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Heterospecific Prey and Trophic Polyphenism in Larval Tiger Salamanders

HOWARD H. WHITEMAN, JUDY P. SHEEN, ERIC B. JOHNSON, ANNA VANDEUSEN, ROBIN CARGILLE AND TYSON W. SACCO

Polyphenisms (environmentally cued polymorphisms) are ubiquitous, yet the specific proximate mechanisms producing alternative morphs are generally not well known. We tested hypotheses for the role of large heterospecific prey in the cannibalistic polyphenism within larval tiger salamanders, *Ambystoma tigrinum nebulosum*, to determine whether heterospecific prey directly or indirectly influence the production of cannibal morphs. Field surveys suggested, and laboratory experiments confirmed, that macroinvertebrate prey induce cannibals via an increase in body size variation within larval salamander populations. Dietary data and laboratory foraging experiments revealed that cannibals preferred conspecifics even when their capture success was greater on macroinvertebrates. Typical morphs, in contrast, consumed only macroinvertebrate and other prey and never successfully cannibalized conspecifics. Our results support the indirect hypothesis that cannibals are induced via increased body size variation within a population of larvae, as a result of differential consumption of large heterospecific prey, and do not rely on consumption of heterospecific prey once they are cannibals. The cannibalistic polyphenism is one example of phenotypic plasticity in which the functional significance and the proximate mechanisms producing the two morphs are becoming clearer, allowing further study of the molecular and physiological basis of the alternative phenotypes.

UNDERSTANDING the proximate mechanisms that induce plastic phenotypes is fundamental to gaining insight into the evolution of such plasticity (Schlichting, 1986; Scheiner, 1993; Schlichting and Pigliucci, 1998). Polyphenism, the production of alternative morphs in varying environments, provides an ideal situation in which to evaluate such questions, because the effects of different environments can be tested against discrete rather than continuous phenotypes (Caswell, 1983; Smith-Gill, 1983). Previous theoretical and empirical research has shown that polyphenism is prevalent in environments that vary temporally and spatially, and/or when trade-offs occur in the fitness payoffs to each morph (Roff, 1996; Smith and Skúlason, 1996; Schlichting and Pigliucci 1998). Yet, our understanding of the precise mechanisms that induce alternative morphs is still poor for many polyphenic species (Whiteman, 1994; Roff, 1996; Smith and Skúlason, 1996). This is unfortunate, given the important role polyphenism is thought to play in population divergence, speciation, and other macroevolutionary change (West-Eberhard, 1986, 1989; Via 2001).

Such is true of the cannibalistic polyphenism in tiger salamanders. Cannibalistic salamander larvae (herein ''cannibals'') have enlarged heads and vomerine teeth compared to the more typical larval morphology (''typicals''; Powers, 1907; Collins and Cheek, 1983). As their name suggests, the large head and teeth of cannibals are used to consume other salamander larvae, whereas typicals often concentrate on invertebrate prey types (Collins and Holomuzki, 1984; Holomuzki and Collins, 1987). Cannibal morphs are most prevalent in temporary ponds, where they attain larger sizes than typicals and thus likely accrue advantages in terms of size at and survival to metamorphosis (Lannoo and Bachmann, 1984; Reilly et al., 1992). The cannibal morphology reduces intraspecific competition between individuals by exploiting a higher trophic level (open niche) that is unattainable by typical morphs (Holomuzki and Collins, 1987; Maret and Collins, 1997).

A number of proximate environmental factors have been shown to influence the production of cannibalistic morphs and similar trophic polyphenisms in salamanders, including density, kin structure, prey type and density, and population size-structure (e.g, references herein plus Pfennig and Collins, 1993; Wakahara, 1995; Pfennig et al. 1999). However, the importance of heterospecific prey in the production of cannibals has yet to be fully explored. In some populations, large heterospecific prey make up the majority of cannibal stomach contents (Lannoo

and Bachmann, 1984; Loeb et al., 1994; Maret and Collins 1997), suggesting an important role for heterospecific prey in the production and/or maintenance of cannibalistic morphs. In other populations, however, large heterospecific prey represent only a small percentage of cannibal diets (Rose and Armentrout, 1976; Collins and Holumuzki, 1984; Maret and Collins, 1997). Based on these observations, we asked two related questions about the role of heterospecific prey in the cannibalistic polyphenism: 1. How do heterospecific prey influence cannibal production? 2. Are heterospecific prey utilized by cannibals once they are produced?

If heterospecific prey affect cannibal production, they might do so directly, with the morph produced when high densities of such prey are available, perhaps because of the energetic benefits cannibal morphs accrue by foraging on large heterospecific prey. Alternatively, heterospecific prey might indirectly lead to cannibals via increased body size variation. Under this hypothesis, large heterospecific prey provide an energy boost to some larvae, increasing their body size and thus size variation within the salamander population. This, in turn, leads to exploitation of smaller larvae by larger larvae via cannibalism (sensu Maret and Collins, 1994), and the production of the cannibal morph.

Four types of data sets have been used in previous studies to explore the cannibalistic polyphenism (field surveys, induction experiments, dietary analyses, and foraging experiments), and the above hypotheses make specific predictions regarding each data type. Both the direct and indirect hypotheses predict a positive relationship between large heterospecific prey density and cannibal frequency in field and experimental populations, but the indirect mechanism predicts a positive correlation between cannibal frequency and larval body size variation as well. The direct hypothesis also predicts that, controlling for prey availability, cannibal diets will primarily contain large heterospecific prey, whereas the indirect hypothesis predicts that conspecifics should make up the majority of cannibal diets. Finally, the direct hypotheses predicts that large heterospecific prey will be preferred by cannibals over conspecifics during foraging experiments, and that cannibals will be more efficient foragers than typicals on heterospecific prey. In contrast, the indirect mechanism predicts that conspecifics will be preferred by cannibals over large heterospecific prey, and that cannibals will be more efficient foragers than typicals on conspecifics.

