

WHEN SPECIALISTS COMPETE: INCREASED COMPETITION AS A COST OF
RESOURCE POLYMORPHISM

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ABSTRACT

JEFFREY SCOTT PAULL: When specialists compete: increased competition as a cost of resource polymorphism
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Resource polymorphisms—the occurrence within a single population of alternative morphs showing differential resource use—are spectacular examples of diversity within species. Here, we empirically evaluate a potential constraint to resource polymorphism in spadefoot toad tadpoles. We characterize the dietary differences between alternative carnivore and omnivore morphs and assess the potential ecological consequence of any such differences. We found that, as a group, the ancestral omnivore morph is a trophic generalist, whereas the derived carnivore morph is a trophic specialist. Furthermore, we show that these specialist carnivores experience greater intramorph competition for their distinctive resources than do the generalist omnivores. In contrast to the situation in omnivores, functional limitations associated with the evolution of trophic specialization may preclude carnivores from switching to alternative resources when the resource for which they are adapted is depleted. Generally, such costs of resource specialization may often constrain the evolution of resource polymorphism.

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CHAPTER I

STABLE ISOTOPE ANALYSIS OF POPULATION DIET STRUCTURE

Introduction

Stable isotope analysis presents a promising tool for ecologists and evolutionary biologists to investigate the consequences of individual variation in resource-use strategies and potentially clarify the evolution of novel resource use. Here I discuss how stable isotopes can be used to investigate the diet structure within populations. I begin with an overview stable isotope analysis and then describe examples of how stable isotopes can be applied to individual level dietary research.

What are stable isotopes

Stable isotopes are a different form of a chemical element that differs in the number of neutrons in their nucleus. The extra neutron in the nucleus makes stable isotopes heavier than their elemental form. For example, the stable isotope of carbon, ^{13}C , is heavier than ^{12}C . Heavy and light isotopes differ in the rate in which they undergo biochemical reactions, with the lighter isotopes reacting faster (Urey 1947). For instance, C_3 plants are isotopically lighter compared to C_4 plants due to differences in their photosynthetic pathways (Lajtha & Michener 1994). The differential rates of reactions cause the heavy and light isotopes to accumulate at different rates in the tissue of organisms where these reactions are taking place. By measuring the ratio of the heavy to light isotopes, researchers can identify different types of primary producers, and also, the composition of an individual's diet.

Isotopic ratios in substances are measured via mass spectrometry and expressed in delta notation (e.g. $\delta^{13}\text{C}$). Stable isotopes are measured relative to a common international standard material. They are calculated as differences from a given standard material ($\delta X = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$) and expressed in per mil units (‰). Here, X is the stable isotopic form of an element, and R is the ratio of the heavy to light isotope in X. Comparing the R_{sample} with R_{standard} allows for isotope comparisons to be made across the stable isotope facilities that measure their signatures (I.E. This is a calibration technique).

How are stable isotopes used in ecology

Stable isotope ecologists can identify the resources of consumers by measuring the stable isotopes of their tissue. Consumer tissue is synthesized from their resources and the stable isotopic composition of their tissue predictably reflects that of their resources. Carbon and nitrogen are commonly used in stable isotopic studies in aquatic systems. In particular, $\delta^{13}\text{C}$ is enriched in the tissue of consumers that rely on atmospheric sources of CO_2 vs. consumers that rely on dissolved sources of CO_2 (Fry 2006). This means that in a pond or lake, $\delta^{13}\text{C}$ reflects a consumer's use of littoral vs. limnetic resources, respectively. $\delta^{15}\text{N}$ is commonly used in diet studies because it becomes highly enriched in the tissue of consumers relative to their resources (about 3.4‰ enrichment), thus providing a measure of trophic level (Post 2002). This enrichment occurs because, during digestion, the lighter isotope is excreted faster than heavier isotope. Thus the ratio of the heavy to light isotope increases in the tissue of consumers relative to their resources.

Stable isotope ecologists can sometimes even link consumers to their specific resources by measuring the isotopes of both consumers and their potential resources. Consumer tissue is a mixture of the stable isotopic signatures of their prey. Stable isotopic mixing models can be used to convert the isotopic mixtures into proportional amounts of different prey items utilized by consumers. In practice the use of mixing models can be quite challenging and researchers should

take precautions to avoid the potential pitfalls associated with this technique (Phillips & Gregg 2001, 2003).

