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Effects of Variation in Natural Algal and Detrital Diets on Larval Anuran (*Hyla regilla*) Life-History Traits

SARAH J. KUPFERBERG, JANE C. MARKS, AND MARY E. POWER

The quality of algae as food for larval Pacific treefrogs, *Hyla regilla*, varies among algal taxa. Tadpole diets were manipulated in enclosures in the South Fork Eel River, northern California. Enclosed tadpoles were fed ad libitum one of the dominant filamentous green algae (*Cladophora, Zygnema, Mougeotia,* or *Oedogonium*), the dominant cyanobacterium in the habitat (*Nostoc*), flocculent detritus, or a commercial reptile/amphibian food. Tadpoles in a control treatment were not fed but had access to seston which deposited in all enclosures. Diet treatment significantly affected weekly growth rates, as well as time to and weight at metamorphosis. Tadpoles grew most rapidly on filamentous green algae with diatom epiphytes and on the commercial food. Tadpoles fed epiphytized *Cladophora* metamorphosed 27.1% (11.7 days) sooner and weighed 38.9% (70 mg) more than tadpoles fed *Cladophora* cleaned of diatoms. In comparison to tadpoles fed *Mougeotia*, which does not support epiphytes, tadpoles fed epiphytized *Cladophora* metamorphosed 37.4% (19.7 days) sooner and weighed 31.6% (50 g) more. Tadpoles with the lowest growth rates were those filtering deposited seston or flocculent detritus. Observed changes in life-history traits with diet are assessed in relation to models which predict the optimal timing of metamorphosis.

HERBIVOROUS tadpoles consume many different taxa and growth forms of algae including filamentous green algae (Jenssen, 1967), epiphytic algae (Dickman, 1968), epibenthic algae (Calef, 1973), planktonic diatoms, unicellular chlorophytes, and cyanobacteria (Hendricks, 1973; Seale and Beckvar, 1980; Johnson, 1991) as well as algae in tadpole fecal pellets (Steinwachser, 1978a). Despite the taxonomic diversity represented in "herbivorous" tadpole diets, there has been no analysis of the effect of qualitative differences among algal taxa on tadpole growth and development.

Quantitative differences in the abundance (concentration) of phytoplankton between ponds with similar species composition do affect larval size (Johnson, 1991). Perhaps differential effects resulting from qualitative variation in natural diets have gone unstudied because, for filter-feeding tadpoles in ponds and lakes, no selective feeding has been observed (Farlowe, 1928; Jenssen, 1967; Seale and Beckvar, 1980). In these studies, species composition of algae present in tadpole guts was in equal proportion to algal abundance in well-mixed pond plankton. In rivers and streams, however, periphyton is patchy, with different algal taxa dominant in different patches. Therefore, selective feeding by tadpoles is possible, and choices among algal taxa may affect frog fitness. Factors affecting food consumption rates have been suggested as the most important parameters for predicting the size and timing of metamorphosis (Pandian and Marian, 1985a).

The purpose of this study was to determine how variation in algal and detrital food resources available to tadpoles in a northern California river affects three larval life-history traits: growth rate, length of larval period, and size at metamorphosis. These three traits affect survivorship and reproductive potential, hence fitness, in amphibians (Smith, 1987; Semlitsch et al., 1988; Berven, 1990). We assess our results in relation to growth and metamorphosis models (Wilbur and Collins, 1973; Collins, 1979) which assume that amphibian larvae adjust their developmental rate in response to resource levels in the environment. When larvae reach a minimum size necessary for metamorphosis, they may continue growing to some maximal larval size, if the environment is favorable as indicated by high previous growth rates, or initiate metamorphosis, if the aquatic environment is poor. This predicted trade-off between premetamorphic growth and development results in a negative correlation between size and time to metamorphosis such that large tadpoles will transform earlier than smaller individuals.

STUDY SYSTEM AND SITE

The food value of algal and detrital resources was assessed by field manipulations of diets of tadpoles of the Pacific treefrog, *Hyla regilla*. *Hyla*

regilla breeds in a wide variety of aquatic habitats, including lentic areas and stranded sidepools of rivers during low-flow season. Experiments were conducted in the South Fork Eel River, near Branscomb, California (39°44'N, 123°39'W) where *H. regilla* commonly breeds from mid to late May through early Aug. During the rest of the year, *H. regilla* is dispersed in the forests and meadows surrounding the study site at the Northern California Coast Range Preserve. Adult *H. regilla* use algae mats and sedges (*Carex nudata*) as oviposition sites, in particular submerged sedge roots and blades that overhang the water surface.