We evaluated the role of large heterospecific prey in the production of cannibals in *Ambystoma tigrinum nebulosum* (Hallowell) from south-central Colorado, using a combination of field surveys, stomach content sampling, and laboratory experiments. We interpret our results in the context of a simple cost-benefit model for the role of heterospecific prey in the cannibalistic polyphenism. Finally, we utilize this and previous studies to formulate a coherent ecological mechanism for cannibal production.

Materials and Methods

Study organism and area.—Ambystoma tigrinum nebulosum is a western tiger salamander subspecies whose range extends south from western Colorado and Utah to south-central New Mexico and central Arizona (Conant and Collins, 1998). This subspecies is well known to exhibit cannibalistic larval morphs (Collins and Cheek, 1983; Pfennig and Collins, 1993).

We studied *A. t. nebulosum* larvae in cattle-watering ponds in the Gunnison Basin region of south-central Colorado during June–August 1995–96. Each pond was surrounded by pasture, had a silt and mud bottom, and contained abundant emergent vegetation. None of the ponds exceeded 2 meters in depth although there was variation in size, shape, hydrology, and elevation.

Field surveys.—Surveys were conducted on a total of 12 ponds in the Gunnison Basin area during July and August 1995. Densities of larval salamanders, tadpoles, and macroinvertebrates ($\geq$ 2.5cm) were estimated at each pond using a drop-box consisting of a wooden frame and 0.5cm mesh hardware cloth. Two boxes were utilized: for shallow ponds, we used a $0.67 \times 0.64 \times 0.71$m (length $\times$ width $\times$ depth) box; for deeper ones, we used a $0.75 \times 0.75 \times 1.22$m box. Each box was carefully and quickly positioned in the pond 1 m ahead of a researcher to minimize the loss of any target organisms. Drops were made along a transect oriented along the widest portion of the pond. A D-net was used to sweep the enclosed volume of water within the box and the number of organisms captured was recorded. The sample was considered empty when three consecutive sweeps yielded zero target organisms (Loeb et al. 1994). A meter stick was used to estimate the water depth at each drop to the nearest cm. Each pond was sampled with at least five drops (mean = 6.5; range 5–9). For each drop, the densities of each target organism were calculated by dividing the total number of animals captured by the total volume of water enclosed by

the drop, and the mean number of animals per drop was used as the response variable.

After the drops were completed, a seine was pulled across each of the ponds to capture salamander larvae for measurement. The larvae captured in the sweeps were identified to morph using characters described by Powers (1907) and Collins and Cheek (1983), and were separated by morph to prevent individuals from consuming each other. Each morph was counted, and a subset of individuals was measured for snout–vent length (SVL), total length (TL), and gape width (all cannibals and a maximum of 20 haphazardly chosen typicals per pond). The frequency of cannibals was estimated for each pond by dividing the number of captured cannibals by the total number of larvae captured.

We seined ten of the twelve ponds several weeks after the initial sample to determine if the frequency of cannibal morphs had changed. In all cases there was a higher proportion of cannibals in the second sampling period, and we used the highest cannibal frequency for all subsequent analyses. Two ponds were not resampled because few larvae had been captured during the first sampling period. For each pond, we estimated elevation from USGS topographic maps in order to test for elevation effects on cannibal frequency.

Dietary analysis.—We analyzed salamander diets from four of the study ponds during late July to early August 1996. Larval salamanders were captured using a seine and held in large buckets filled with pond water. We used a modified gastric-lavage technique to obtain stomach contents of each morph (Zerba, 1989; Whiteman et al., 1996). Body size measurements (SVL and TL) of each larva were made using a measuring board and metric ruler. After processing, larvae were released into the pond from which they had been collected. Although we did not measure prey densities in the field, typicals and heterospecific prey were abundant in all four ponds.

Stomach contents were stored in 90% EtOH and later separated into the following taxonomic categories using a Leica dissecting microscope: *Ambystoma tigrinum* larvae, *Pseudacris triseriata* tadpoles, caddisfly (Limnephilidae) larvae, damselfly (Coenigrionidae) larvae, zooplankton, dipteran larvae, mollusca, or other (mostly vegetation, hemipterans, coleopterans, or terrestrials). The volume of each prey type was estimated following Hynes (1950) and Collins and Holomuzki (1984).

Foraging behavior experiment.—The foraging experiment was designed to test the relative effects of morph and body size on prey preference and predation efficiency. We presented predator larvae of varying SVL and morphology with three prey types: tiger salamander larvae (mean ± 1 SE = 35 ± 1 mm SVL), damselfly larvae (*Coenagrion resolutum* and *Ennallagma cyathigerum*; 20 ± 2 mm total length (TL)), and caddisfly larvae (*Limnephilus* sp.; 18 ± 2 mm TL). All three prey types were naturally present in the ponds from which the predators were collected. Sizes of all prey types were held constant, and all prey were collected at approximately the same time as the predator larvae. All experiments took place during July and August 1996.

Predator larvae were collected from three ponds as described above. Captured salamander larvae were sorted by morphology and body size, and were transported back to the Rocky Mountain Biological Laboratory (RMBL) for experiments.

Predator larvae were starved for 24 hours and then put individually into a 48L clear, plastic rectangular tank with six typical larvae, six caddisfly larvae, six damselfly larvae, and three twigs (for structural complexity). Prey were added a few minutes before the predator was introduced. The foraging behavior of the predator larva was recorded for one hour after the first attempted capture by the predator. The number of attempted and successful captures on each prey type was recorded. Prey density was kept constant throughout the trial by replacing any prey that were eaten with an individual of the same type. If the predator larva failed to eat either salamander larvae or a macroinvertebrate within one hour from entry into the apparatus, the experimental trial was terminated.

