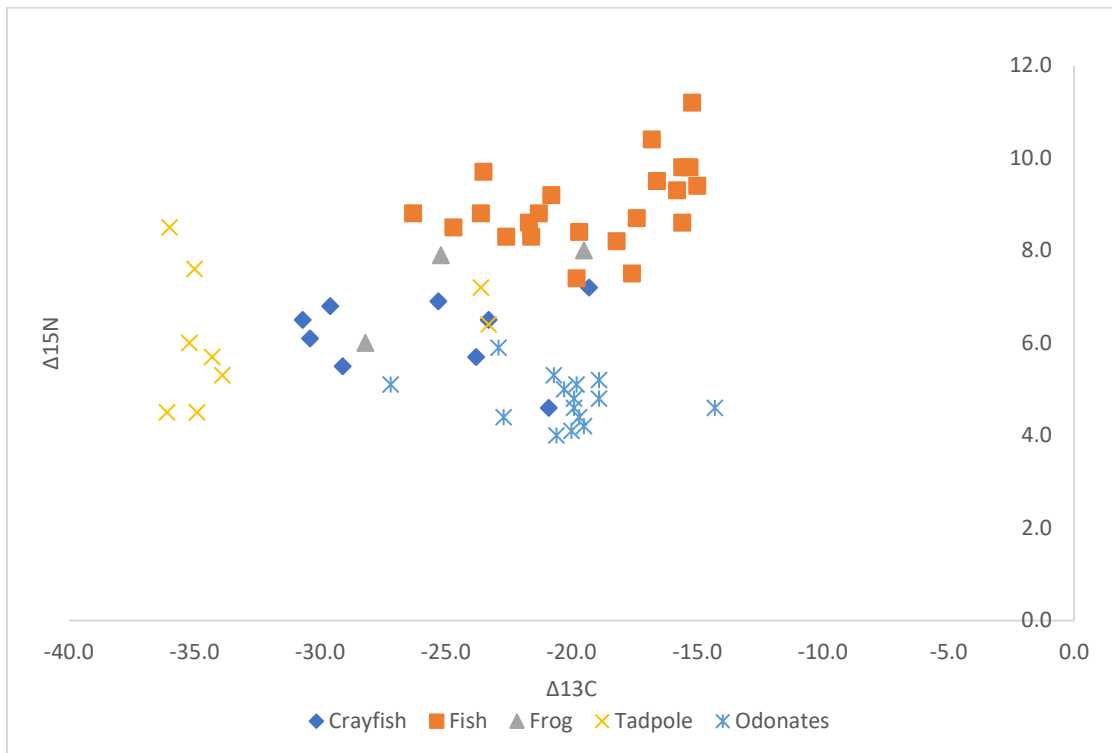


Figure 4: The  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  levels of each specimen, where the x-axis represents  $\delta^{13}\text{C}$  and the y-axis represents  $\delta^{15}\text{N}$

Levels of  $\delta^{15}\text{N}$  as a function of dry weight in grams of sampled tadpoles, *Lepomis* spp., and crayfish varied among taxa (Figure 5-7). All three linear models had an  $R^2$  value of less than 0.5 and p-values that indicated that the relationship was not statistically significant for tadpoles (likelihood ratio test  $\chi^2 = 2.8$ ,  $p = 0.21$ ) and *Lepomis* spp. (likelihood ratio test  $\chi^2 = 1.6$ ,  $p = 0.15$ ) but was statistically significant for crayfish (likelihood ratio test  $\chi^2 = 2.8$ ,  $p = 0.02$ ). The respective organisms sampled in this study showed no significant change in  $\delta^{15}\text{N}$  with size, likely because most individuals were of equivalent size classes, except crayfish which had sufficient size ranges to potentially have more prey diversity. Odonate larvae were not assessed due to insufficient weight data and there were not enough individual adult anurans to justify a regression.

