Diet comparison in three tadpole species, *Rana sylvatica*, *Bufo americanus*, and *Pseudacris crucifer*, in a northern temperate climate

Jennifer K. Quammen and Dr. Richard D. Durtsche

Department of Biological Sciences
Northern Kentucky University
Highland Heights, KY 41099

Abstract

The natural diet of northern temperate tadpoles is a largely neglected area of study. We investigated the natural diets in three anuran larvae, the wood frog (*Rana sylvatica*), the American toad (*Bufo americanus*), and the spring peeper (*Pseudacris crucifer*), from several ephemeral ponds in northern Minnesota. Previous laboratory studies suggest that these species, as well as others with similar mouthparts, are herbivorous suspension or filter feeders. Our results suggest that all three species are active grazers upon the periphyton (aüfwichs or algal material) found on aquatic vegetation and submerged substrates and detritus. The diet of these tadpoles was primarily composed of detritus: 73.3 % in *B. americanus*, 73.9 % in *R. sylvatica*, and 82.1 % in *P. crucifer*. *R. sylvatica* consumed a statistically greater proportion (by volume) of invertebrate foods than the other tadpole species, lending support to their suggested predacious feeding behavior. However, this pattern is most likely biologically insignificant, as these foods made up < 2 % of the diet. The remainder of the identifiable organic foodstuffs were counted and analyzed to find possible interspecific trends. Diatoms made up the highest proportion of these identifiable items; diatoms contain large quantities of lipids that tadpoles may utilize as an energy source. The green and blue-green algae comprised the majority of the remaining identifiable foodstuffs. These data bring into question the nutritional contribution made by detritus and periphyton to the development of tadpoles in the life history of the frog, and the trophic positioning of anuran larvae in ephemeral aquatic ecosystems.

Introduction

The natural diet of common northern temperate frog and toad (anuran) larvae is a topic that has received little attention. There have been many studies into the feeding strategies of tadpoles under laboratory conditions (e.g., Seale *et al* 1980); however, few have considered the natural diet of these organisms (e.g., Jenssen 1967, Dickman 1968). The general view has been that anuran larvae are herbivorous filter or suspension feeders (Duellman and Trueb 1986). Diet is especially important in tadpoles because many species are in short-lived aquatic environments (i.e., ephemeral ponds). This places additional constraints on these tadpoles to consume foods that will ensure they reach metamorphosis prior to the drying of the pond. Some tadpoles rely on carnivory of other tadpole species to reach their metamorphic state. Petranka *et al* (1994) suggest that *R. sylvatica* tadpoles are facultative predators on *B. americanus* tadpoles, resulting in differential breeding pond selection by adult *B. americanus*. More recently, McDiarmid and Altig (1999) have suggested that many tadpoles are grazers, feeding from the substrates in aquatic systems. We studied the natural larval diets of species from three different families: the wood frog (*Rana sylvatica* - Ranidae); the American toad (*Bufo americanus* - Bufonidae); and the spring peeper (*Pseudacris crucifer* - Hylidae). Understanding the diet and natural history of these organisms is crucial for gaining insight into their complex life histories and interactions. We also investigated pond use among tadpole species and whether these species had differing diet compositions.

Materials and Methods

The following described field methods were carried out at Long Lake Conservation Center (LLCC) in Pallisade, Minnesota in May and June, 2001. LLCC comprises 760 acres of pine forests and peat bogs

which provided an excellent source of tadpoles during our field studies.

There were 10 different ephemeral ponds used for water quality analyses in these investigations, which we refer to as LLCC1 through LLCC10. The main sites from which specimens were taken are LLCC1, LLCC5, and LLCC7. Global positioning system (GPS) of these ponds are as follows: LLCC1 is N4638.881' W9327.884', LLCC5 is N4638.778' W9327.840', and LLCC7 is N4638.742' W9327.811'. The GPS was measured using a Garmin GPS III Plus compass.

