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## Effect of Diet on Bullfrog (*Rana catesbeiana*) Tadpole Growth and Development

Alexander H. Michajliczenko, Geoffrey R. Smith, and Jessica E. Rettig

Among tadpoles, diet can determine growth and developmental rates (e.g., Martínez, Herráez and Álvarez, 1994; Kupferberg, 1997; Babbitt and Meshaka, 2000; Álvarez and Nicieza, 2002; see also review in Alfrod, 1999). In particular, diets with high protein content relative to carbohydrate content result in higher growth and development rates (e.g., Cabrera Peña & Salinas, 1989; Martínez et al., 1993; Carmona-Osalde et al., 1996). Understanding the role of diet in the growth and development of anuran larvae, particularly Ranids, has a practical application. In many regions of the world, frogs are raised as food items. Thus any information that could maximize the production of frogs might be of economic importance (e.g., Martínez et al., 1993, 1994; Carmona-Osalde et al., 1996).

### Methods

We experimentally examined the effects of diet (“carnivorous,” “herbivorous,” or mixed diet; *sensu* Álvarez and Nicieza, 2002) on growth and development of bullfrog tadpoles (*Rana catesbeiana* Shaw). We predicted the highest performance in tadpoles raised on a “carnivorous” diet, followed by those raised on a mixed diet, and that the lowest performance would be for those tadpoles raised on a “herbivorous” diet.

Tadpoles were collected from a local pond (Liberty, Clay Co, Missouri), and haphazardly assigned to a treatment combination. We had three diet treatments: (1) “carnivorous” diet (Pond care® Summer Staple Pond Food: Crude Protein (min) 36.2%; Crude Fat (min) 3.5%; Crude Fiber (max) 4.2%; Moisture (max) 7.4%), (2) mixed diet (1:1 ration by mass of “carnivorous” and “herbivorous” diets), and (3) “herbivorous” diet (Kaytee Natural Alfalfa Cubes: Crude Protein (min) 12%; Crude Fat (min) 1.5%; Crude Fiber (max) 30.0%; Moisture (max) 12.0%). All diets were homogenized into a powder using a blender. There were 20 replicates of each treatment combination.

Tadpoles were kept individually in plastic containers (15 cm X 15 cm X 9 cm) filled with de-ionized water. Tadpoles were fed ( $\approx$  10% of body mass), containers cleaned, and water replaced every fourth day. Tadpoles were kept at room temperature (19°C) and on a 12:12 day:night photoperiod.

Tadpoles were all Gosner stage 25 (Gosner, 1960) when the experiment began, and were matched for size, with no differences in initial body mass among treatment groups ( $F_{2,57}=0.47$ ,  $P=0.62$ ). Tadpoles were weighed approximately every four weeks throughout the experiment which ran from 16 July 1999 through 29 March 2000 (257 d). At each weighing, developmental stage of each tadpole was determined using Gosner (1960).

Mortality of some tadpoles precluded the use of a repeated measures analysis. Therefore, we analyzed the data (body mass and development stage) from the first weighing (i.e., week 4 of the experiment), the data from the last weighing prior to the first metamorph being observed, survivorship (number of days survived; metamorphs were said to have survived the entire experiment since in nature they would have successfully survived the aquatic environment), and Gosner stage at the conclusion of the experiment (after 257 d) using separate one-way ANOVAs.

### Results

Tadpoles on the "carnivorous" diet were significantly larger than tadpoles on the mixed or "herbivorous" diets after only 4 weeks (Table 1;  $F_{2,53}=14.9$ ,  $P<0.0001$ ). Fisher's Protected LSD tests found that all three diets were significantly different from each ( $P\leq 0.009$  in all three comparisons). At this time, all tadpoles were Gosner Stage 26.

After 26 weeks, tadpoles on the "carnivorous" diet were substantially larger than those grown on the mixed or "herbivorous" diet (Table 1;  $F_{2,35}=26.7$ ,  $P<0.0001$ ). All three treatments were significantly different from each other (Fisher's Protected LSD:  $P\leq 0.0006$  in all three comparisons). After 26 weeks, tadpoles on the "carnivorous" diet were more advanced developmentally than those raised on the other diets (Table 1;  $F_{2,35}=37.3$ ,  $P<0.0001$ ). Developmental stage for each treatment was significantly different from each other (Fisher's Protected LSD:  $P\leq 0.031$  in all three comparisons).

At the end of the experiment, tadpoles on protein diets were more developmentally advanced than those raised on the other diets (Table 1;  $F_{2,31}=37.3$ ,  $P<0.0001$ ). All treatment means were significantly different from each other (Fisher's Protected LSD:  $P\leq 0.023$  in all three comparisons). Indeed, 50% of the surviving protein diet tadpoles metamorphosed, whereas 20% of the mixed diet and none of the "herbivorous" diet tadpoles metamorphosed. Diet did not significantly affect survivorship ( $F_{2,57}=0.37$ ,  $P=0.69$ ).

Table 1. Body mass and developmental stage (Gosner, 1960) of bullfrog tadpoles raised on different diets (see text) at various points in the experiment. Metamorphosed individuals were given a value of 41 as this is the stage at which they were considered to have metamorphosed and were removed from the experiment. Means are give  $\pm 1$  SE with  $n$  in parentheses.

	"Carnivorous"	Diet Mixed	"Herbivorous"
<b>Body Mass</b>			
4 weeks	0.631 $\pm$ 0.034 g (18)	0.511 $\pm$ 0.034 g (19)	0.396 $\pm$ 0.020 g (19)
26 weeks	5.526 $\pm$ 0.428 g (15)	3.989 $\pm$ 0.369 g (11)	2.278 $\pm$ 0.242 g (12)
<b>Developmental Stage</b>			
26 weeks	33.2 $\pm$ 0.84 g (15)	30.7 $\pm$ 0.95 g (11)	25.4 $\pm$ 0.42 g (12)
End of Experiment <sup>1</sup>	38.2 $\pm$ 0.91 g (13)	34.5 $\pm$ 1.7 g (9)	26.2 $\pm$ 0.55 g (12)

### Discussion.

Our results for the effects of diet on growth and development of bullfrog tadpoles are generally consistent with previous findings, and with our predictions. "Carnivorous" diets appear to provide the best opportunity for tadpole growth and development (Cabrera Peña and Salinas, 1989; Martínez et al., 1993; Carmona-Osalde et al., 1996; review in Alford, 1999; this study), although this may depend on other conditions (e.g., temperature, Álvarez and Nicieza, 2002). However, there may be a limit to the benefit such a diet can provide tadpoles. Carmona-Osalde et al. (1996) found that the optimal protein content for *Rana catesbeiana* is around 45%. Our results suggest that an "herbivorous" diet is suboptimal and does not allow for tadpole growth and development. Our results, and the results of the other studies mentioned above, suggest diet quality may be more important than diet quantity in many species of anurans (assuming that the amount of food meets the minimal requirements for survival). Indeed, Steinwascher and Travis (1983) found that a diet's protein to carbohydrate ratio influenced *Hyla chrysoscelis* tadpole growth more than the amount of food, but interestingly not in *Rana clamitans*, although protein level and food level did have an effect.

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