Rainfall is highly seasonal, and the Eel River has a winter flood/summer drought hydrograph. During the summer low-flow period when the rock-bedded river is clear and sunlit, large algal blooms occur. Early in the season, diatoms and cyanobacteria appear on rock surfaces. Then a macroalgal flora develops which comes to be dominated by *Cladophora glomerata*, a filamentous green alga that grows attached to rock substrates and then sloughs off to form floating mats. In more lentic side pools and under other conditions when *Cladophora* is not abundant, filamentous species from the order Zygnematales, such as *Mougeotia* and *Spirogyra*, dominate. A key difference for tadpole consumers between *Cladophora* and the Zygnematales is the presence of epiphytic diatoms and bacteria. The thick, rough cell walls of *Cladophora* provide attachment sites for diatoms (Lowe et al., 1982), but the thick mucous sheath of the Zygnematales species prevents the attachment of epiphytic diatoms. For further descriptions of algal phenology and this algal-based food web, see Power (1990, 1992). *Hyla regilla* tadpoles at the Eel River have diverse foraging modes which include scraping periphyton off rocks, scraping and biting filamentous algae and epiphytic diatoms in floating mats, bottom feeding on benthic detritus, as well as surface feeding on films of diatoms and pollen (SJK, pers. obs.).

Materials and Methods

Experimental design.—*Hyla regilla* egg masses were collected in side pools of the river in June and July of 1990. Eggs were left attached to plant or algal substrates and maintained until hatching in flow-through field enclosures. Enclosures were 12.7-liter white plastic buckets (30 cm diameter) with two windows (12 cm × 16 cm) covered with 1.0 mm mesh screening. Within two days after hatching, when yolks were reabsorbed, tadpoles were randomly assigned to replicate enclosures. One large cleaned cobble was placed in each bucket to anchor and stabilize the enclosures. Food treatments were randomly interspersed among enclosures. We, therefore, expected variation in larval growth and development caused by maternal and genetic effects (Travis, 1980, 1981; Travis et al., 1987) or by complex interactions among egg size, food level, and density (Berven and Chadra, 1988), to be similar across all replicates and treatments.

Densities were maintained at five larvae per enclosure. In a sidepool with abundant tadpoles, ambient *H. regilla* density, as measured by sampling with a bottomless bucket enclosure, was 2.53 ± 2.53/encl (range = 0–8/encl, n = 15). If tadpoles in buckets died, they were replaced within one day by tadpoles of similar size and dietary history, which had been maintained in separate stock buckets for this purpose. Mortality replacement was curtailed once metamorphosis began in a replicate. Buckets were kept in a shallow lentic area of the river and maintained at a constant depth (15 ± 3 cm) by moving them when necessary to compensate for falling water level in the river. Water temperatures in experimental and replacement buckets were measured midday with a hand-held thermometer. No significant differences among treatment mean temperatures were observed in Experiment I (maximum difference among treatment means = 1.3 C, $F = 0.633$, $df = 5,36$, $P = 0.68$). Treatments in Experiment II were interspersed in the same area of river such that temperature differences among treatments were likely to be similar to Experiment I. Tadpoles were fed ad libitum algal or detrital diets from hatching until metamorphosis. Algae or detritus were added to maintain a visually conspicuous surplus of food resources. Buckets were monitored and maintained daily for food abundance, depth, and metamorphosing frogs, from the initiation of Experiment I on June 27 until Sept. 8, after which buckets were checked twice a week until Oct. 20, when the experiments were terminated. No fouling was observed in buckets.

Growth was measured in the field by weighing tadpoles weekly, July 11–Aug. 29, 1990. Each replicate group of five tadpoles was weighed together to the nearest 0.1 g using an OHAUS Portogram field balance, and a bucket mean per capita mass was calculated. Group weighing was curtailed once metamorphosis began in any given replicate. Each time that tadpoles were removed from their enclosures for weighing, fecal samples were collected, and enclosures were scrubbed and rinsed in the river to remove any algae that might have grown on the walls of the

bucket and to remove any accumulation of detritus. Initial weight of tadpoles was estimated from the average weight measured on a batch of sacrificed hatchlings. Estimation was necessary because recent hatchlings were so small and fragile that it was difficult to weigh them without causing injury.

The time to metamorphosis for each individual was recorded, and each metamorph was weighed in the laboratory to the nearest mg. Individuals were considered fully metamorphosed when tails were completely reabsorbed, which occurred approximately four days after front limb eruption. This measure was used rather than eruption of the first front leg when the metamorphs were still aquatic for two reasons: (1) They were easier to transport when metamorphosis was complete; and (2) Because they cannot eat during this last phase of metamorphosis, this protocol assesses diet effects on complete larval history which includes growth to a peak weight and decline to a final weight. For *Rana tigrina* tadpoles, significant effects of food quality were manifest during the energy depletion process of metamorphosis rather than during the energy accumulation process of larval growth (Pandian and Marian, 1985b).

Diet treatments.—Algae used in diet treatments represent the dominant taxa found in different environments within the South Fork Eel River. Observations regarding algal distribution, macroscopic growth form, and microscopic counts of epiphytes, are summarized in Table 1.