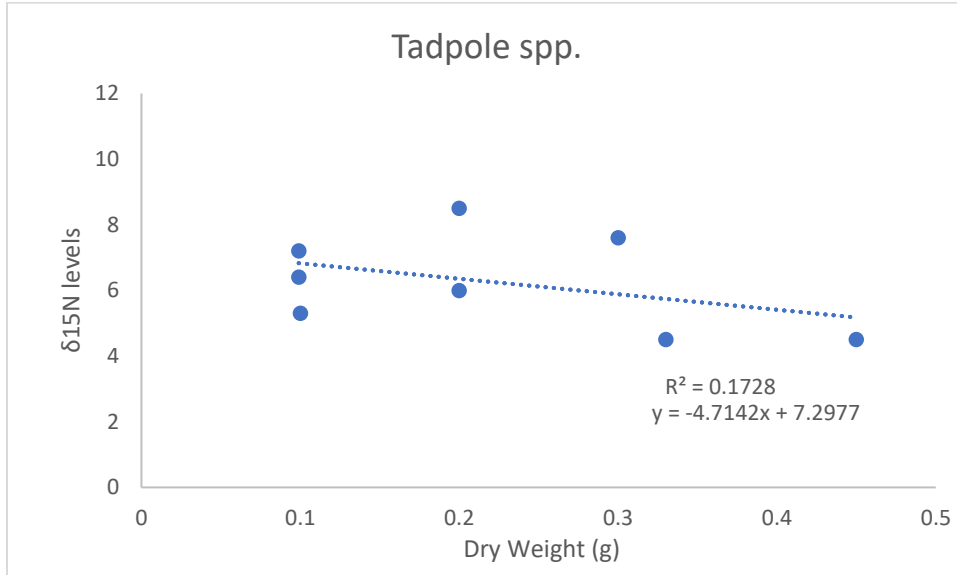


Figure 5: A figure depicting the  $\delta^{15}\text{N}$  levels of the tadpoles as a function of dry weight in grams, where the x-axis is the dry weight (g) and the y-axis is  $\delta^{15}\text{N}$ . The  $R^2$  value is 0.1728. (alpha)=7.2977 (beta)=-4.7142

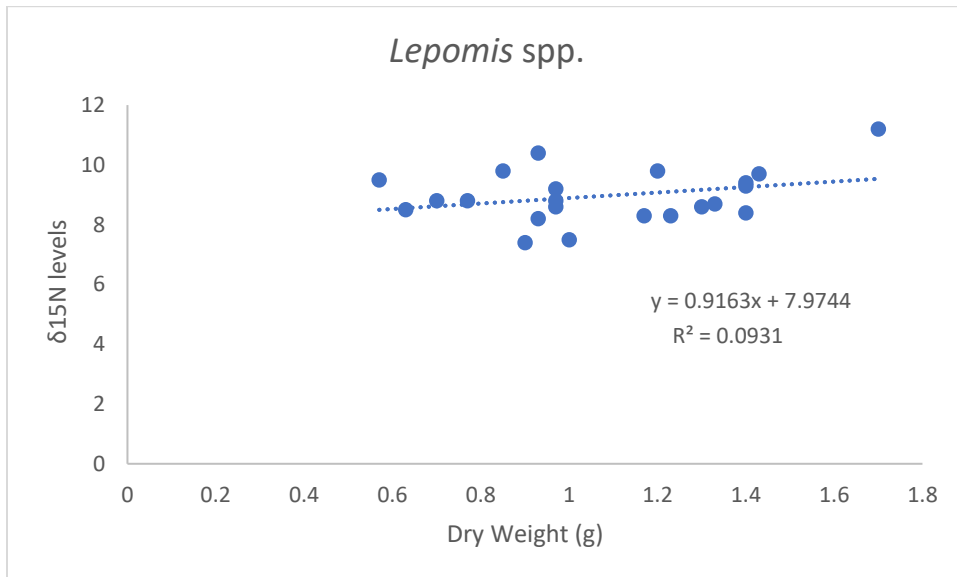


Figure 6: A figure depicting the  $\delta^{15}\text{N}$  levels of *Lepomis* spp. as a function of dry weight in grams, where the x-axis is the dry weight (g) and the y-axis is  $\delta^{15}\text{N}$ . The  $R^2$  value is 0.0931. (alpha)=7.9744 (beta)=0.9163x