Water chemistry measurements

Water from each of the study sites was tested at weekly intervals for nitrite, nitrate, ammonia, total hardness, and phosphate. Simultaneous with water samples were collection, dissolved oxygen, temperature, conductivity, and total dissolved solids, which were measured with Hach equipment (for temperature, conductivity, and total dissolved solids) and Yellow Springs Institute (YSI) model 52 (for dissolved oxygen and temperature). The water chemistry analyses were performed using Hach equipment and chemicals. Physical parameters of the ponds were observed, including perimeter distance, average and maximum depth, and the GPS location. The perimeter was measured using a roll-a-meter device. The depths were measured with weighted rope, marked at every 10 cm. In addition to these measurements, StowAway TidbiT Temperature data-loggers were used at varying depths in several ponds to record the thermal profile of these aquatic sites.

Climatic data

The relative humidity, minimum and maximum temperature, and rainfall were measured on a daily basis at a central location at LLCC. The equipment used for these measurements were a barometer, thermometer, and rain gauge. The outdoor relative humidity and temperature were also recorded using Hobo data loggers at the same location where the other climatic measurements were taken.

Morphology of specimens

The measurements taken of specimens were total body length, snout-vent-length (SVL), body height, body width, body mass, and developmental stage. Total body length was measured with digital calipers from the snout to the tail tip (straightened), SVL from snout to vent, body height as the greatest distance from ventral to dorsal surfaces, body width as the greatest distance from right to left sides. Body mass was determined with a digital scale (Acculab Model PP2060D 0.001g). Species (Altig *et al* 1999) and developmental stage (Gosner 1960) were determined for each specimen. Further measurements were taken for specimens used for dissection and their stomach contents. These measurements included total gut mass, total gut length, and average gut diameter and were taken using the digital scale and the digital calipers.

Separation and quantification of digesta

Tadpoles were cold anesthetized and pithed prior to dissection. Tadpoles were dissected using a dissection microscope, small forceps, and small scissors. Once the digestive system was removed from the specimen (from esophagus to anus), the mass was taken. Next, the gut was uncoiled and straightened to obtain accurate measurements of gut length and diameter. For an initial set of tadpoles (n = 4), the gut was partitioned into five sections (stomach/esophagus, foregut, midgut, hindgut, and colon) to determine if the portion of identifiable materials decreased as they progressed through the digestive tract due to assimilation. In all specimens studied, there was a decline in the volumetric percentage of identifiable materials, which led us to limit analysis to the first one-third of the digestive tract.

Once the gut was measured and sectioned, the gut was opened and the digesta removed, again under a dissecting microscope. This material was then transferred to a slide to prepare a wet mount of the materials. To identify and quantify the digesta, slides were examined under a compound microscope at 100x and 400x magnifications. The total percentage of unidentifiable detritus, dead organic matter, was determined with an ocular grid through both random and transect counting methods. The average of these individual percentages was reported as the percentage detritus for that specimen. Also, transects of the slides were completed and counts (frequencies) of food items were determined. We chose to divide the foodstuffs into ten separate categories: filamentous green algae, colonial green algae (including desmids), unicellular green algae, diatoms, blue-green algae, dinoflagellates, euglenoids, macrophyte pieces, pollen grains, and invertebrates.

Table 1: Descriptive Statistics for Climatic Data

Descriptive Statistics	Mean	Std. Dev.	Std. Error	Count	Min.	Max.	# Missing
Minimum temperature	9.396	4.616	0.942	24	2.000	20.000	3
Maximum temperature	19.667	5.767	1.177	24	6.500	29.000	3
Daily rainfall (cm)	0.829	1.412	0.277	26	0.000	5.715	1

-

Table 2: Descriptive Statistics for LLCC1 Water Chemistry

Inclusion Criteria: LLCC1 from LLCC Water Chemistry	Mean	Std. Dev.	Std. Error	Count	Min.	Max.	# Missing
Temp from DO meter	13.967	2.046	0.528	15	11.400	17.200	0
Temp from pH meter	14.020	2.067	0.534	15	11.400	17.300	0
Temp from conductivity meter	13.973	2.191	0.566	15	11.400	17.200	0
Dissolved oxygen (mg/L)	8.069	4.597	1.187	15	4.420	16.960	0
pH	5.700	0.177	0.046	15	5.500	6.000	0
Total dissolved solids	38.927	7.933	2.048	15	23.200	45.700	0
Conductivity	73.467	23.853	9.738	6	51.000	95.600	9