Two food treatment experiments were conducted sequentially. The first experiment covered the wide range of foods available to tadpoles and the second experiment focused on green filamentous algae only. Experiment I consisted of six food treatments: two filamentous green algae, *Cladophora glomerata,* and *Zygnema* sp.; a cyanobacterium, *Nostoc* sp.; flocculent detritus; and a commercial reptile/amphibian food, Tetra Reptomin®. Seston (suspended organic matter including detritus and algal cells), deposited naturally into all buckets; in our sixth (control) food treatment, no other food was provided. Algae were harvested from the river and added to enclosures in a loose form. Flocculent detritus was collected by suction, and equal aliquots were provided to replicate buckets. Samples of the flocculent detritus were examined using light microscopy to monitor composition of its algal flora as the experiment and season progressed. For a list of taxa in flocculent detritus, see Appendix I. Each treatment was replicated six times. Sample size of the seston treatment declined to five over the course of the experiment because of mortality in excess of the supply of replacement tadpoles.

Experiment II was conducted to evaluate differences among filamentous green algae. This set of five treatments consisted of *Cladophora* with a heavy load of epiphytic diatoms; *Cladophora* from which epiphytes were removed by vigorous rinsing; *Mougeotia* sp., *Oedogonium* spp.; and ambient seston as in Experiment I. For both *Cladophora* treatments in Experiment II, the algae were left connected to rocks, and the whole rock was placed in the enclosure. For other treatments, loose algae were provided. The efficacy of diatom removal by rinsing was monitored by examining *Cladophora* filaments under 100× magnification. Each treatment was replicated eight times. Sample size of the seston treatment declined to six over the course of the experiment because of mortality in excess of the supply of replacement tadpoles. Two replicates of the *Oedogonium* treatment, and one replicate of the *Mougeotia* treatment, were lost because of a combination of tipped over buckets and mortality in excess of replacement tadpoles.

To verify qualitative differences among natural food types, samples of algae and detritus used in diet treatments were collected, freeze-dried, and sent to a laboratory (Agricultural and Forestry Research Experiment Station–Palmer Research Center, University of Alaska, Fairbanks) for analysis of crude protein (Isaac and Johnson, 1976). For taxa with sufficiently large samples, carbohydrate (Smith, 1969) and fat (Randall, 1974) content were also assessed (Table 2). For nonaugmented and detrital treatments, quantity and quality may be confounded to the extent that there are low concentrations of algal cells in seston and that composition of edible material in detritus changes as summer progresses. A given diatom cell, e.g., *Epithemia,* is likely to have the same nutritional content whether it occurs in seston, in detritus, or as an epiphyte, but part of the "quality" of a seston diet is its dilute nature. To determine whether tadpoles used the algae provided as food, fecal samples from tadpoles in each treatment were examined microscopically for undigested, partially digested, and empty algal cells.

Statistical analyses.—Analysis of variance (ANOVA) was used to test the effect of larval diet on three response variables: average larval growth rate, length of larval period (number of days from hatching until metamorphosis), and metamorph size (body mass). Bucket means of per capita size were used for analysis of growth rate

TABLE 1. CHARACTERISTICS OF ALGAE USED IN DIET TREATMENTS.

Treatment	Type of algae	Morphology	Substrate	Habitat	Mucus	Epiphyte community
Cladophora glomerata	Division: Chlorophyta Order: Cladophorales	*Branched filaments *Thick cell walls *Turfs up to 2 m	Epilithic Sloughs off to form large floating mats	Lotic or Lentic	−	32 epiphytic diatoms/cell; Primarily *Epithemia* (with cyanobacteiral endosymbiont); also *Cocconeis, Synedra*
Zygnema sp.	Division: Chlorophyta Order: Zygnematales	*Unbranched filaments *Turfs 10–40 cm	Epilithic	Lotic	+	0 epiphytes
Mougeotia sp.	Division: Chlorophyta Order: Zygnematales	*Unbranched filaments	Epilithic, epiphytic, free floating	Lentic	+	0 epiphytes
Oedogonium spp.	Division: Chlorophyta Order: Oedogoniales	*Unbranched filaments	Epilithic, epiphytic, free floating	Lotic or Lentic	−	<1 epiphytic diatom/cell
Nostoc sp.	Division: Cyanophyta Order: Hormonogales	*Membranous or globular colony of unbranched filaments	Epilithic, or free floating	Lotic or lentic	+	0 epiphytes
Flocculent material (mostly detritus with >17 species of algae)	Divisions: Chlorophyta, Chrysophyta, Euglenophyta, Cyanophyta	*Single cells *Colonies *Filaments	Free floating, epiphytic, or in sediment	Warm, shallow pools	−	n/a

TABLE 2. NUTRITIONAL QUALITY OF ALGAL TAXA USED IN MANIPULATIONS OF *Hyla regilla* DIET.

Treatment	Crude protein (%)	Total non-structural carbohydrates (%)	Crude fat (%)
Epiphytized *Cladophora*	11.25	2.00	1.07
Cleaned *Cladophora*	5.88	2.90	0.56
Zygnema sp.	21.13	—*	—
Mougeotia sp.	11.56	—	—
Oedogonium spp.	7.81	10.8	—
Nostoc sp.	34.13	—	—
Flocculent detritus	0.44	<1	0.18

* Missing values indicate insufficient amount of sample for fat and carbohydrate analysis.

because all tadpoles in a bucket were weighed as a group. Because the same individuals were weighed at weekly intervals, size at one week was inherently correlated to size the week before. Growth rates within a bucket could not be treated as independent observations, so a repeated measures ANOVA model (Gurevitch and Chester, 1986) was used to test the effect of diet on larval growth rates. This method allowed comparison of growth trajectories, as whole units, by calculating a single weighted sum of the repeated measures on each replicate group of tadpoles. The time intervals for weighing tadpoles in both experiments were not evenly spaced (0, 2, 3, 4, . . . weeks), so the linear contrast coefficients were computed according to the methods of Grandage (1958). The weighted sums of repeated size measurements were log-transformed to meet homogeneity of variance assumptions.