The size of predator typicals was based upon the size range of cannibals that were used in the experiment. Because cannibals tend to be larger than typicals (Collins and Holomuzki, 1984; Sheen and Whiteman, 1998), few typicals were collected within the cannibal size range. A total of twenty cannibals (SVL range = 30–72 mm) were collected, while only six typicals from this size range (SVL = 35–53 mm) were used in the experiment. Each larva was returned to its natal pond within four days after it was used in a foraging trial.

Predation attempts were used as a measure of prey preference. Because some of the cannibals were sated after consuming a salamander larva, we calculated the predation attempt rate (per minute) until a salamander larva was caught. To determine predation efficiency, we divided the

number of captures by the number of attempted captures for each predator.

Induction experiment methods.—In the following two experiments (Experiments I and II), we manipulated variables to assess their respective effects on growth and development of the cannibal morphology. Although each experiment differs slightly, general methodology for each is described below.

All experiments were conducted at the RMBL in a weatherport (Weatherport, Inc., Delta, CO), an outdoor chamber lined with foam insulation and warmed with a portable electric heater. The water within the weatherport was allowed to fluctuate between 15 and 32 C, but daily temperatures averaged 22.3 C. These temperatures do not differ from those found in the study site ponds (Whiteman and Buschhaus, 2002; Whiteman unpubl. data).

In each experiment, recently-hatched salamanders were captured with a seine in one or more ponds near the RMBL. The SVL of each larva was measured and individuals were sorted into 2mm size categories (e.g., 16–17mm, 18–19mm, etc.). After sorting, animals of similar size were transferred into experimental tanks. ANOVA revealed that there was no significant difference in initial SVL between treatments (Exp. I: all $F < 0.85$, all $P > 0.43$); Exp. II: all $F < 0.03$, all $P > 0.87$). During the first week of each experiment a few animals died and were replaced with individuals of identical size.

Experimental tanks consisted of 13.2-liter plastic storage boxes filled with eight liters of aged spring water. Tanks were stacked in blocks of six (Exp. I) or four (Exp. II) with each block containing one tank of each treatment. On a daily basis, blocks were rotated within the weatherport and within each block from top to bottom to avoid microclimate effects. Blocks were separated by cardboard partitions to avoid any visual cues between adjacent tanks, and opaque lids blocked visual cues from above or below. Tanks were cleaned with a siphon every three to four days for the first 27 days of each experiment, and thereafter cleaned weekly. New water was added to replace the lost volume during cleaning.

Salamander larvae were fed brine shimp (*Artemia* sp) daily. Each experimental tank was allocated 0.015 g (dry wt) of brine shrimp eggs per animal, which were hatched daily. The resulting naupilii were rinsed and resuspended in 2.5 ml of aged spring water per larvae for each feeding (Collins and Cheek, 1983; Collins pers. comm.).

At the end of each experiment, surviving salamander larvae were measured and scored for the cannibal morphology by a person that was blind to the treatment that each salamander experienced. An animal was considered a cannibal when it showed qualitatively significant enlargement of the head and vomerine tooth patch relative to typical larvae, as described in Powers (1907) and Collins and Cheek (1983).

Induction experiment I: Effect of salamander and tadpole density on cannibal production.—This experiment simultaneously tested the effects of conspecific and *Pseuadacris triseriata* tadpole density on cannibal production in a 2×3 factorial design. The experiment utilized two salamander densities (six and 12 larvae per tank) and three tadpole densities (zero, two, and six tadpoles per tank), with 10 replicates of each treatment.

Hatchling salamander larvae were captured on 6 and 8 July 1995 and processed as described above. Tadpoles were collected using a dipnet on 15 and 16 July. The tadpoles were sorted by size into three visual classes, but no measurements were taken. Tadpoles were then added to each tank in accordance with the treatment density, and care was taken to distribute the sizes of tadpoles evenly across treatments and replicates. Dead or eaten tadpoles were replaced daily through 29 July, at which point the replacement tadpole supply was depleted. Besides brine shrimp, no additional food was added to the treatments containing tadpoles because our expectation was that the tadpoles would feed on the detritus that accumulated in the tanks.

The number of salamanders in each tank was counted daily and any changes in morphology and behavior of individuals were noted. On 14 August (day 29, the end of the experiment), each salamander was scored for presence of the cannibal morphology.

Induction experiment II: Effect of salamander and macroinvertebrate density on cannibal production.—This experiment simultaneously tested the effects of conspecific density and the presence of macroinvertebrates on cannibal production in a 2×2 factorial design. The experiment utilized two salamander densities (six and 12 larvae per tank) and two macroinvertebrate densities (zero and eight insects per tank), with 10 replicates of each treatment.

Hatchling salamander larvae were collected on 26 and 28 June 1996 using a seine and processed as described above. In addition to brine shrimp prey, macroinvertebrate treatments received four caddisfly (*Limnephilus* sp.) and four damselfly (*Coenagrion resolutum* and *Ennallagma*

cyathigerum) larvae that were replenished as needed daily.

The number of salamanders in each tank was counted daily and any changes in morphology and behavior of individuals was noted. On day 20 and 36 (the end of the experiment), each salamander was weighed and measured for SVL. All tanks were scored for cannibals after 23 days and at the conclusion of the experiment.

Statistical analyses.—For field surveys, we used simple and multiple regression to analyze the effect of estimated densities of salamander larvae, tadpoles, macroinvertebrates, both the mean and coefficient of variation (CV) in typical morph SVL, and elevation on cannibal morph frequency. Polynomial regressions were performed to better understand relationships when they appeared curvilinear. Dietary samples were analyzed with Mann-Whitney U tests, while the effects of body size, morph, and prey type on foraging behavior was tested using simple regression, *t*-tests and Kruskal-Wallis. Finally, the effect of treatment on frequencies of cannibal morphs in our induction experiments was analyzed with log-linear analysis and chi-square tests. We analyzed differences in body size among treatments using ANOVA; block effects were removed from the analysis when they were non-significant. When multiple tests were conducted on the same variable, we reduced α based on the number of tests performed (Rice, 1989). All statistics were conducted using StatView, SuperANOVA, and SYSTAT.