Shape approximations for linear to volumetric conversion

The food items in the digestive tracts were enumerated and their volume was determined through a volumetric conversion from the linear dimensions. The geometric shapes were chosen based on their ability to most accurately approximate the general shape displayed by these food items. A rectangular based box was chosen to represent the volume of the pieces of macrophyte found in the tadpoles. A sphere was chosen for the unicellular algae, the colonial algae, the dinoflagellates, and the pollen grains. A cylinder was used for volumetric approximations of blue-green algae, filamentous green algae, the euglenoids, and the invertebrates. The diatoms were volumetrically approximated with an elliptical cylinder.

Control

Tadpoles were collected from several of our sites for dissection, morphology information. We collected specimens at different times of the day and night and preserved them in 10% formalin immediately upon capture to avoid any loss of digesta through active digestmatic breakdown.

Statistics

The statistical procedures used in these analyses include descriptive statistics and measures of central tendency.

Results

Climatic data indicate that temperatures during this study ranged from 2° to 29° C (Table 1). Rainfall was sporadic but common with a mean daily rainfall of 0.829 cm. Water chemistry parameters among all ten ponds sampled were not significantly different, and the mean values for these parameters at ponds 1, 5, and 7 are found in Tables 2, 3, and 4, respectively.

In all three tadpole species detritus made up over 70% of the diet (*R. sylvatica* had 73.9%, *B. americanus* had 73.3%, and *P. crucifer* had 82.1%). Figures 1 through 3 provide the diet breakdown for the propor.

Table 3: Descriptive Statistics for LLCC5 Water Chemistry

Inclusion Criteria: LLCC5 from LLCC Water Chemistry	Mean	Std. Dev.	Std. Error	Count	Min.	Max.	# Missing
Temp from DO meter	16.767	3.763	2.173	3	13.800	21.00	0
Temp from pH meter	16.867	3.745	2.162	3	13.700	21.000	0
Temp from conductivity meter	16.667	4.148	2.395	3	13.300	21.300	0
Dissolved oxygen (mg/l)	6.083	1.765	1.019	3	4.200	7.700	0
pH	5.667	0.208	0.120	3	5.500	5.900	0
Total dissolved solids	43.067	6.955	4.016	3	35.200	48.400	1
Conductivity	87.800	16.122	11.400	2	76.400	99.200	1

.

Table 4: Descriptive Statistics for LLCC5 Water Chemistry

Inclusion Criteria: LLCC5 from LLCC Water Chemistry	Mean	Std. Dev.	Std. Error	Count	Min.	Max.	# Missing
Temp from DO meter	16.767	3.763	2.173	3	13.800	21.00	0
Temp from pH meter	16.867	3.745	2.162	3	13.700	21.000	0
Temp from conductivity meter	16.667	4.148	2.395	3	13.300	21.300	0
Dissolved oxygen (mg/l)	6.083	1.765	1.019	3	4.200	7.700	0
pH	5.667	0.208	0.120	3	5.500	5.900	0
Total dissolved solids	43.067	6.955	4.016	3	35.200	48.400	1
Conductivity	87.800	16.122	11.400	2	76.400	99.200	1

tion of the identifiable diet that was not detritus in these species.

Diatoms made up the greatest proportion of the live organic material in all three species. Invertebrate contributions to the diet were significantly higher in *R. sylvatica* than either of the other two tadpole species. *Bufo americanus* tadpoles used for this study were Gosner stages 28.2 (SE = 0.123), *Rana sylvatica* were stages 34.7 (SE=0.151), and *Pseudacriscrucifer* tadpoles were stages 27.4 (SE=0.073).