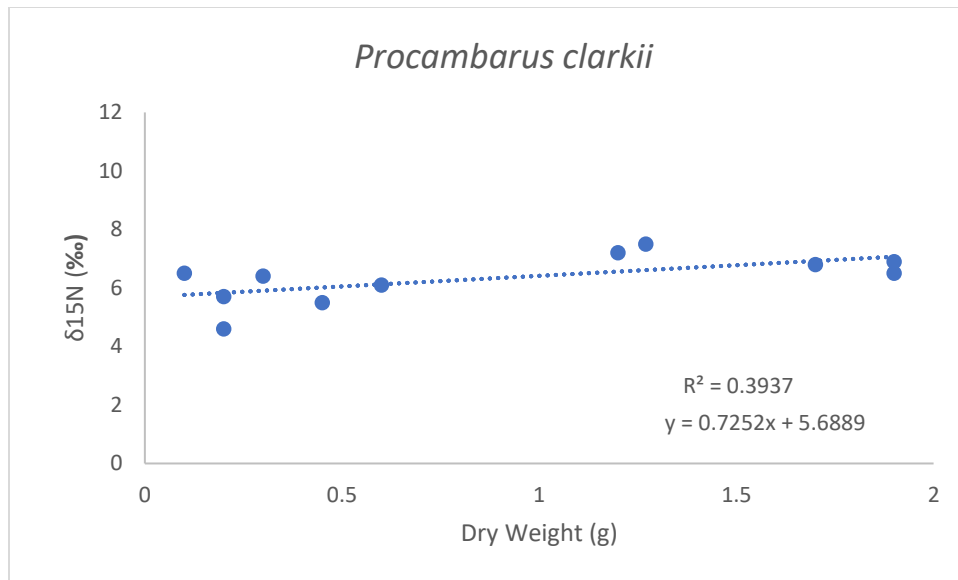


Figure 7: A figure depicting the  $\delta^{15}\text{N}$  levels of crayfish as a function of dry weight in grams, where the x-axis is the dry weight (g) and the y-axis is  $\delta^{15}\text{N}$ . The  $R^2$  value is 0.3937. ( $\alpha$ )=5.6889 ( $\beta$ )=0.7252x

## Discussion

The results of this study did not support my hypothesis and showed minimal niche overlap between the targeted taxa. Most amphibians were captured in the Ditch, whereas the Ditch supported the fewest fish and odonates. This was unexpected, considering that it was the coldest waterbody with the most canopy coverage. Colder temperatures are known to reduce growth rates of tadpoles, while increased canopy coverage leads to a general decrease in the nutritional quality of food sources, also reducing growth rates. Reduced growth rates significantly increase the risk of mortality in tadpoles (Schiesari 2006). This was likely because there was more overhead canopy cover, longer drying periods, and, as a result, less fish presence at this site than the other two ponds (Werner *et al.* 2007). Less fish presence likely decreased predation, competition, and increased activity level of the tadpoles, making capture more likely (Richter-Boix *et al.* 2006).

This study did provide evidence supporting the niche plasticity and non-herbivorous trophic positioning of tadpoles. The results support the idea proposed by Altig *et al.* (2007) that most tadpoles exhibit omnivorous tendencies, and more comprehensive studies focusing on the assimilation rather than the ingestion of materials are needed to better understand tadpole trophic ecology. The  $\delta^{15}\text{N}$  ratios of sampled tadpoles indicate that some animal material is consumed, even to the same degree as the *Lepomis* spp. in some individuals (Figure 4). Even at the lowest  $\delta^{15}\text{N}$  ratio value of a single tadpole, it occupies a similar trophic level to odonate larvae and crayfish, both of which are species known to consume animal material as part of their regular diets. Understanding this diet selection by tadpoles in various environmental circumstances will be key to creating and managing environments that produce healthy, viable adult individuals. It