The use of these mixing models is becoming very popular in stable isotope ecology because it allows the researcher to identify the specific dietary differences between individual organisms. With these techniques, stable isotope ratios of carbon ($\delta^{13}\text{C}$), and nitrogen ($\delta^{15}\text{N}$), are often used to describe the niches among organisms (Hobson & Clark 1992; Newsome et al. 2007), and the structure of food webs (Van Zander 1999).

Use of stable isotope analysis to investigate population diet structure

Diet studies are increasingly considering the ecological causes and consequences of individual differences in diet and foraging behavior (Bolnick et al. 2003; Bolnick et al. 2011; Araújo et al. 2011). Using stable isotopes to study individual level diet variation allows researchers to characterize the dietary structure of a population and better understand the importance of frequency dependent effects in addition to density dependent effects. For instance, stable isotope analysis may help to clarify how populations might respond to competition. Studies of intraspecific competition often rely on population size as a proxy for the intensity of intraspecific competition. However, differences in the population's diet structure can greatly alter the outcome of intraspecific competition. If the diet variance among individuals is high, then competition may not be as severe as the population size would suggest. This is because the severity of competition decreases as the diet distance between individuals also increases (Martin & Pfennig 2009; Bolnick et al. 2003; Bolnick & Paull 2009). Additionally if individuals within a population form dietary clusters, the severity of competition may differ within different clusters. In some dietary clusters, population-wide competition may be less intense than intra-cluster competition and therefore competition may have different consequences for different individuals depending upon the subset of the population they belong. If individual level diet variation is

ignored then researchers might miss important frequency dependent interactions and potentially mischaracterize the causes and consequences of resource competition (Bolnick et al. 2003; 2011).

Investigating population diet structure may be especially useful in studies of the evolution of resource polymorphism. In a resource polymorphism, a single population may consist of alternative morphs that utilize alternative resources. Resource polymorphism is thought to be an adaptive response to lessen competition (reviewed in Smith and Skúlason 1996), thus it may be useful to investigate how resources are used within each alternative morph. The consequences of resource competition may differ between morphs, especially if they use resources differently.

For instance, in chapter II of this thesis, I use stable isotope analysis to better understand the dietary structure of a resource polymorphism in *Spea multiplicata* tadpoles. I do this as a first step to understanding the consequences of alternative resource-use strategies. I find the potential for individual level diet specialization in the omnivore morph whereas the carnivore morph exhibits little dietary variation between individuals. Additionally, I discover that the omnivore morph is a trophic generalist, whereas carnivore morph is a trophic specialist. This leads to the hypothesis that carnivores might experience increased intra-morph competition compared to omnivores as a result of the performance trade-offs that come with specialized carnivore morphology. I find support for this hypothesis and conclude that such heightened competition may pose a cost to carnivory during the evolution of resource polymorphism. By studying the diets of individuals, I demonstrate that competition may be more severe for certain individuals depending on whether they are generalists who compete with other generalists, or whether they are specialists who compete with other specialists. Thus, it is important to consider the diet structure of a population when trying to understand the role of competition, and stable isotopes are highly useful for this.

The advantages of stable isotope analysis

Using stable isotope analysis to investigate population diet structure has the potential to uncover some fundamental consequences of diet variation. However, many individual level diet studies rely on stomach content analysis of diet alone. This can sometimes be problematic. In particular, stomach content analysis only reveals what individuals ate recently. Thus, such analyses can be subject to stochastic sampling effects that reflect, e.g., differences in the amounts of different food types present in different locations or at different times. Additionally, stomach content analyses do not take into account potential lifetime dietary differences between individuals. Stable isotope analysis on the other hand, records dietary information at multiple temporal scales depending on the tissue used for analysis (Tieszen et al., 1983; Dalerum & Angerbjorn, 2005). This not only allows the researcher to control for stochastic effects, but it also allows researchers to measure other aspects of individual foraging behavior, such as individual specialization or ontogenetic diet shifts (Bolnick 2003; Phillips & Eldridge 2006).

Isotopes should be interpreted with caution and ideally, they should be bolstered with gut content analyses (Jardine et.al. 2003). However they remain to be an invaluable tool for researchers interested in population diet structure and its potential ecological and evolutionary consequences.