Discussion

Results from our study indicate that northern temperate anuran larvae, as represented by species from three different families, are predominately detritivores and most likely feed off the periphiton or aüfwich from aquatic vegetation or submerged substrates. This contradicts the views presented by Duellman and Trueb (1986) that tadpoles are predominantly filter feeding herbivores. Observations of tadpole feeding and analysis of their mouthparts indicate that they tend to scrape or graze their food off substrates. The role of detritus presents a new field for energy acquisition that has not been investigated before in tadpole feeding studies. This will open future research into what nutrients and energy they are extracting from this material, and what the origin of the detritus, be it animal or plant, might say about the position of tadpoles in the food chain.

Our study confirms the suggested separation of tadpole populations due to predatory influence. The

ponds used for our collections had a clear population separation between *R. sylvatica* and *B. americanus* tadpoles. However, ponds with *R. sylvatica* also had an increased overall tadpole diversity, including two smaller *Pseudacris* species, while *B. americanus* appeared to be the singular tadpole species in the ponds it inhabited. The ponds studied showed no major differences in water chemistry or physical features that would otherwise lead to this clear species separation. Since it has been proposed that *R. sylvatica* tadpoles are facultative predators, it is possible that *R. sylvatica* tadpoles would require an increased consumption of dietary protein, which was quantifiable through the identification and relative composition of the ingested materials.

Acknowledgements

This research was funded through Greaves Undergraduate Research funding and the Center for Integrative Natural Sciences and Mathematics at Northern Kentucky University. We would like to extend our thanks to all the staff, naturalists, and administrators of Long Lake Conservation Center for providing an environment conducive to research, for housing, and of course for feeding us during May and June 2001. We thank Taya Dickman and Andy Pfaeler for their assistance in specimen and water sample collections.

References

Altig R, McDiarmid RW, Nichols KA, Ustach PC. 1999. Tadpoles of the United States and Canada: A Tutorial and Key.

Dickman, M. 1968. The effect of grazing by tadpoles on the structure of a periphyton community. Ecology 49: 1188-1190.

Duellman WE, Trueb L. 1986. Biology of amphibians. New York: McGraw-Hill. 670 p.

Gosner KL. 1960. A simplified table for staging anuran anuran embryos and larvae with notes on identification. Herpetologica 16: 183-190.

Jenssen TQ. 1967. Food habits of the green frog, *Rana clamitans*, before and during metamorphosis. Copeia: 214-218.

McDiarmid RW, Altig R. 2000. Tadpoles: the biology of anuran larvae. Chicago: The University of Chicago Press. 444 p.

Petranka JW, Hopey ME, Jennings BT, Baird SD, Boone SJ. 1994. Breeding habitat segregation of wood frogs and American toads: The role of interspecific predation and adult choice. Copeia: 691-697.

Seale DB, Beckvar N. 1980. The comparative ability of anuran larvae (genera: *Hyla*, *Bufo*, and *Rana*) to ingest suspended blue-green algae. Copeia: 495-503.

Stevens CE, Hume IA. 1996. Comparative physiology of the vertebrate digestive system. Second Edition. New York: Cambridge University Press. 400 p.

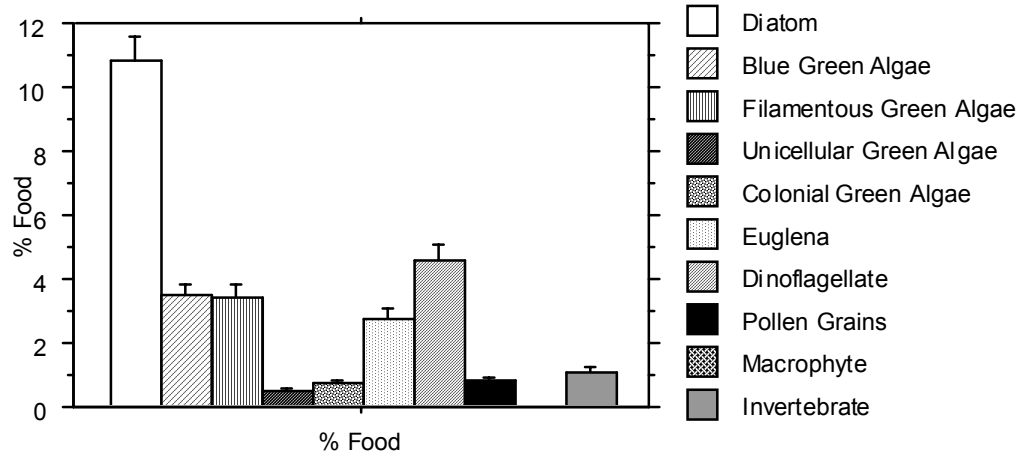

Figure 1. Diet breakdown of identifiable foodstuffs for *Rana sylavatica* (n=23). Unidentifiable detritus (not indicated) made up the vast majority of food in the gut comprising 73.9% of the diet.