Results

Field surveys.—Field observations revealed that cannibal frequency was positively correlated with macroinvertebrate density (Fig. 1A; $F_{1,9} = 16.0$, $P = 0.003$, $R^2 = 0.64$; $\alpha = 0.00156$). A curvilinear equation fit this relationship better ($F_{1,9} = 38.4$, $P < 0.0001$, $R^2 = 0.91$). A marginally significant positive relationship was found between cannibal frequency and the CV of typical larvae SVL (Fig. 1B; CV: $F_{1,9} = 9.5$, $P = 0.013$, $R^2 = 0.51$), and this relationship was significant using a 2nd order equation ($F_{1,9} = 20.8$, $P = 0.0007$, $R^2 = 0.84$). Mean typical SVL showed a weak and non-significant effect on cannibal frequency (Fig. 1C; $F_{1,9} = 3.8$, $P = 0.08$, $R^2 = 0.30$), and polynomial regression did not lead to substantial improvement ($F_{1,9} = 4.5$, $P = 0.048$, $R^2 = 0.53$). There was no clear relationship between cannibal frequency and salamander larval density, tadpole density, or elevation (Figs. 1D–F; all $R^2 < 0.05$, $P > 0.54$). However, cannibals were only found above a certain threshold of salamander larvae density (> 7 per m^3; Fig. 1D).

Stepwise regression results corresponded to those found using simple linear regression. A multiple regression model of macroinvertebrate density, typical CV, and typical mean SVL explained the majority of variance in cannibal frequency (final $R^2 = 0.87$). Macroinvertebrate density entered the model first, explaining 64% of the variance in cannibal frequency. The CV of typical SVL added another 13% of explanatory power to the regression, and typical mean SVL contributed an additional 10% to the final R^2-value (final model: $F_{3,7} = 15.9$, $P = 0.002$). No other variables were robust enough to enter the model.

Dietary Analysis.—Cannibals and typicals differed substantially in diet (Fig. 2). Only cannibals consumed other *A. t. nebulosum* larvae, and on average salamander larvae made up 62.3 ± 0.08% (mean ± SE) of cannibal gut contents. In contrast, typical diets were composed primarily of molluscs (44.4 ± 0.08%; mostly bivalves), dipterans (28.1 ± 0.06%), and several species of zooplankton (17.6 ± 0.04%), and all three were consumed in greater proportion in typicals than in cannibals (Mann-Whitney *U*, all $P \leq 0.0002$). Cannibal and typical diets did not differ in the proportion of caddisfly larvae (16.4 ± 0.05% in cannibals vs 5.8 ± 0.03% in typicals), damselfly larvae (6.2 ± 0.03% vs. 2.7 ± 0.02%), or miscellaneous other prey (7.3 ± 0.03% vs 0.7 ± 0.01%; mostly vegetation, hemipterans, coleopterans, or terrestrials).

Foraging behavior experiment.—Cannibals and typicals had significantly higher predation attempts per minute on salamander larvae when compared to caddisfly or damselfly larvae (cannibals: $H = 19.5$, $P < 0.0001$, df = 2; typicals: $H = 6.6$, $P = 0.036$, df = 2, Mann-Whitney *U* used to separately analyze prey types). Predation attempts did not differ between the latter prey types for either morph. There were very few attempts made on caddisfly larvae, and no successful captures of caddisfly larvae were observed for either morph, thus analysis of efficiency was reserved for salamander and damselfly prey. Cannibals did not differ in efficiency between prey types, catching both salamanders and damselflies approximately 20% of the time ($t = 0.10$, $P = 0.92$, df = 28). Typicals never successfully captured a salamander (0%), but captured damselfly prey 42% of the time.

Predation attempts per minute by cannibals increased slightly with SVL for salamander prey, but not damselfly larvae (Fig. 3A; salamander