has been previously established that there is a phenotypic trade-off between growth and development in tadpoles dependent on predation and competition (Reylea and Auld 2005). In circumstances where tadpoles are facing intense aquatic predation, having access to high-protein food sources like animal material will allow them to develop more quickly to escape aquatic predators. Limited access to animal material or other high-protein food sources in this circumstance of intense predation could also limit tadpole recruitment, which would have population-wide effects. Conversely, in situations of limited predation but intense competition, growth is likely more important as to be able to out-compete other individuals for resources. This effect has also been shown to induce changes in tadpole mouthparts, tail size, and body size (Reylea and Auld 2005). In addition to these documented phenotypical adaptations, it would be beneficial to conduct further research assessing whether there are behavioral adaptations regarding food selectivity in various environmental circumstances (i.e., are they selecting higher protein foods when under intense predation). This knowledge will be important for both captive breeding programs and management of wild populations, particularly for threatened and endangered species.

The results of the stable isotope analysis also indicate that the tadpoles of *Lithobates* spp. in this system are utilizing a different carbon pathway than the other taxa included in this study. One possible explanation for this is the presence of *Procambarus clarkii* in the system. Arribas *et al.* (2015) found that the presence of crayfish in a system influenced the diet of local tadpoles by forcing them to consume more detritus and other  $\delta^{13}C$  depleted food sources. Moreover, the highly negative  $\delta^{13}C$  values suggested less autochthonous inputs in tadpole diets (France 1995), compared with other organisms. Therefore, crayfish may be forcing tadpoles to use more macrophyte-based food, possibly to avoid the crayfish. This phenomenon is also evidence of potentially significant niche plasticity (e.g., Dangles 2002), as it shows the ability of tadpoles to shift their diets in the presence of an influencing environmental factor.

Surprisingly, there was little evidence of *Lithobates* spp. tadpole predation by the sampled taxa, despite being in a relatively closed system and the knowledge that all sampled organisms are known to be predators of tadpoles. There are a few potential explanations for this, the first being that the individuals sampled were simply too small for the tadpoles to be of ingestible size. Conversely, this could be potential evidence of documented behavioral plasticity by tadpoles in the presence of predators (Richter-Boix *et al.* 2006). The *Acris* spp. tadpoles, however, did not exhibit this change, and were utilizing the same carbon pathway. This could be a result of the general difference in size between the *Acris* spp. and *Lithobates* spp. tadpoles, as the dry weight of all *Acris* spp. tadpoles were <0.1g and the average dry weight of the *Lithobates* spp. tadpoles was 0.27g and ranged from 0.1g to 0.45g. This could also be explained as a difference in the behavioral plasticity and ability to respond to the of individual species. Another explanation for this is the difference in habitat usage by respective species. Both *Acris* spp. tadpoles and one *Lithobates* spp. tadpole were captured in Pond 1, where the concentration of *Lepomis* spp. was highest, whereas the majority of tadpoles were captured in the Ditch, where the density of *Lepomis* spp. was the lowest. It is possible that there was no predation of *Lithobates* spp. tadpoles by *Lepomis* spp. due to this low level of co-occurrence in this system. However, even with the number of individuals present, one would still expect to see some degree of overlap in carbon pathways between these organisms, as they are both assumed to be eating animal material, macrophytes, detritus, and algae in a small, closed system that limits resource



availability. This provides more evidence of niche plasticity in tadpoles, potentially in response to competition and displacement by the *Lepomis* spp.

A major hindrance during the sampling portion of this study was difficulty in procuring amphibian samples and excluding basal food resources. This may have been in part due to less-than-ideal weather conditions in the early spring after an uncharacteristic freeze in February. It is also possible that there were fewer amphibians in the area than anticipated based on habitat, as there was no previous density estimate for the area. It is also likely that there was trap predation, as Broad-banded Watersnakes (*Nerodia fasciata confluens*) were found inside the galvanized minnow traps on three separate occasions when no other taxa were found at the trap site. However, more appropriate sampling methods likely would have produced better results for more adult amphibians. Visual surveys along established transects targeting herpetofauna would increase the likelihood of producing amphibian samples and should be incorporated in further research. This study also solely procured anurans. If resources allow for it, implementing a drift fence and pitfall traps would also increase the likelihood of producing amphibian samples, as well as increase the diversity of amphibians captured (Greenberg *et al.* 1994). Additional tadpoles and adult amphibians could have been captured by including electrofishing (Dgebuaдзе and Baashinskiy 2017; Gilbert *et al.* 2017; Strain and Raesly 2012), although this method would have been impractical in these locations. Though it was outside of the scope of this study, it would also be beneficial to also measure the basal food sources in the system for a more intensive study, which would have allowed for more sophisticated and nuanced analyses.