Data on metamorphs were subjected to nested ANOVAs in which values for individual tadpoles were nested within buckets, and buckets nested within treatments, because the size at metamorphosis and the length of the larval period of each tadpole within a bucket were not statistically independent (Underwood, 1981). With this two-factor ANOVA model, we tested for both bucket and diet treatment effects. To meet assumptions of normality and homogeneity of variance, time to metamorphosis data were log-transformed. Multiple comparisons among treatment means were made using Tukey's highest significant difference test (alpha = 0.05).

To examine the relationship between size at metamorphosis (body mass) and length of larval period [ln(days) to metamorphosis], Pearson's correlation coefficients were calculated on individual tadpoles. Analyses and correlations were conducted using Systat (Wilkinson, 1989).

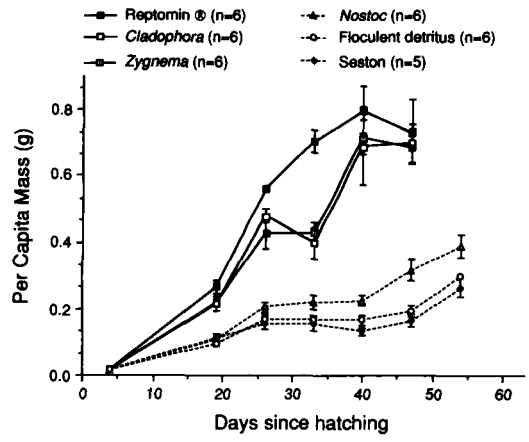

Fig. 1. Growth of *Hyla regilla* larvae fed different algal and detrital diets in Experiment I. Treatments represented by solid, dashed, and dotted lines are significantly (Tukey HSD, $P < 0.001$) different from each other. Error bars are one standard error and sample sizes indicate number of bucket replicates per treatment.

RESULTS

Effect of a wide array of diets (Experiment I).—Diet had a significant effect on growth as revealed by the repeated measures analysis (Table 3). These differences clearly split the treatments into two categories of high and low quality diets (Fig. 1). Larvae fed Reptomin®, and the two filamentous green algae, *Cladophora* and *Zygnema,* had faster growth rates than larvae fed *Nostoc,* flocculent detritus, and larvae with nonaugmented diets. Among the low quality foods, however, the trajectory of tadpoles fed *Nostoc* was significantly ($P = 0.001$) higher than the growth trajectory of tadpoles in the nonaugmented seston treatment.

Fecal analysis revealed that diatoms growing as epiphytes on the *Cladophora* were consumed by tadpoles along with filaments of the macroalga. Empty, broken, and unbroken diatom frustules were the dominant component of feces of tadpoles fed *Cladophora.* The most common diatom genus found in the feces was *Epithemia.* Empty and full *Cladophora* cells were also observed in the feces. Tadpole feces from the *Zygnema* treatment had empty and full *Zygnema* cells and very few diatoms. For tadpoles in the *Nostoc* treatment, there was a marked absence of *Nostoc* cells in their feces even though tadpoles were observed with their mouthparts attached to the balls of *Nostoc.* Feces of tadpoles in the detrital and seston treatments contained mostly amorphous material.

Diet treatment was associated with significant

TABLE 3. REPEATED MEASURES ANALYSIS OF VARIANCE IN GROWTH OF *Hyla regilla* TADPOLES FED DIFFERENT ALGAL AND DETRITIAL DIETS (EXPERIMENT I), AND FILAMENTOUS GREEN ALGAL DIETS (EXPERIMENT II).

		Contrast[a]			
		Size		Growth	
Source	df	MS	F[b]	MS	F[c]
Experiment I					
Grand mean	1	—			1745****
Diet treatment	5	2.19	178****	85.5	77.6****
Error	28	0.0123		3.80	
Total	34			0.049	
Experiment II					
Grand mean	1	—		0.517	5.02*
Diet treatment	4	2.85	50.0****	3.79	36.8****
Error	32	0.057		0.103	
Total	37				

* $P < 0.05$, **** $P < 0.0001$.

[a] Size and growth contrasts are generated from the repeated measures of body mass to test for treatment differences in size and change in size over time, or growth.

[b] The F statistic associated with diet treatment for the size contrast tests the significance of the main effect of diet on larval body mass.

