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DAVID K. SKELLY[1,4] AND JENNIFER GOLON[2,3]

[1]*School of Forestry & Environmental Studies, Department of Ecology & Evolutionary Biology,
Yale University, 370 Prospect Street, New Haven, CT 06520, USA*
[2]*Department of Ecology & Evolutionary Biology, University of Connecticut, Storrs, CT 06269, USA*

ABSTRACT: Larval anurans are known for having diverse diets, but there have been relatively few studies examining their assimilation of natural foods or how they cope with variation in available resources. In our study, we estimated assimilation of food resources of two species of larval anurans that were presented with benthic substrates from a set of natural ponds. Assimilation was measured during a week-long experiment during which we renewed substrates and collected feces daily. Larvae of wood frogs (*Rana sylvatica*) and spring peepers (*Pseudacris crucifer*) were given benthic substrates from ponds shaded by deciduous forest vegetation as well as from unshaded ponds. We measured organic content in the foregut of tadpoles and from their feces. Estimates of assimilation efficiency were lower than those reported for tadpoles consuming artificial food. Both species experienced reduced assimilation efficiency on closed canopy food resources, but did not differ in their abilities to assimilate organic matter from either type of food resource. However, wood frogs processed more food during the experiment. Our results suggest that natural variation in composition of food resources among ponds could have important effects on tadpole performance and provide some support for interspecific differences in food utilization consistent with relative patterns of performance and distribution.

Key words: Anura; Assimilation; Canopy cover; *Pseudacris*; *Rana*; Tadpole

LARVAL anurans are known for the cosmopolitan nature of their diet. Tadpoles ingest an impressive array of substances, including attached and planktonic algae, senesced vegetation, bacteria, fungi, zooplankton, and animal flesh, as well as inorganic substances such as sand (Calef, 1973; Diaz-Paniagua, 1985; Dickman, 1968; Farlowe, 1928; Hendricks, 1973; Jenssen, 1967; Petranka et al., 1998). It has been presumed that the diverse diets of most tadpoles reflect a nonselective mode of feeding (e.g., Jenssen, 1967). In fact, early researchers went so far as to suggest that tadpole guts represented an unbiased sample of resident algal species (Farlowe, 1928). Perhaps as a consequence of this nonselective view, variation in food resource composition among aquatic habitats has not been viewed as an important factor for patterns of performance and distribution of tadpoles (reviewed by Skelly, 2001). However, more recent research suggests that tadpoles may be selective

and that food composition may affect performance (Kupferberg, 1997; Kupferberg et al., 1994).

In our study, we examined patterns of assimilation by two species of tadpoles, wood frogs (*Rana sylvatica*) and spring peepers (*Pseudacris crucifer*). While several previous studies have estimated assimilation by tadpoles (e.g., Altig and McDearman, 1975), virtually all such studies have used artificial food (see Waringer-Loschenkohl and Shagerl, 2001 for an exception). The purpose of our study was to present information on the utilization of natural food sources by tadpoles.

Additionally, we were interested in the consequences of food resource variation on tadpole performance and distribution. Our focal species are broadly sympatric across much of eastern North America, but differ sharply in their distribution across an important environmental gradient. Spring peeper tadpoles are largely restricted to ponds that do not have fully developed overhead forest canopies while wood frogs are often found in such closed canopy ponds (Skelly et al., 1999). Historical data suggest that forest succession

[3] PRESENT ADDRESS: Department of Zoology, University of Oklahoma, Norman, OK 73109, USA.
[4] CORRESPONDENCE: e-mail, david.skelly@yale.edu

leading to canopy closure can be associated with local extinctions of spring peeper populations (Skelly et al., 1999). While the forest canopy gradient is associated with a number of potentially salient variables (e.g., water temperature; Skelly et al., 2002), food resource composition has received little attention.

Benthic substrates are known to be important food resources for tadpoles (e.g., Kupferburg, 1997; Wassersug, 1975). The forest canopy gradient is associated with strong changes in benthic substrates; benthos of open canopy ponds are often dominated by senesced emergent grasses and sedges, while substrates in closed canopy ponds are often composed of senesced deciduous leaves (Skelly et al., 2002). In both cases, senesced vegetation has an associated periphytic community that can also differ between open and closed canopy environments; periphyton from open canopy ponds tends to be more productive and more diverse (Skelly et al., 2002).

We hypothesized that food resources from closed canopy ponds could be a factor in limiting the performance and distribution of tadpoles. We predicted that the assimilation efficiency of both species would be decreased in closed canopy ponds and that spring peepers would be relatively poor at utilizing food resources from closed canopy ponds as compared with wood frogs. Below, we report the results from an experiment in which tadpoles of both species were presented with controlled amounts of benthic substrate from natural ponds with either closed or open canopies. For each tadpole species, we measured the organic fraction of foregut contents and feces, estimated assimilation efficiency, and calculated the total amount of feces produced.