Furthering our understanding of tadpole trophic ecology prepares us to combat the spread of chytrid fungus to amphibians. Based on my model, these taxa are not interacting extensively beyond predation. However, these organisms have the potential to be hosts for the fungus. More research should be conducted to establish whether they create an indirect path of infection by contaminating the environment or basal food sources of tadpoles. While chytrid fungus is not typically lethal to tadpoles, it does reduce foraging efficiency, which negatively impacts growth, survival, and recruitment (Hanlon *et al.* 2015). However, even on the occasion that these species are determined to be direct or indirect sources of infection, a deep understanding of their ecological interactions will be needed to assess the best course of action. Eliminating or reducing tadpole predators could be catastrophic. The presence of predators often results in density-dependent population regulation (Anholt and Werner 1995), without which would likely result in population booms followed by resource scarcity. It would be a difficult and delicate task to balance limiting the sources of chytrid exposure and maintaining stable populations within an ecosystem, and it can only be done with a comprehensive understanding of the trophic ecology of all organisms involved.

## ***Conclusion***

With the rapid widespread decline of amphibian species, it is becoming increasingly urgent to understand the trophic ecology of amphibians, particularly at the larval stage. Based on my data, there is no significant niche overlap between taxa. Interestingly, however, I found that most tadpoles were utilizing a different carbon pathway than the other sampled taxa and had

unexpected variation in  $\delta^{15}\text{N}$  levels, indicating variation in trophic position. This data supported the proposed ideas that tadpoles exhibit the potential for niche plasticity and have omnivorous tendencies. Considering these trends were evident in a small, closed system with limited sampled organisms, more research needs to be dedicated to uncovering exactly what trophic relationships influence tadpoles in other systems, varying climates, temporal differences, and how these relationships might differ between various species.

## ***Acknowledgements***

I would like to thank everyone at the LSU Freshwater Ecology lab for continually supporting and teaching me the past four years. I would specifically like to thank Mariah Noveh and Dr. Kaller for always answering even my most mundane and obvious questions. I am particularly grateful to Dr. Kaller for sharing all of your knowledge and experience with me, particularly when it came to program R. I would also like to thank Erin Thayer for always encouraging me and giving advice when I needed it. Thank you to Angela Stahl for your patient instruction in sample preparation and to Dr. Polito for kindly allowing me to use your lab for isotope analysis. Finally, I am thankful to the LSU School of Renewable Natural Resources and the Roger Hadfield Ogden Honors College for all the opportunities and support that has been provided to me.

## ***Literature Cited***

- Akin, S., and K. O. Winemiller. 2008. Body size and trophic position in a temperate estuarine food web. *Acta Oecologica* 33:144–153.
- Albecker, M., D. Kramer, M. Albecker, and H. D. Vance-chalcraft. 2015. Distributed under creative commons CC-BY 4.0 OPEN ACCESS.
- Altig, R., M. R. Whiles, and C. L. Taylor. 2007. What do tadpoles really eat? assessing the trophic status of an understudied and imperiled group of consumers in freshwater habitats. *Freshwater Biology* 52:386–395.
- Anholt, B. R., and E. E. Werner. 1995. Interaction between food availability and predation mortality mediated by adaptive behavior. *Ecology* 76:2230–2234.
- Arribas, R., C. Díaz-Paniagua, S. Caut, and I. Gomez-Mestre. 2015. Stable isotopes reveal trophic partitioning and trophic plasticity of a larval amphibian guild. *PLOS ONE* 10:e0130897.
- Bennett, S. H., J. L. Waldron, and S. M. Welch. 2012. Light bait improves capture success of aquatic funnel-trap sampling for larval amphibians. *Southeastern Naturalist* 11:49–58.
- Brannelly, L., T. McMahan, M. Hinton, D. Lenger, and C. Richards-Zawacki. 2015. *Batrachochytrium dendrobatidis* in natural and farmed Louisiana crayfish populations: prevalence and implications. *Diseases of Aquatic Organisms* 112:229–235.
- Bright, E. 1999. Sampling Protocol for Odonata Larvae. Michigan Odonata Survey Technical Note