[c] The F statistic associated with the grand mean for the growth contrast tests the hypothesis that there is no linear trend in growth averaged across all diet treatments during the course of each experiment. The F statistic associated with diet treatment for the growth contrast tests the hypothesis that there is no treatment × time interaction (equivalent to the hypothesis that the linear trends of size over time have equal slopes).

differences in weight at metamorphosis (nested ANOVA $F = 37.091$, $P < 0.0001$) as well as time to metamorphosis (nested ANOVA $F = 59.376$, $P < 0.0001$). There were no significant differences among bucket replicates within a food treatment with respect to weight at or time to metamorphosis. Tadpoles eating Reptomin® (426 ± 14 mg) were significantly heavier than tadpoles eating *Zygnema* (294 ± 17 mg) and *Cladophora* (251 ± 9 mg). Tadpoles on poor quality diets metamorphosed at significantly smaller sizes than those on the high quality diets. Live mass at metamorphosis for tadpoles fed *Nostoc* (144 ± 14 mg) and flocculent detritus (132 ± 16 mg) did not differ significantly from the weights of tadpoles in the nonaugmented seston treatment (152 ± 32 mg). Developmental rates of tadpoles were similarly influenced by diet, with a clear break between high and low quality diets. Time to metamorphosis for tadpoles fed *Cladophora* (41.5 ± 1.3 days), *Zygnema* (43.15 ± 2.1 days), and Reptomin® (39.1 ± 9.1 days) did not differ significantly. Development was much slower for tadpoles fed *Nostoc* (69.5 ± 4.5 days). For flocculent detritus (81.3 ± 2.7 days) and seston (78.8 ± 3.4 days), the larval period was almost twice as long as filamentous green algal treatments.

Differences in survival to metamorphosis among diet treatments are summarized in Table 4. These data are underestimates because they refer to the percent of tadpoles surviving after mortality replacement was curtailed. Generally, tadpoles fed higher quality foods in Experiment I also had higher survivorship.

Overall, larger and heavier tadpoles developed faster than did smaller, lighter individuals, thus creating a negative relationship between time to metamorphosis and weight at metamorphosis across all treatments (Fig. 2, $r = -0.529$, $n = 95$, $P < 0.001$). The value and sign of the correlation, however, was not consistent within individual treatments. In high food quality treatments, tadpoles that developed more

TABLE 4. SURVIVORSHIP TO METAMORPHOSIS OF *Hyla regilla* TADPOLES FED ALGAL AND DETRITAL DIETS.

Experiment I		Experiment II	
Treatment	Survivorship	Treatment	Survivorship
Reptomin	80%	Epiphytized *Cladophora*	85%
Cladophora	80%	Cleaned *Cladophora*	67.5%
Zygnema	53%	*Mougeotia*	71%
Nostoc	40%	*Oedogonium*	40%
Flocculent detritus	40%	Ambient seston	40%
Ambient seston	28%		

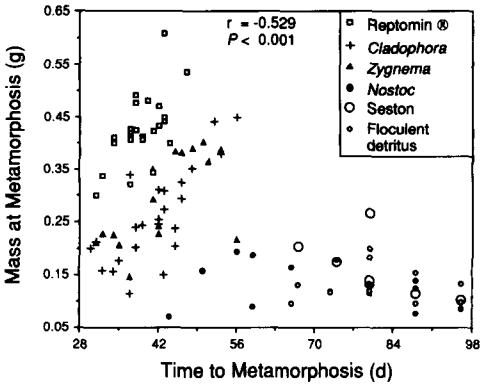

Fig. 2. Relationship between mass at metamorphosis and length of larval period for all tadpoles in Experiment I (n = 95 metamorphs).

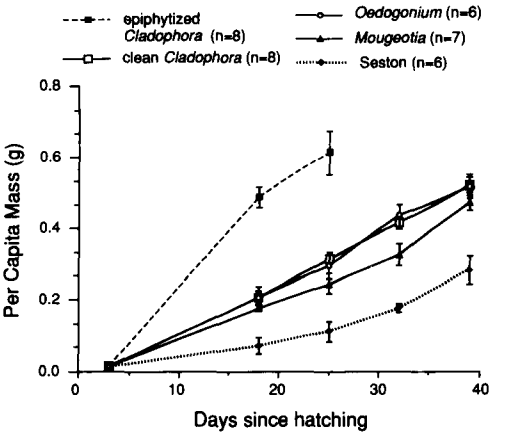

Fig. 3. Growth of *Hyla regilla* larvae fed green filamentous algal diets in Experiment II. Symbols as in Figure 1.

slowly metamorphosed at greater weights (Reptomin®, r = 0.621, n = 24, $P < 0.001$; *Zygnema*, r = 0.638, n = 16, $P < 0.005$; and *Cladophora*, r = 0.747, n = 24, $P < 0.001$), but tadpoles in low food quality treatments did not (*Nostoc*, r = −0.181, n = 12, $P > 0.05$; flocculent detritus, r = 0.053, n = 12, $P > 0.5$; and seston, r = −0.595, n = 7, $P > 0.1$).