Methods

Our study was conducted at the Yale Myers Forest (3500 ha) in northeastern Connecticut. We performed a 7-d experiment during which we provided two species of tadpoles, spring peepers and wood frogs, access to one of two types of food resources, closed or open canopy (2 × 2 design). Closed canopy benthic substrates consisted of senesced red maple (*Acer rubrum*) and red oak (*Quercus rubra*) leaves from the bottom of one of two ponds (Blacksmith, Woodpile) categorized as having a closed forest canopy above the basin. Open canopy benthic substrates consisted of senesced burr reed (*Sparganium eurycarpum*) or cattail (*Typha latifolia*) stems from the bottom of one of two ponds (Centre Pike, Dentist) denoted as having open canopy above the basin.

Ponds were categorized as open or closed canopy based on summer spherical densiometer measurements. We measured cover in the center of each pond and 2 m from the shoreline at each of the four cardinal compass points relative to the pond center. Results from the five measurements were averaged to yield a canopy cover estimate for each pond. Open canopy ponds each had <25% cover, while closed canopy had >75% cover.

Tadpoles for the experiment were collected from natural populations at Yale Myers Forest. Wood frogs were collected as eggs from two ponds (Blacksmith, Dentist) on 10 April 1998. Eggs were placed outdoors in wading pools filled with about 125 l of aged well water and, upon hatching, tadpoles were fed a mixture of rabbit chow and senesced deciduous leaves. Spring peepers (which lay their eggs singly) were collected as larvae from two ponds (Dentist, Morse Bog), placed in wading pools, and also fed a mixture of rabbit chow and leaves. Wood frogs typically oviposit and hatch earlier and develop more rapidly than spring peepers. In order to obtain size matched wood frogs and spring peepers, wood frog embryos were collected soon after hatching and placed in wading pools under 50% shade cloth to protect embryos from overheating. At Yale Myers Forest, spring peepers began breeding within 1–2 wk after wood frogs; spring peeper larvae were collected from the earliest cohorts (on 10 May 1998).

Prior to the start of the experiment, individual tadpoles were placed in an experimental container consisting of two 500-ml plastic cups nested together and holding 460 ml of aged well water. The bottom of

the upper cup was removed, and a piece of black fiberglass window screening was placed between the cups to allow feces to fall through. Containers were placed outdoors on a table under shade cloth that allowed about 50% of incident light. Thus, tadpoles were exposed to ambient conditions of day length and air temperature. For example, at 1330 h on 4 June 1998, a cloudless day, temperatures in a subset of 15 cups averaged 18.6 C. Simultaneous measurements of water temperature in closed (Blacksmith) and open (Dentist) canopy ponds were 13.7 and 18.6 C, respectively. Each of the four treatments was replicated 14 times for a total of 56 containers. Location of each container was randomized within the array.

Food was withheld from tadpoles for 48 h to allow them to clear their guts. Then, beginning on 3 June 1998, pieces of benthic substrate were placed in each container and renewed daily. For each food treatment, a daily food ration consisted of a 1 × 6 cm piece of senesced vegetation. Each piece of vegetation was cut to these dimensions using a razor blade. Thus, the total surface area of vegetation presented to each tadpole during the experiment was the same.

Once per day, we collected feces from the space below the screening using a pipette. All collected feces were placed in a plastic vial and frozen at −30 C. At the conclusion of the experiment, each tadpole was weighed and then frozen at −30 C. Subsequently, the gut of each tadpole was dissected out, its length measured, and the contents of the anterior one-third of the tract removed. These foregut contents were then re-frozen. We chose to use one-third of the gut in order to obtain sufficient gut contents upon which to base an assessment of organic content.

After dissections were complete, foregut contents and feces were subject to a drying and ashing regime. Each sample was placed in a pre-weighed aluminum foil cup and placed in a drying oven for ≥45 h at 80 C. After weighing (dry mass), each sample was placed in a muffle furnace for 4 h at 500 C. Samples were then placed in a drying oven for ≥45 h at 80 C and then re-weighed (ash mass). The resulting masses were measured to the nearest 0.001 mg using a Cahn Model C-33 microbalance. In nine cases (ranging between 1 and 3 cases for each of the four treatments), we obtained negative estimates of foregut ash mass (i.e., [ash + vessel] < vessel). In one case, a fecal ash sample was compromised due to a handling error. These tadpoles were excluded from analyses requiring ash mass estimates.