- Collins, J. P. 2010. Amphibian decline and extinction: what we know and what we need to learn. *Diseases of Aquatic Organisms* 92:93–99.
- Connelly, S., C. M. Pringle, R. J. Bixby, R. Brenes, M. R. Whiles, K. R. Lips, S. Kilham, and A. D. Huryn. 2008. Changes in stream primary producer communities resulting from large-scale catastrophic amphibian declines: can small-scale experiments predict effects of tadpole loss? *Ecosystems* 11:1262–1276.
- Cummins, K. W., and M. J. Klug. 1979. Feeding ecology of stream invertebrates. *Annual Review of Ecology and Systematics* 10:147–172.
- Dangles, O. 2002. Functional plasticity of benthic macroinvertebrates: implications for trophic dynamics in acid streams. *Canadian Journal of Fisheries and Aquatic Sciences* 59: 1563-1573.
- Deniro, M. J., and S. Epstein. 1981. Influence of diet on the distribution of nitrogen isotopes in animals. *Geochimica et Cosmochimica Acta* 45:341–351.
- Dgebuadze, Y. Y. and I. V. Bashinkskiy. 2017. Electrofishing method improves evaluation of amphibian larvae abundance: a case of "beaver rivers." *Integrative Zoology* 12: 345-350.
- Evans-White, M. A., W. K. Dodds, and M. R. Whiles. 2003. Ecosystem significance of crayfishes and stonerollers in a prairie stream: functional differences between co-occurring omnivores. *Journal of the North American Benthological Society* 22:423–441.
- France, R. 1995. A critical examination of stable isotopes analysis as a means for tracing carbon pathways in stream ecosystems. *Canadian Journal of Fisheries and Aquatic Sciences* 53: 651-656.
- Gamradt, S. C., L. B. Kates, and C. B. Anzalone. 1997. Aggression by non-native crayfish deters breeding in California newts. *Conservation Biology* 11: 793-796.
- Gilbert, E. I., J. C. Dean, and R. M. Maglothin. 2017. Responses of American Bullfrog, *Lithobates catesbeianus*, and Southern Leopard Frog, *Lithobates sphenoccephalus*, to low voltages in uniform aquatic electrical fields. *Southeastern Naturalist* 62: 148-154.
- Gleason, F. H., M. Kagami, E. Lefevre, and T. Sime-Ngando. 2008. The ecology of chytrids in aquatic ecosystems: roles in food web dynamics. *Fungal Biology Reviews* 1:17–25.
- Glooschenko, V., W. F. Weller, P. G. R. Smith, R. Alvo, and J. H. G. Archbold. 1992. Amphibian distribution with respect to pond water chemistry near Sudbury, Ontario. *Canadian Journal of Fisheries and Aquatic Sciences* 49:114–121.
- Greenberg, C. H., D. G. Neary, and L. D. Harris. 1994. A Comparison of Herpetofaunal Sampling Effectiveness of Pitfall, Single-Ended, and Double-Ended Funnel Traps Used with Drift Fences. *Journal of Herpetology* 28:319–324.
- Hanlon, S., K. Lynch, J. Kerby, and M. Parris. 2015. Batrachochytrium dendrobatidis exposure effects on foraging efficiencies and body size in anuran tadpoles. *Diseases of Aquatic Organisms* 112:237–242.
- Kaller, M.D., C.C. Green, and A.H. Haukenes. In press. Growth and Development. *Methods for Fish Biology*, 2nd edition. S. Midway, C. Hasler, and P. Chakrabarty, editors. American Fisheries Society. Bethesda, MD.
- Kitchell, J. F., and J. T. Windell. 1970. Nutritional value of algae to bluegill sunfish, *Lepomis macrochirus*. *Copeia* 1970:186–190.
- Laurila, A., J. Kujasalo, and E. Ranta. 1998. Predator-induced changes in life history in two anuran tadpoles: effects of predator diet. *Oikos* 83:307–317.
- Liew, N., M. J. Mazon Moya, C. J. Wierzbicki, M. Hollinshead, M. J. Dillon, C. R. Thornton, A. Ellison, J. Cable, M. C. Fisher, and S. Mostowj. 2017. Chytrid fungus infection in zebrafish