Effect of various filamentous green algal diets (Experiment II).—Variation in quality among different types of filamentous green algae also had significant effects on larval size as well as significant differences in slope of growth rate trajectories (Table 3). Tadpoles fed *Cladophora* with epiphytic diatoms (approx. 32 diatoms/host cell) had a dramatically higher growth rate than tadpoles fed the other three filamentous green algal diets (Tukey HSD multiple comparison, $P = 0.001$; Fig. 3). Tadpoles fed *Oedogonium* (<1 diatom/host cell) and cleaned *Cladophora*, which also had far fewer diatoms than epiphytized *Cladophora*, had very similar growth trajectories. The trajectory for tadpoles fed *Mougeotia*, which lacks epiphytes completely, was consistently below but not statistically different from trajectories of tadpoles fed *Oedogonium* and cleaned *Cladophora*.

Presence or absence of epiphytic diatoms among the treatments influenced size at metamorphosis as well as time to metamorphosis. Weights of tadpoles eating epithytized *Cladophora* (250 ± 8 mg) and *Oedogonium* (240 ± 6 mg) were significantly higher than weights of tadpoles eating *Mougeotia* (190 ± 2 mg) or cleaned *Cladophora* (180 ± 2 mg). The overall treatment effects on weight (nested ANOVA F = 20.70, $P < 0.0001$) and ln(time) (nested ANOVA F = 44.77, $P < 0.0001$) were highly significant. Tadpoles eating epiphytized *Cladophora* had the shortest time to metamorphosis (31.6 ± 1.30 days, n = 34), almost 12 days sooner than tadpoles eating *Cladophora* from which epiphytes had been removed (43.37 ± 1.35 days, n = 27). Significant variation in weight at metamorphosis also occurred among replicates of epiphytized *Cladophora* (F = 12.4, $P < 0.001$) and *Oedogonium* (F = 2.52, $P = 0.037$). Similarly, significant variation in time to metamorphosis occurred among replicates of epiphytized *Cladophora* (F = 2.413, $P = 0.027$) and *Mougeotia* (F = 4.362, $P = 0.001$). Despite this within-treatment variation, treatment differences were highly significant.

Survivorship to metamorphosis (Table 4) was highest in the epiphytized *Cladophora* treatment. Tadpoles in treatments associated with longer larval periods had correspondingly lower survivorship.

The correlations between size and time to metamorphosis (Fig. 4) were similar to, although not as strong as, those in the first experiment. Across all treatments, there was a negative correlation between length of larval period and weight at metamorphosis (r = −0.287, n = 110, $P < 0.005$), whereas within individual filamentous green algae treatments correlations were positive (epiphytized *Cladophora* r = 0.202, r = 34, $P < 0.25$; clean *Cladophora* r = 0.211, $P < 0.27$; *Oedogonium* r = 0.887, n = 12, $P < 0.0005$; and *Mougeotia* r = 0.237, n = 25, $P < 0.25$). The low quality control, ambient seston, had a negative correlation coefficient value consistent with the results of Experiment I (r = − 0.585, n = 12, $P < 0.05$).

Discussion

Wide array of natural diets (Experiment I).—Within a river habitat, larval *H. regilla* face a complex array of algal and detrital foods offering widely varying nutritional benefits. Dramatic differences in growth and development were associated with diet treatments representative of the resources available at the field site. The most nutritionally beneficial natural foods in terms of growth, development, and survivorship were the filamentous green algae, *Cladophora* and *Zygnema*. The numerous advantages of large larval size and quick development include decreased risk of predation (Calef, 1973; Kruse and Francis, 1977; Semlitsch and Gibbons, 1988) and decreased mortality resulting from habitat desiccation (Tevis, 1966; Newman, 1988; Crump, 1989).

Tadpoles in the *Nostoc* treatment may have grown poorly for several reasons. Although *Nostoc* had the highest crude protein content of all algal taxa, tadpoles may have been unable to break open the tough globular colonies. Alternatively, *Nostoc* may have been digested but of low nutritional value otherwise. *Nostoc* may be somewhat toxic to tadpoles, as has been documented with other genera in the Nostocaceae for fish and livestock (Gorham, 1964).

Tadpoles in the ambient seston and flocculent detritus treatments had the lowest growth and developmental rates, as well as lowest survivorship. Concentrations of suspended seston in the South Fork Eel River may be lower than the threshold at which measurable ingestion and filtration by hylid tadpoles occurs (Seale and Beckvar, 1980). Although the flocculent material initially contained a diversity of algal cells, including 17 taxa, as the season progressed, its algal component declined as its detrital component increased. Its crude protein content was an order of magnitude lower than the other algal treatments. Poor larval quality of *H. regilla* fed detritus or seston is consistent with the growth response of *Bufo americanus* tadpoles fed similar diets (Ahlgren and Bowen, 1991). Flocculent detritus and precipitates of dissolved organic matter collected from river water provided some nutritional value to *B. americanus* tadpoles but did not support growth. Ahlgren and Bowen postulated that lack of growth could arise because detritus has lower levels of protein and amino acids than other foods available to aquatic organisms (Bowen, 1987). Steinwachser and Travis (1983) found that the ratio of protein to carbohydrate was more important than overall food quantity for promoting high growth and developmental rates in *H. chrysocelis*. Protein content may explain some of the differences caused by food treatment in our experiments; however, a direct correspondence between high protein content and high growth/developmental rates was not observed across all treatments.