..

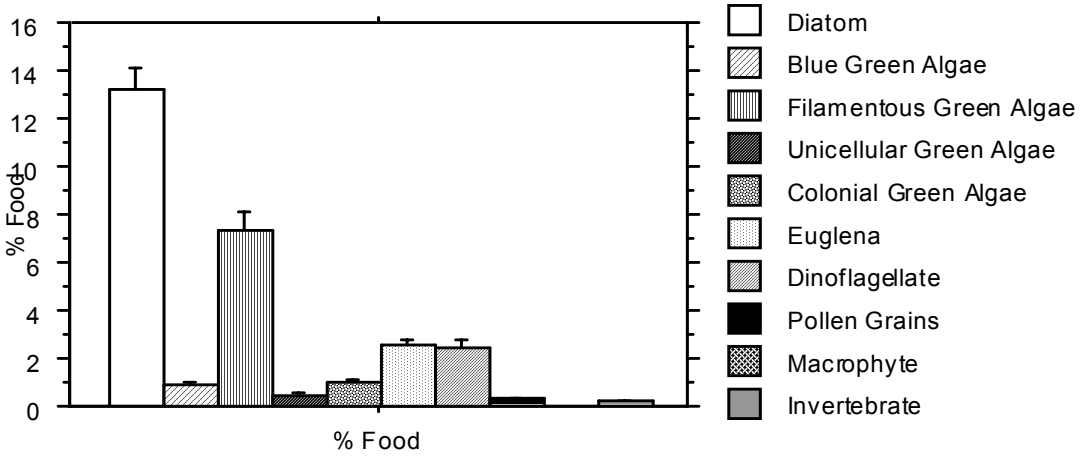

Figure 2. Diet breakdown of identifiable foodstuffs for *Bufo americanus* (n=21). Unidentifiable detritus (not indicated) made up the vast majority of food in the gut, comprising 73.3% of the diet.

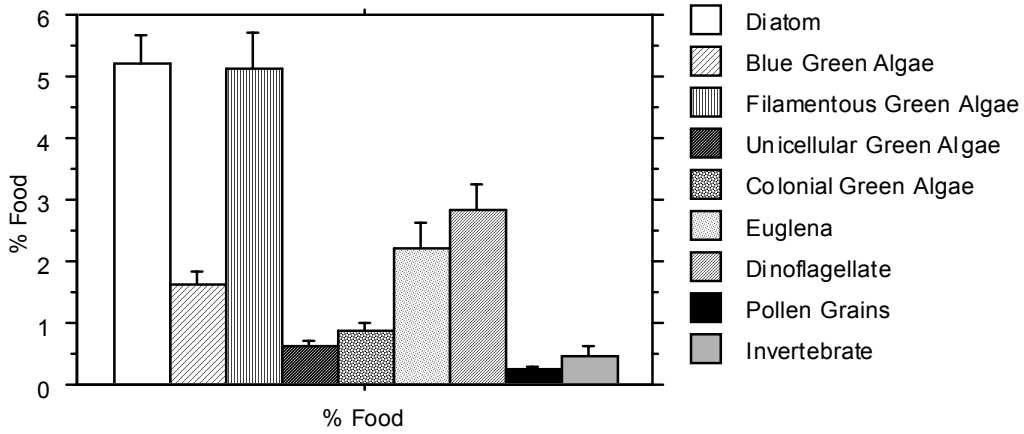

Figure 3. Diet breakdown of identifiable foodstuffs for *Pseudacris crucifer* (n=9). Unidentifiable detritus (not indicated) made up the vast majority of food in the gut, comprising 82.1% of the diet.