- demonstrates that the pathogen can parasitize non-amphibian vertebrate hosts. *Nature Communications* 8:15048.
- Lips, K. R. 2016. Overview of chytrid emergence and impacts on amphibians. *Philosophical Transactions of the Royal Society B: Biological Sciences* 371:20150465.
- Montaña, C. G., S. D. G. T. M. Silva, D. Hagyari, J. Wager, L. Tiegs, C. Sadeghian, T. A. Schriever, and C. M. Schalk. 2019. Revisiting “what do tadpoles really eat?” a 10-year perspective. *Freshwater Biology* 64:2269–2282.
- McCallum, M. L. 2007. Amphibian decline or extinction? current declines dwarf background extinction rate. *Journal of Herpetology* 41:483–491.
- McMahon, T. A., L. A. Brannelly, M. W. H. Chatfield, P. T. J. Johnson, M. B. Joseph, V. J. McKenzie, C. L. Richards-Zawacki, M. D. Venesky, and J. R. Rohr. 2013. Chytrid fungus *Batrachochytrium dendrobatidis* has nonamphibian hosts and releases chemicals that cause pathology in the absence of infection. *Proceedings of the National Academy of Sciences of the United States of America* 110:210–215.
- Minagawa, M., and E. Wada. 1984. Stepwise enrichment of  $^{15}\text{N}$  along food chains: further evidence and the relation between  $\delta^{15}\text{N}$  and animal age. *Geochimica et Cosmochimica Acta* 48:1135–1140.
- Nystrom, P., W. Axelsson, J. Sidenmark, and C. Bronmark. 1997. Crayfish predation on amphibian eggs and larvae. *Amphibia-REptilia* 18: 217-228.
- Oficialdegui, F. J., M. I. Sánchez, C. Monsalve-Carcaño, L. Boyero, and J. Bosch. 2019. The invasive red swamp crayfish (*Procambarus clarkii*) increases infection of the amphibian chytrid fungus (*Batrachochytrium dendrobatidis*). *Biological Invasions* 21:3221–3231.
- Oksanen, J., F. G. Blanchet, M. Friendly, R. Kindt, P. Legendre, D. McGlenn, P. R. Minchin, R. B. O'Hara, G. L. Simpson, P. Solymos, M. H. H. Stevens., E. Szoecs, and H. Wagner. 2020. vegan: Community Ecology package. R package version 2.5-7. <https://CRAN.R-project.org/package=vegan>
- Parkyn, S. M., K. J. Collier, and B. J. Hicks. 2001. New Zealand stream crayfish: functional omnivores but trophic predators? *Freshwater Biology* 46:641–652.
- Pereira, K. E., B. I. Crother, D. M. Sever, C. L. Fontenot Jr, J. A. Pojman Sr, D. B. Wilburn, and S. K. Woodley. 2018. Skin glands of an aquatic salamander vary in size and distribution and release antimicrobial secretions effective against chytrid fungal pathogens. *Journal of Experimental Biology* 221:jeb183707.
- Peterson, B. J., and B. Fry. 1987. Stable isotopes in ecosystem studies. *Annual Review of Ecology and Systematics* 18:293–320.
- Ranvestel, A. W., K. R. Lips, C. M. Pringle, M. R. Whiles, and R. J. Bixby. 2004. Neotropical tadpoles influence stream benthos: evidence for the ecological consequences of decline in amphibian populations. *Freshwater Biology* 49:274–285.
- R Core Team. 2021. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.
- Relyea, R. A., and J. R. Auld. 2005. Predator- and competitor-induced plasticity: how changes in foraging morphology affect phenotypic trade-offs. *Ecology* 86:1723–1729.
- Richter-Boix, A., G. A. Llorente, A. Montori, and J. Garcia. 2007. Tadpole diet selection varies with the ecological context in predictable ways. *Basic and Applied Ecology* 8:464–474.
- Robinson, D., A. Hayes, and S. Couch. 2022. broom: Convert statistical objects into tiny tibbles. R package version 0.7.12. <https://CRAN.R-project.org/package=broom>