For each sample, dry and ash masses were used to calculate organic content (ash free dry mass/dry mass). Foregut and fecal samples from a given tadpole were used to estimate assimilation using Conover's index (Conover, 1966):

$$U' = [(F' - E') \times (1 - E')^{-1} \times F'] \times 100$$

where F' is the ash-free dry mass per dry mass ratio of food and E' is the corresponding ratio for feces. This measure quantifies changes in the fraction of organic matter between food and feces. A positive value is consistent with assimilation of organic matter. A negative value indicates that organic content of feces exceeded that for food. In this experiment, a negative value could arise for two reasons. First, feces were collected for seven consecutive days and are the cumulative result of many feeding bouts. Of necessity, the food sample for each tadpole was estimated using the contents of the foregut at the particular moment when the experiment ended. It is possible that the tadpole's final meal comprised food that was of lower organic content than previous feeding bouts, possibly resulting in organic content of food that is exceeded by the organic content in feces. Second, because tadpoles secrete mucus that is ingested along with their food (Wassersug, 1975), it is possible that feces could contain more organic matter than their food.

The organic content of foregut and fecal samples, fecal mass, total gut length, final body mass, and U' were analyzed using univariate, two-way analyses of variance. For each of these tests, species (spring peeper or wood frog) and benthic substrate origin (open or closed canopy)

TABLE 1.—Results from analyses of variance of foregut and fecal organic content from tadpoles of wood frogs and spring peepers that were given access to natural benthic substrates from open or closed canopy ponds.

Content	Source	df	Sum of squares	F	P
Foregut	Species	1	0.018	0.55	0.461
	Canopy	1	0.053	1.67	0.204
	Species × canopy	1	0.010	0.32	0.576
	Error	43	1.032		
Feces	Species	1	0.001	0.01	0.988
	Canopy	1	0.072	6.46	0.013
	Species × canopy	1	0.005	0.34	0.564
	Error	51	0.690		

served as factors. Prior to analysis, proportions (organic content, U′) were angular transformed. Final Gosner Developmental Stages were compared between species using a Kruskal-Wallis Test.

RESULTS

Each of the 56 tadpoles survived the duration of the experiment. At the conclusion of the experiment, wet body mass of the two species was approximately equal (spring peepers: $\bar{x} \pm SE = 93.8 \pm 4.8$ mg, wood frogs: 92.4 ± 4.2 mg; $F_{1,52} = 0.045$, $P = 0.833$), but median Gosner Stage (Gosner, 1960) of spring peepers was somewhat more advanced (Gosner Stages: 28 for spring peepers versus 25 for wood frogs, Mann-Whitney U test: $U = 519$, $P = 0.001$). Total gut length (mm) of wood frogs was greater than that of spring peep-

ers (47.1 ± 1.3 versus 41.8 ± 1.5, $F_{1,49} = 7.738$, $P = 0.008$).

Organic content of tadpole foregut material did not differ between wood frog and spring peeper tadpoles, nor did it vary as a function of food treatment (Table 1; Fig. 1). In contrast, organic content of feces varied with food treatment (Table 1; Fig. 1). Tadpoles given access to open canopy food resources tended to yield feces lower in organic content ($\bar{x} = 55\%$) than their counterparts fed on closed canopy food ($\bar{x} = 62\%$). There was no evidence that organic content of feces differed between species; a separate analysis, however, showed that wood frogs produced substantially more feces than spring peepers during the experiment (4.6 ± 0.4 versus 2.7 ± 0.3 mg dry mass), but that fecal production did not vary with food treatment (Table 2; Fig. 2).

We estimated assimilation of organic matter by each individual using Conover's index (U′). We found that U′ did not differ between species, but that tadpoles given open canopy food assimilated more organic material (Table 3). Estimated assimila-
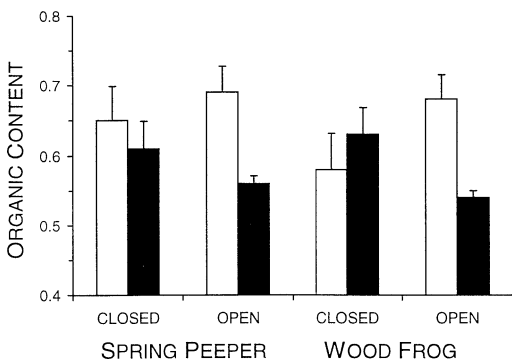

FIG. 1.—Organic content (ash free dry mass/dry mass) of foregut material (open bars) and feces (filled bars) collected from spring peeper and wood frog tadpoles that were given access to natural benthic substrates from open or closed canopy ponds. Bars represent treatment means + 1 SE.