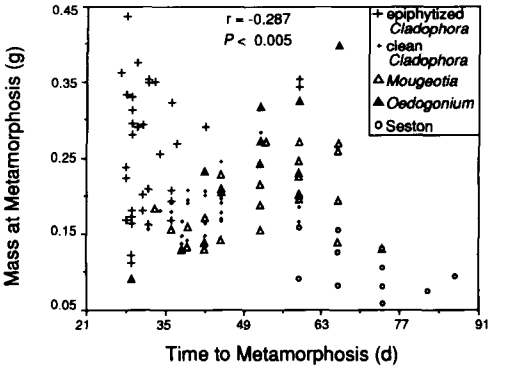

Fig. 4. Relationship between mass at metamorphosis and length of larval period for all tadpoles in Experiment II (n = 110 metamorphs).

Effect of filamentous green algal diets (Experiment II).—The main differences in larval quality among treatments appear to be a result of the presence or absence of epiphytes, consisting primarily of diatoms but also including bacteria. Tadpoles fed *Mougeotia*, which does not support an epiphyte community, and tadpoles fed *Cladophora*, from which epiphytes were removed, grew more slowly and were smaller at metamorphosis than tadpoles grazing epiphytic diatoms off of filaments in the epiphytized *Cladophora* and *Oedogonium* treatments. Diatoms may be richer in calories, fat, or protein than green algae, or all of the above. Additionally stores of nutrients in diatoms may be more accessible to tadpoles than in filamentous green algae. Diatoms store excess photosynthate in the form of lipids whereas Chlorophyte taxa store excess photosynthate as carbohydrate (Bold and Wynne, 1985). A very early study of tadpole nutrition (Emmet and Allen, 1919) found that *Rana pipiens* fed high fat diets were significantly smaller than those fed low fat diets, but the fats used were butterfat and lard and are probably not comparable to the lipids found in diatoms. Essential fatty acid requirements of aquatic consumers may differ among taxa and be met by certain foods but not by others (Dadd, 1981; Cowey and Tacon, 1981). The difference in protein content between *Cladophora* with epiphytes and cleaned *Cladophora* is attributable

to *Epithemia*, the most abundant epiphyte, which contains a cyanobacterial endosymbiont capable of fixing atmospheric nitrogen (Floener and Bothe, 1980). The important role of diatoms is consistent with the results of tadpole gut analyses of two ranid species. Diatoms constitute approximately one-third the gut content biomass of *R. pipiens* (Hendricks, 1973) and 97% of the gut contents of *R. clamitans* in winter months (Jenssen, 1967). Bacteria attached to algae and detritus may also be important food for tadpoles.

The magnitude of the effects resulting from differences in diet composition can be contrasted to those reported for studies in which life-history traits varied in response to manipulations of food quantity. Because significant interactions between larval density and food quantity exist (Wilbur, 1977b; Murray, 1990), we compare our investigation to experiments conducted at similar densities, in terms of number of tadpoles per container. Because these containers were closed systems and smaller than our flow-through enclosures, exact equivalency is impossible; but the overall trends of the comparisons are striking. Wilbur (1977b) reported that for *R. sylvatica* tadpoles raised at a density of four per enclosure there was an approximate 45% increase in weight at metamorphosis with a fourfold increase in rabbit chow, and a 35% increase in weight with a sixfold increase in rations (fouling of the water occurred at this high food level). We observed a similar increase in weight at metamorphosis resulting from diatoms alone. There was a 38.8% increase in weight at metamorphosis between tadpoles fed *Cladophora* cleaned of its epiphytes and tadpoles fed *Cladophora* with epiphytic diatoms. For a more northern population of *R. sylvatica* larvae, Murray (1990) reported a 70% increase in weight at metamorphosis with a sixfold increase in lab rations. We observed a 116.67% increase in weight at metamorphosis between tadpoles in the seston treatment and those fed epiphytized *Cladophora*. The effects of qualitative variation among algal taxa comprising the diet can be as great or greater than the effects of variation in food quantity. Thus, the diversity of algal and detrital resources may account for a significant portion of the variation in tadpole growth and developmental rates observed in the field.

Correlations between size and time.—The observed correlations between size at metamorphosis and length of larval period can be compared to the correlations predicted by existing models of the timing of amphibian metamorphosis. The Wilbur-Collins model predicts that there should be an overall negative correlation between size at metamorphosis and time to metamorphosis. Larger individuals should metamorphose before smaller individuals. This prediction has been upheld for *B. americanus* in laboratory manipulations of diet quantity and timing (Alford and Harris, 1988) as well as in the field for two ponds of wood frog tadpoles (Berven, 1990). In both of our experiments, negative correlations were observed across all treatments, supporting the hypothesis of trade-offs between growth and development.