- Rüppell, G., D. Hilfert-Rüppell, B. Schneider, and H. Dedenbach. 2020. On the firing line – interactions between hunting frogs and Odonata. *International Journal of Odonatology* 23:199–217.
- Schiesari, L. 2006. Pond canopy cover: a resource gradient for anuran larvae. *Freshwater Biology* 51:412–423.
- Shoup, D. E., M. A. Nannini, and D. H. Wahl. 2012. The effect of vegetation density on juvenile bluegill diet and growth. *Journal of Freshwater Ecology* 27:199–209.
- Sparling, D.W. 2009. Amphibian water quality. Part 2.7 in C. K. Dodd, Jr. editor. *Amphibian ecology and conservation: a handbook of techniques*. OUP Oxford.
- Stockwell, M. P., J. Clulow, and M. J. Mahony. 2015. Evidence of a salt refuge: chytrid infection loads are suppressed in hosts exposed to salt. *Oecologia* 177:901–910.
- Strain, G. F. and R. L. Reasely. 2012. Amphibian sampling techniques along Maryland coastal-plain streams. *Northeastern Naturalist* 19: 229-248
- Toledo, L. F., J. Ruggeri, L. Leite Ferraz de Campos, M. Martins, S. Neckel-Oliveira, and C. P. B. Breviglieri. 2021. Midges not only sucks, but may carry lethal pathogens to wild amphibians. *Biotropica* 53:722–725.
- Werner, E. E., D. K. Skelly, R. A. Relyea, and K. L. Yurewicz. 2007. Amphibian species richness across environmental gradients. *Oikos* 116:1697–1712.
- Whiles, M. R., R. O. Hall, W. K. Dodds, P. Verburg, A. D. Huryn, C. M. Pringle, K. R. Lips, S. S. Kilham, C. Colón-Gaud, A. T. Rugenski, S. Peterson, and S. Connelly. 2013. Disease-Driven Amphibian declines alter ecosystem processes in a tropical stream. *Ecosystems* 16:146–157.
- Van der Sluijs, J. P. 2020. Insect decline, an emerging global environmental risk. *Current Opinion in Environmental Sustainability* 46:39–42.
- Wickham, H., M. Averick, J. Bryan, W. Chang, L. D. McGowan, R. François, G. Grolemond, A. Hayes, L. Henry, J. Hester, M. Kuhn, T. L. Pedersen, E. Miller, S. M. Bache, K. Müller, J. Ooms, D. Robinson, D. P. Seidel, V. Spinu, K. Takahashi, D. Vaughan, C. Wilke, K. Woo, and H. Yutani. 2019. Welcome to the tidyverse. *Journal of Open Source Software* 4:1686.
- Wilson, N. J., and C. R. Williams. 2014. A critical review of freshwater crayfish as amphibians predators: Capable consumers of toxic prey? *Toxicon* 82: 9-17.
- Willson, J. D., and W. Gibbons. 2010. Drift fences, coverboards and other traps. Part 4.13. C. K. Dodd, Jr. editor. *Amphibian ecology and conservation: a handbook of techniques*. OUP Oxford.
- Zanden, M. J. V., and J. B. Rasmussen. 1999. Primary consumer  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  and the trophic position of aquatic consumers. *Ecology* 80:1395–1404.