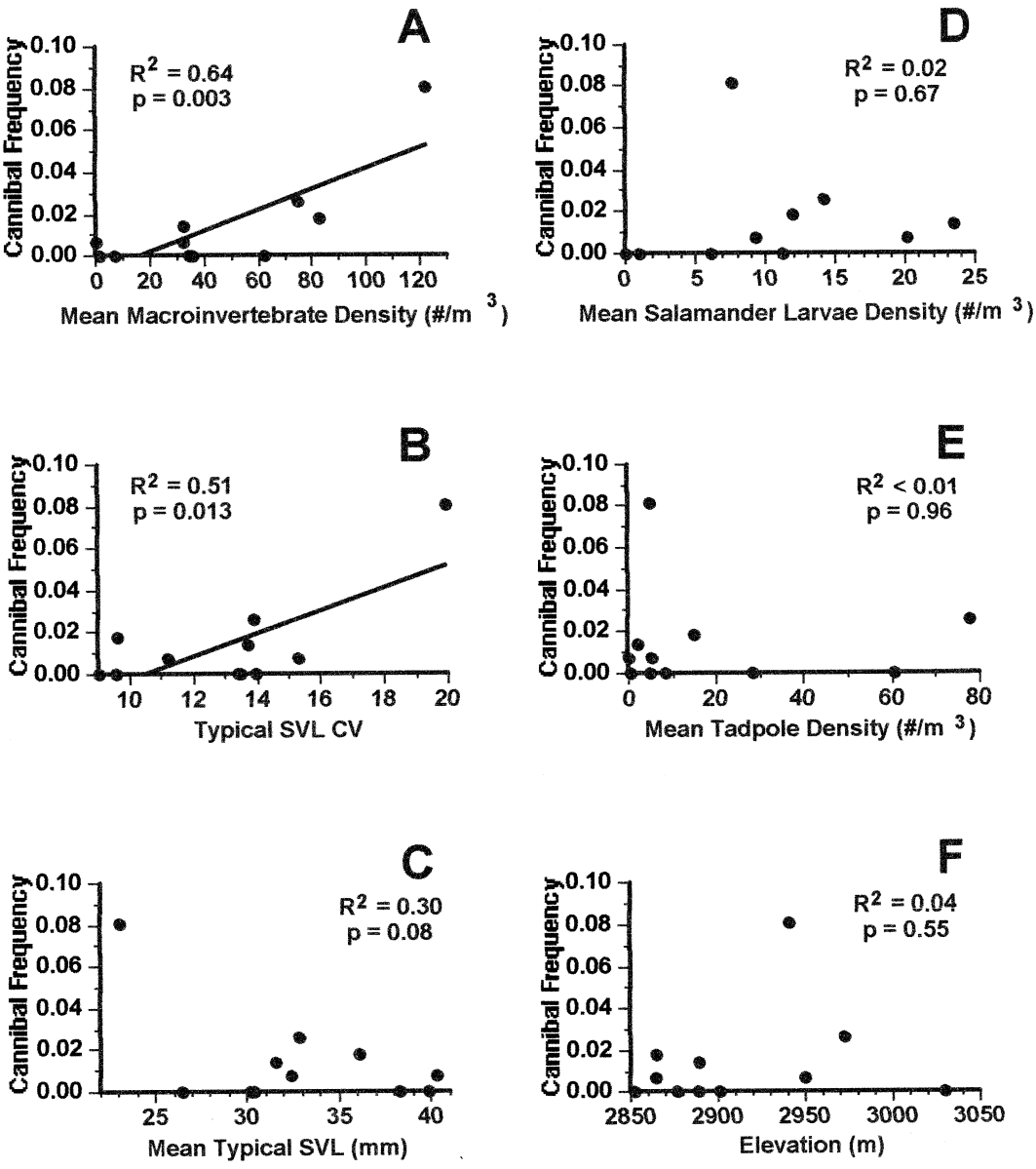

Fig. 1. Effects of environmental variables on cannibal frequency in field populations. Each point represents a single study population. (A) Mean macroinvertebrate density; (B) coefficient of variation (CV) in typical morph snout–vent length (SVL); (C) mean typical SVL; (D) mean salamander larvae density; (E) mean tadpole density; (F) elevation.

prey: $F_{1,18} = 5.12$, $P = 0.04$, $R^2 = 0.22$; damselfly prey: $F_{1,18} = 0.15$, $P = 0.71$, $R^2 = 0.08$; $\alpha = 0.025$). Similarly, cannibal efficiency increased significantly with SVL for salamander, but not damselfly, prey (Fig. 3B; salamanders: $F_{1,15} = 20.7$, $P < 0.001$, $R^2 = 0.58$; damselflies: $F_{1,11} = 0.04$, $P = 0.84$, $R^2 = 0.04$). For typicals, body size did not significantly affect predation attempts or efficiency on either prey type (All $F < 3.9$, all $P > 0.14$).

Because there were significant effects of body size on cannibal foraging on salamander prey, we compared behavior among morphs for this prey type by limiting the analysis to animals of the same size range (35–53 mm SVL). Because size did not influence performance in either morph on damselfly larvae, comparisons between morphs for this prey were made with all individuals included in the analysis. Predation attempts did not differ between morphs for sal-

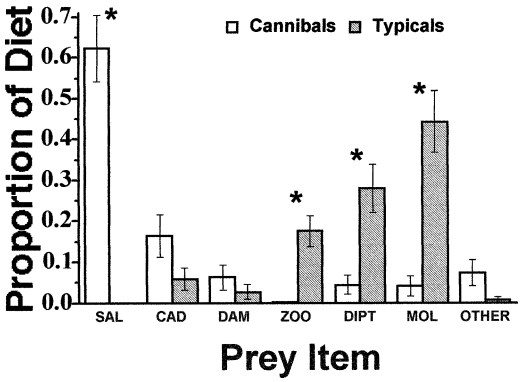

Fig. 2. Stomach contents of cannibal and typical Arizona tiger salamanders. Shown are the mean proportions ± 1 SE. Asterisks denote significant differences at $\alpha = 0.0008$ (seven comparisons).

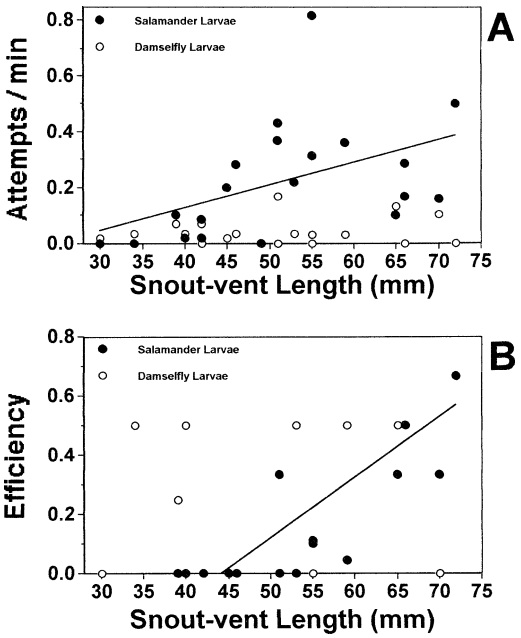

Fig. 3. Effects of snout–vent length on (A) attempted captures per minute and (B) efficiency for cannibal larvae foraging on salamander and damselfly larvae prey. See text for statistics. Regression lines are shown only for salamander larvae.

amander prey, but typicals had a marginally higher number of attempts per minute than cannibals on damselfly prey (Table 1). Cannibals captured about 20% of the salamander prey they attacked, while typicals never captured salamander prey. Thus, only damselfly efficiency could be statistically compared between morphs. Typicals showed a marginally significant increased efficiency on damselfly prey compared to cannibals (42% vs 21%; Table 1).