More complex models also predict negative correlations. These theories for predicting the optimal point of metamorphosis incorporate comparisons of growth rates in the larval aquatic and juvenile terrestrial habitats (Werner, 1986) as well as time constraints for a recent metamorph to reach reproductive status in the following breeding season, such that earlier metamorphosing individuals have higher overall fitness (Rowe and Ludwig, 1991). Seasonal variation in optimal size, resulting in declining sizes at metamorphosis over time, may be the result of an adaptive response to time constraints. Early large individuals gain little advantage by remaining in an aquatic habitat because the "relative benefits of gaining mass decrease with present mass, while smaller larvae early in the season have much to gain by increasing mass" (Rowe and Ludwig, 1991:421).

Within high quality food treatments of this experiment, however, positive correlations were observed, such that tadpoles metamorphosing early were smaller. This trend occurred both within and between buckets. Similar positive correlations in natural populations of bullfrogs, *R. catesbiana*, may be produced by conditions where resources are limiting (Collins, 1979). Once early, large individuals leave the aquatic habitat, the amount of resources for remaining smaller larvae would increase, thus releasing smaller individuals from exploitative competition, increasing mass-specific growth rates, and eventually resulting in metamorphosis at a weight greater than the early individuals. Thus, a positive correlation may reflect a response to environmental fluctuation rather than an underlying optimization strategy. The observed within-bucket trend matched this expected pattern. When the first tadpole metamorphosed, it was the largest amongst the five, and then the smaller individuals subsequently grew and transformed at larger sizes than those achieved by the first to metamorphose. This was not likely to have been caused by exploitative competition, however, because algae was supplied ad libitum. This trend could have been caused by

a release from interference competition. Chemical interference via growth inhibitors by large individuals (Richards, 1962; Licht, 1967; Steinwachser, 1978b) is a possible mechanism but seems unlikely because enclosures exchanged water with the open river. Direct physical interference, in which large individuals monopolize resources (Wilbur, 1977a), may have occurred in algal or Reptomin® treatments. In flocculent detritus and seston treatments, however, food resources were not clumped into defensible patches and may have been below threshold concentrations for ingestion of suspended particles. The nonsignificant correlations between size and length of larval period in those treatments may have occurred because removal of a tadpole under such conditions would not free up any available resources that are suspended or dissolved in the water, whereas patches of algae may become more available when tadpole density declined.

The competitive release hypothesis has been criticized (Alford and Harris, 1988) because tadpoles may experience declining productivity as the season progresses independent of density (Wassersug, 1975; Wilbur, 1980). However, in some systems, primary productivity depends on tadpole density and grazing intensity. As *R. pipiens* complex tadpoles metamorphosed out of a Michigan pond in June, particulate nitrogen and suspended particles, including algae, increased, and intermediate levels of grazing enhanced algal biomass specific production (Seale, 1980). The algal phenology at the Eel River may also create a situation in which tadpoles that stay in the aquatic environment longer experience increases in diatom abundance, and hence in food quality, as *Cladophora* becomes overgrown with epiphytic diatoms.

The effects of algal diet on tadpoles are relevant to amphibian conservation, particularly in light of recent reports of worldwide amphibian declines (Blaustein and Wake, 1990). Human impacts on freshwater habitats such as nutrient loading (Eminson and Phillips, 1978; Schindler, 1990), and acidification (Schindler, 1990), can alter composition of the algal flora. Hydrologic regimes in rivers also strongly influence the composition and biomass of attached algae (Tett et al., 1978; Fisher et al., 1982; Power and Stewart, 1987). A natural absence of flooding can cause replacement of epiphyte loaded *Rhizoclonium* by epiphyte free *Spirogyra* (Power and Stewart, 1987). This shift in algal resources would represent a decline in food quality for herbivorous tadpoles. When hydrologic regimes are altered by dams or diversions, changes in the abundance and taxonomic composition of algae also follow (Ward and Stanford, 1979). Our study suggests that land use which alters the availability of various algal taxa to grazing tadpoles could have important consequences for frog populations via recruitment of new individuals.

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DEPARTMENT OF INTEGRATIVE BIOLOGY, UNIVERSITY OF CALIFORNIA, BERKELEY, CALIFORNIA 94720. Submitted 6 Jan. 1993. Accepted 9 May 1993. Section editor: W. J. Matthews.

APPENDIX. COMPOSITION OF FLOCCULENT MATERIAL, AS DETERMINED BY EXAMINATION WITH A LIGHT MICROSCOPE, 40–400× MAGNIFICATION.

Algal flora	Microfauna	Other
Bacillariophyta (diatoms)		
Amphora sp.	ciliates	detritus
Cymbella sp.	rotifers	tadpole feces
Epithemia sp.		
Frustulia sp.		
Nitzschia sp.		
Rhopalodia sp.		
Chlorophyta (green algae)		
Ankistrodesmus sp.		
Chlamydomonas sp.		
Cosmarium sp.		
Mougeotia sp.		
Oedogonium sp.		
Oocystis sp.		
Pediastrum sp.		
Scenedesmus sp.		
Staurastrum sp.		
Cyanophyta (bluegreen algae)		
Nostoc sp.		
Euglenophyta (euglenoids)		
Euglena sp.		