TABLE 2.—Results from analyses of variance of total dry mass of feces produced during a 7-d experiment with tadpoles of spring peepers and wood frogs that were given access to natural benthic substrates from open or closed canopy ponds.

Source	df	Sum of squares	F	P
Species	1	49.779	17.573	<0.001
Canopy	1	3.348	1.18	0.282
Species × canopy	1	1.498	0.53	0.470
Error	52	147.302		

FIG. 2.—Total dry mass of feces collected daily from individual tadpoles over the course of a 7-d experiment. Tadpoles were either spring peepers or wood frogs and were given access to natural benthic substrates from open canopy (open bars) or closed canopy (filled bars) ponds. Bars represent means + 1 SE.

tion efficiency on closed canopy food tended to be small or negative because of extremely negative estimates for a few individuals of both species. In fact, for both species, most individuals given closed canopy substrates yielded positive estimates of assimilation efficiency.

DISCUSSION

The assimilation efficiency of tadpoles given access to natural benthic substrates varied, but was generally lower than that found for tadpoles given access to artificial food (Altig and McDearman, 1975). We found that assimilation efficiency of tadpoles exposed to open canopy substrates was higher than that for closed canopy substrates, averaging 38% for wood frogs and 30% for spring peepers. In contrast, Altig and McDearman (1975) reported assimilation efficiencies between 54 and 86% among four species given access to rabbit chow. These results should not be taken to suggest that natural foods are inherently less nutritious than artificial food. A recent study (Waringer-Loschenkohl and Schagerl, 2001), in which larvae of *R. dalmatina* were exposed to cultures of single phytoplankton species, showed that assimilation efficiency ranged from extremely low (2%, *Chlamydomonas*) to values as high as those found for artificial food (80%, *Spyrogyra*). These authors conclude that variation in efficiency is likely to reflect differences in the constitution of the

TABLE 3.—Results from analysis of variance of assimilation efficiency (U′) estimated from individual tadpoles of spring peepers and wood frogs that were given access to natural benthic substrates from open or closed canopy ponds.

Source	df	Sum of squares	F	P
Species	1	9.926	0.01	0.985
Canopy	1	140078.103	4.96	0.031
Species × canopy	1	827.361	0.03	0.865
Error	42	1185737.851		

cell wall and could be enhanced by the simultaneous ingestion of sand and other inorganic substances that may help to break down cell walls.

Our results suggest that tadpoles are affected by natural variation in the composition of benthic food resources. Tadpoles of both species were less able to assimilate substrates collected from closed canopy ponds. This finding is consistent with a growing number of results that suggest closed canopy ponds can be challenging to larval anurans, possibly owing to differences in light, water temperature, dissolved oxygen, primary productivity, and the composition of periphyton (Skelly et al., 1999, 2002; Werner and Glennemeier, 1999).

We predicted that, if food resources contribute to anuran distributional patterns, then the tadpoles of an open canopy specialist species (the spring peeper) would be less able to utilize closed canopy food resources. In terms of assimilation efficiency, this prediction was rejected. There was no evidence that the species differed in their ability to assimilate organic matter from either resource type. However, we did find evidence that wood frog tadpoles processed more food than spring peepers (i.e., rate of egestion was 70% higher; Fig. 2). Higher processing rates, possibly associated with a longer gut (Altig and Kelly, 1974; Altig and McDearman, 1975; Wassersug, 1975), could aid wood frogs in coping with food resources in closed canopy ponds. Results from a common garden experiment comparing growth on closed versus open canopy resources show that wood frogs can grow as fast or faster on closed canopy food resources (Skelly et al., 2002). This pattern is oppo-

site that observed for spring peepers, which tend to grow faster on open canopy food resources.

In contrast to previously held convictions, it appears (1) that tadpoles are selective in their diet (Kupferburg et al., 1994; Waringer-Loschenkohl and Schagerl, 2001) and (2) that variation in food resources within (Kupferburg et al., 1994; Waringer-Loschenkohl and Schagerl, 2001) and among (our study) ponds is likely to have important consequences for tadpoles. Together, these results raise the possibility that patterns of performance and distribution of tadpoles may be influenced by food resource composition, as has been found among more extensively studied grazing invertebrates (reviewed by Feminella and Hawkins, 1995). A number of researchers have posited that resource based competition between species is widespread, of strong effect, and frequently asymmetric (e.g., Morin and Johnson, 1988). If this is the case, then a mechanistic understanding of resource use by tadpoles is likely to be highly illuminating in trying to understand how competition and other interactions influenced by resource use affect the performance and distribution of anuran larvae.

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