Induction experiment I: Effect of salamander and tadpole density on cannibal production.—Log-linear analysis revealed no significant interaction between the effect of salamander density and tadpole density on cannibal frequency ($G = 0.00$, $P = 1.00$, df = 8). Salamander density significantly increased cannibal frequency ($G = 8.86$, $P = 0.003$, df = 1, Fig. 4A) while tadpole density had no impact ($G = 0.44$, $P = 0.80$, df = 2).

Induction experiment II: Effect of salamander and macroinvertebrate density on cannibal production.—Log-linear analysis revealed no significant interaction between the effects of salamander density and macroinvertebrate density on cannibal frequency ($G = 0.00$, $P = 1.00$, df = 4). Treatments containing macroinvertebrate prey produced significantly more cannibals than treatments without this prey type ($G = 8.53$, $P = 0.004$, df = 1, Fig. 4B). In contrast, salamander density had no significant effect on cannibal induction ($G = 0.53$, $P = 0.47$, df = 1).

To investigate the mechanism behind cannibal production in this experiment, we analyzed variation in SVL and mass. There were signifi-

Table 1. Experimental Comparisons of Foraging Behavior between Morphs. For predation attempts per minute, $\alpha = 0.025$ (two comparisons).

Response variable	Prey	Mean ± 1 SE (*n*) Cannibal	Mean ± 1 SE (*n*) Typical	*t*	*P*	df
Pred. attempts/min	Sal	0.17 ± 0.05 (10)	0.17 ± 0.06 (6)	0.05	0.48	14
	Damsel	0.04 ± 0.01 (20)	0.08 ± 0.02 (6)	1.8	0.04	24
Efficiency	Sal	0.20 ± 0.07 (17)	0.0 ± 0.0 (5)	—	—	—
	Damsel	0.21 ± 0.07 (7)	0.42 ± 0.13 (5)	1.5	0.07	10

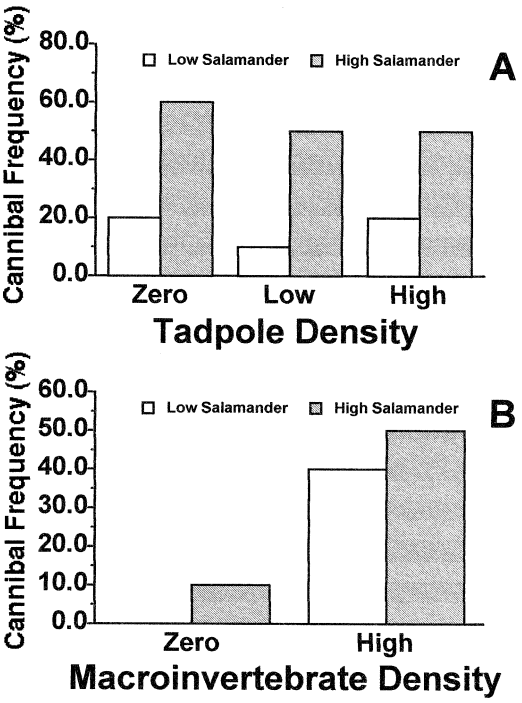

Fig. 4. (A) Effect of tadpole and salamander larvae density on cannibal frequency in Induction Experiment I. (B) Effect of macroinvertebrate and salamander larvae density on cannibal frequency in Induction Experiment II. See text for statistics.

cant effects of macroinvertebrate prey on mean SVL and mass on day 20 of the experiment (Table 2). In addition, significant increases were found in the CV of SVL and mass in macroinvertebrate treatments versus those without macroinvertebrates. However, salamander density and the macroinvertebrate × salamander density interaction had no effect on the mean or CV of SVL and mass (all $F < 0.82$, all $P > 0.37$; Table 2). Comparisons of tanks producing cannibals versus those without them revealed no differences in CV of SVL ($F_{1,16} = 1.2$, $P = 0.28$), but significant differences in the CV of mass ($F_{1,16} = 7.0$, $P = 0.02$). Comparisons of body size at the end of the experiment were biased because of the presence of single cannibals in many tanks.

Discussion

Previous studies have shown that heterospecific prey can induce cannibalistic morphs by increasing size variation of a population (Maret and Collins, 1994, 1996). Our data support this indirect mechanism for the role of large heterospecific prey in the production of the cannibalistic polyphenism. Field observations revealed that cannibal frequency was significantly correlated with both the density of macroinvertebrate prey and the CV in typical SVL. Laboratory induction experiments showed that macroinvertebrates increase the CV of larval SVL and subsequently increased the production of cannibals. These data are consistent with both the direct and indirect hypotheses for cannibal production, in that increased levels of large heterospecific prey were related to increased frequencies of cannibals. However, the increased body size variation found in both field and lab studies supports only the indirect mechanism.

Our dietary and foraging results are also consistent with the indirect hypothesis. Dietary data revealed that cannibals primarily consumed

Table 2. Effect of Macroinvertebrates and Salamander Density on Body Size Parameters (SVL, Mass, CV-SVL, and CV-Mass) Measured during Experiment II. Shown are means ± 1 SE; sample size is 20 for each mean. Macroinvertebrate × salamander density interactions are not shown. See text for details. Asterisks denote significant differences at $\alpha = 0.00625$ (four comparisons).

	Macroinvertebrate density			
	Zero	High	*F*	*P*
SVL	21.2 ± 0.3	23.3 ± 0.7	7.2	0.01
Mass	0.53 ± 0.02	0.80 ± 0.07	12.4	0.001*
CV-SVL	10.0 ± 1.2	19.9 ± 0.9	40.9	<0.0001*
CV-Mass	26.8 ± 3.7	66.3 ± 4.3	48.0	<0.0001*
	Salamander density			
	Low	High	*F*	*P*
SVL	22.6 ± 0.6	21.9 ± 0.6	0.68	0.42
Mass	0.68 ± 0.06	0.64 ± 0.06	0.39	0.54
CV-SVL	15.3 ± 1.6	14.6 ± 1.5	0.21	0.65
CV-Mass	44.5 ± 6.0	48.6 ± 6.0	0.50	0.48

conspecifics, and consumed heterospecific prey in low frequencies. Cannibals preferred to feed on conspecifics rather than macroinvertebrates, even though at some body sizes their success rate on these heterospecific prey was much higher (Fig. 3B). Typical larvae had marginally higher preference for damselfly prey than did cannibals, and were marginally better at capturing damselflies during experiments, but did not differ from cannibals in consumption of macroinvertebrates in the field. Although they preferred to attack conspecifics during experiments, typicals were never successful at consuming them. These data support the idea that cannibals do not specialize on macroinvertebrate prey, especially when larger conspecifics are available. In contrast, similarly sized typical larvae are not effective at consuming conspecifics and thus should choose the largest heterospecific prey which are available. This conclusion is supported by our foraging experiment, but may be masked by body size effects within our dietary data. That is, our foraging data controlled for size between morphs, whereas in the field typicals were always smaller than cannibals. Because of the large size variation that occurs in ponds with cannibals, typicals may have been too small to actively forage on macroinvertebrates, while the cannibals may have fed on macroinvertebrates or other heterospecific prey earlier in their ontogeny.

However, in some circumstances, particularly when conspecific densities are low relative to heterospecific prey densities, cannibals may specialize on large heterospecific prey. Because amphibian and invertebrate populations often fluctuate considerably from year to year, particularly within the larval stages (Pechmann et al., 1991; Wissinger, 1999; Whiteman and Wissinger, in press) it is likely that the benefit of heterospecific prey changes both spatially and temporally. In our study populations, macroinvertebrate densities have fluctuated over the past decade (Wissinger, pers. comm.). Thus, in some years heterospecific prey may be abundant relative to salamander larvae, which would allow large heterospecific prey to provide resources to cannibals that may be unavailable when tadpoles or macroinvertebrates are less abundant than conspecifics. In support of this hypothesis, Loeb et al. (1994) found that cannibals consumed more heterospecific prey than conspecifics, including *Pseudacris* tadpoles and aquatic insects, under conditions when tadpoles were on average six times more dense than salamander larvae in their study ponds (mean values from Table 1, Loeb et al., 1994; range 0.21–172x). Maret and Collins (1997) found that in populations with low salamander larvae densities, cannibals primarily consumed heterospecific prey, while in populations with higher larval densities, cannibals primarily consumed conspecifics. These studies illustrate that the cannibal morphology is not simply an adaptation for cannibalism, but rather for foraging on the largest available prey (see also Reilly et al., 1992).

Numerous studies have shown that animals will choose the most profitable prey type that maximizes the benefit:cost ratio (B/C; e.g., Stephens and Krebs, 1986). Tiger salamander larvae of each morph follow this same general rule. The cannibal morphology provides a performance advantage in the handling of conspecific prey which typical larvae lack (Reilly et al., 1992; this paper). Thus, the cost (in terms of energy [E], time [t], E/t, or some other currency) of attacking a conspecific is likely much lower for cannibals than typicals. This cost should decrease with increasing cannibal body size, because we found cannibals made more attempted captures on conspecifics as their SVL increased (Fig. 3A), and Maret and Collins (1997) found that the percentage of salamander larvae in cannibals diets increased significantly with body size, at least in some populations. The benefits of different prey types are also clear. Using published data on calories per gram dry mass (Cummins and Wuycheck, 1971) and average dry mass estimates (Whiteman et al., 1996; Whiteman unpubl. data) of salamander larvae, caddisfly larvae, damselfly larvae, and zooplankton, we found that although macroinvertebrates provide five times the energy of zooplankton (approximately 1.8 vs 0.35 calories per individual prey consumed), salamander larvae of similar size to those preyed on by cannibals provide over 200 times the energy of macroinvertebrates (approximately 370 calories). The benefits of macroinvertebrates relative to conspecifics might be further reduced due to decreased digestibility of their chitinous exoskeletons (Ricklefs and Miller, 1999). Thus, the consumption of conspecifics by cannibals and macroinvertebrates by typicals fit the B/C analysis.

Heterospecific prey thus influence the cannibalistic polyphenism by providing extra energy to those salamander larvae able to consume them. These larvae experience faster growth rates than larvae unable to access large heterospecific prey, increasing the size structure of the population (Maret and Collins, 1994). This, in turn, may stimulate the production of cannibals that can experience further increases in growth rate by exploiting smaller conspecifics (Maret and Collins, 1994, 1997). The act of cannibalism is not necessary to produce the can-

nibal morph (Hoffman and Pfennig, 1999), but rather the cannibal morphology provides a way to more efficiently capture and consume large prey per se (Reilly et al., 1992; this paper). Cannibals thereafter preferentially eat the largest prey available, to maximize caloric benefits.

Our field correlations were consistent with our laboratory experiments, because we found significant effects of macroinvertebrates but not *Pseudacris* tadpoles in both studies. The lack of a tadpole effect on cannibal production was unexpected, however, because other research has shown significant effects in the field (Loeb et al., 1994) and the lab (Maret and Collins, 1996). Variation in the timing of breeding between *P. triseriata* and *A. t. nebulosum* has been shown to produce differences in salamander predation rates on tadpoles as well as the production of cannibals (Maret and Collins, 1996). *Pseudacris triseriata* breed at the same time as *A. t. nebulosum* in our populations, possibly because of the short growing season found at high elevations, which constrains amphibians to breed soon after winter ice has melted from the ponds (Whiteman et al., 1994, 1999). This convergence in early breeding between the two species could produce a situation in which *Pseudacris* tadpoles are large enough to avoid predation in the field and in our laboratory experiments, which would reduce their potential impact on cannibal production. In fact, although we provided salamander larvae with the entire range of available tadpole sizes during our experiment, salamanders only consumed 18 tadpoles compared to 54 conspecifics (counting across all treatments with tadpoles present) in Induction Experiment I. In contrast, almost all of the damselflies and approximately half the caddisflies added to Induction Experiment II were consumed, often within an hour of placement in each tank. Thus, the fluctuation and timing of breeding of tadpoles in our populations, in concert with our other data, suggests that macroinvertebrates, rather than tadpoles, appear to be the important heterospecific prey source for the production of cannibals in our Colorado populations. In some Arizona populations, the reverse appears to be true (Loeb et al., 1994; Maret and Collins, 1996), perhaps because of more separation in breeding times of the two species (but see Loeb et al., 1994), or some other factor that decreases the size of tadpoles relative to salamander larvae.

Salamander larval density has been suggested as an important component of the production of cannibal morphs, with higher densities leading to increased cannibal production (Collins and Cheek, 1983; Pfennig et al., 1991). Our field surveys showed no relationship between salamander larval density and cannibal frequency, although there was an apparent minimum density required for cannibal production (Fig. 1D). However, many of our field correlations are strongly influenced by a single population (Fig. 1, cannibal frequency = 0.08), which may mask potential density effects. This population was already close to its maximum cannibal frequency when other environmental variables were sampled (0.075), and thus it is possible that the high cannibal frequency had reduced typical densities, leading to a non-significant effect of salamander larval density on cannibal frequency. Indeed, although omitting this population from the analyses removes all significant field regressions, it also increases the significance level of salamander larval density, such that macroinvertebrate and salamander larval density have more similar impacts on cannibal frequency (mean macroinvertebrate density: $F_{1,8} = 4.87$, $P = 0.058$, $R^2 = 0.38$; mean salamander larval density: $F_{1,8} = 4.6$, $p = 0.065$, $R^2 = 0.36$).

Loeb et al. (1994) also found no clear relationship (beyond threshold effects) between salamander larval density and cannibal frequency in their field correlations. Maret and Collins (1994) found that increased salamander density led to decreased C.V. in body size, which was positively correlated to cannibal frequency. This suggests that cannibal frequency was *negatively* correlated to salamander larval density in this study. However, both Loeb et al. (1994) and Maret and Collins (1994) conducted all of their surveys after cannibals had already been produced, whereas our field sampling was conducted before cannibal production had occurred or had maximized for the pond. Thus, our surveys likely give a more accurate representation of the environmental correlates that *produce* cannibals, rather than the correlates *associated* with cannibal presence. Further, only one of our two induction experiments revealed a significant effect of salamander density on cannibal production. It is possible that variation in experimental methods might have produced these results. For example, we attempted to control for body size effects by categorizing larvae into distinct size categories. In contrast, Collins and Cheek (1983) and Pfennig et al. (1991) used hatchlings without sorting into size classes, which might have produced higher relative size variation than our experiments, with the magnitude of the effect greatest at high densities. Increased size variation, rather than density per se, may therefore be the driving mechanism affecting cannibal production in these studies.

Our data emphasizes the need for under-

standing the proximate mechanisms, not just the proximate cues or correlates, which induce alternative phenotypes. The cannibalistic polyphenism is produced not just through one heterospecific prey type, but rather through a variety of possible large prey, which all have the potential to provide the resources necessary to boost larval growth and allow exploitation of smaller larvae via cannibalism. Furthermore, a number of other proximate factors are known to be important in the induction of cannibals (see Introduction). Thus, the cannibalistic polyphenism has a multi-dimensional developmental reaction norm (sensu Schlichting and Pigliucci, 1998). In many other polyphenisms, the actual mechanisms producing alternative phenotypes are less clear than the functional significance of the polymorphism or the cues associated with its production. The cannibalistic polyphenism is one example of phenotypic plasticity in which both the functional significance and the mechanisms producing the two morphs are becoming clearer, allowing further study of the molecular and physiological basis of the alternative phenotypes.

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(HHW) DEPARTMENT OF BIOLOGICAL SCIENCES, MURRAY STATE UNIVERSITY, MURRAY, KENTUCKY 42071; (HHW, JPS, EBJ, AV, RC, TWS) ROCKY MOUNTAIN BIOLOGICAL LABORATORY, P.O. BOX 519, CRESTED BUTTE, COLORADO 81224; (JPS) DEPARTMENT OF INTEGRATIVE BIOLOGY/MUSEUM OF VERTEBRATE ZOOLOGY, UNIVERSITY OF CALIFORNIA, BERKELEY, CALIFORNIA 94702; (EBJ) DEPARTMENT OF BIOLOGY, LEWIS AND CLARK COLLEGE, PORTLAND, OREGON 97219; (AV) DEPARTMENT OF BIOLOGY, GRINNELL COLLEGE, GRINNEL, IOWA 50112; (RC) DEPARTMENT OF BIOLOGICAL SCIENCES, UNIVERSITY OF NEW ORLEANS, NEW ORLEANS, LOUISIANA 70148; (TWS) DEPARTMENT OF ORGANISMIC BIOLOGY, ECOLOGY, AND EVOLUTION, UNIVERSITY OF CALIFORNIA, LOS ANGELES, CALIFORNIA 90095. PRESENT ADDRESS: (EBJ) DEPARTMENT OF SOCIOLOGY, COLUMBIA UNIVERSITY, NEW YORK, NEW YORK 10027. E-mail: (HHW): howard.whiteman@murraystate.edu. Send reprint requests to HHW. Submitted: 19 Feb. 2002. Accepted: 26 Sept. 2002. Section editor: W. L. Montgomery.