CHAPTER II

INCREASED COMPETITION AS A COST TO SPECIALIZATION DURING THE EVOLUTION OF RESOURCE POLYMORPHISM

Introduction

Resource polymorphisms—the occurrence within a population of alternative morphs showing differential resource use—are taxonomically widespread, and they furnish some of the most striking examples of intraspecific diversity (Smith and Skúlason 1996). Indeed, the phenotypic differences between such alternatives often mirror those between distinct species (e.g., Liem & Kaufman 1984; Hendry et al. 2006; Calsbeek, Smith & Bardeleben 2007; Wund et al. 2008), suggesting that alternative resource-use morphs may represent incipient species (reviewed in Pfennig & McGee 2010). Moreover, such morphs often function ecologically as separate species (Harmon et al. 2009), and their presence may increase the likelihood of species coexistence (Clark 2010). Thus, identifying the factors that promote—or preclude—the evolution of resource polymorphism is crucial for clarifying not only how diversity arises within species, but also, potentially, how species originate and coexist.

Resource polymorphism has long been viewed as an adaptive response to intraspecific competition (reviewed in Smith & Skúlason 1996; Svanback et al. 2008). Specifically, in a population facing intense intraspecific competition for food, frequency-dependent disruptive selection may favor the evolution of alternative resource-use morphs as a means of mitigating this competition (reviewed in Smith & Skúlason 1996; Doebeli 2011; Pfennig & Pfennig 2013). Empirical data largely support this prediction (Smith 1993; Robinson, Wilson & Shea 1996; Bolnick 2004; Svanback et al. 2008; Calsbeek 2009; Hendry et al. 2009; Martin & Pfennig 2009; Cucherousset et al. 2011).

Although intraspecific competition is widespread and frequently strong (Gurevitch et al. 1992; Dybzinski & Tilman 2009), resource polymorphism is far from ubiquitous (Smith & Skúlason 1996). Thus, other factor(s) may often preclude the evolution of resource polymorphism. One such factor is increased fitness costs associated with resource specialization.

The evolution of a resource polymorphism frequently involves increased resource specialization. In some cases, all morphs comprising a resource polymorphism are specialists, such as when alternative morphs differ in “handedness”, as in cichlid fish, *Perissodus microlepis* (Hori 1993), and crossbills, *Loxia* sp. (Benkman 1996). Even in resource polymorphisms containing a dietary generalist, an alternative specialist morph is frequently present (e.g., Liem & Kaufman 1984; Smith 1993; reviewed in Smith & Skúlason 1996; Robinson & Wilson 1998).

Increased specialization often carries an important fitness cost. Specifically, a trait that increases an individual’s ability to harvest one set of resources may limit the same individual’s ability to harvest alternative resources. Such trade-offs may arise if different morphological, physiological, and/or cognitive attributes are required to harvest different resources (Benkman 1996; Robinson, Wilson & Shea 1996; Bolnick et al. 2003; Martin & Pfennig 2009; Ellerby & Gerry 2011). Consequently, resource-use specialists may have lower fitness than generalists in the same population if these resource-use specialists deplete their resource and are forced to switch to another resource for which they are poorly adapted.

Additionally, despite the fact that resource polymorphism is viewed as an adaptive response to intraspecific competition, increased resource specialization may actually intensify competition if the resources that resource-use specialists utilize become scarce. Generally, competition is most severe between the most phenotypically similar individuals (Bolnick & Paull 2009; Martin & Pfennig 2009). Consequently, rather than favoring specialization, competitively mediated selection may favor resource-use generalists that utilize alternative resources when competition for any one resource becomes severe. Under such circumstances, selection may favor in a generalist predator individuals that specialize on different resources through differences in their

foraging behavior (Woo et al. 2008). Thus, although the evolution of a resource polymorphism may reduce overall resource competition, the evolution of novel resource-use specialists may be disfavored if they face increased “intramorph” competition (i.e., competition with individuals of the same morphotype). Such competition could represent an important cost of resource polymorphism, and potentially even constrain its evolution. In short, understanding how resources are differentially used between alternative morphs, and the potential fitness consequences associated with these alternative strategies, is essential for clarifying the conditions that promote—or impede—resource polymorphism’s evolution.

We sought to address the above issues in a natural population of spadefoot toad tadpoles that express a striking resource polymorphism. We began by performing stable isotope analyses to determine if the alternative morphs in this system differ in diet, and also if they were similar or different in trophic breadth (i.e., the trophic range of food items consumed by individuals of each morphotype). These data therefore allowed us to infer whether morphs were trophic generalists (Fig. 1a), specialists (Fig. 1b), a specialist and a generalist (Fig. 1c), or two generalists, with some individual specialization (Fig. 1d). Because our results indicated that one morph was a trophic specialist and the other a trophic generalist (see Results), we then sought to determine experimentally if the trophic specialist experiences greater competition with other trophic specialists than the trophic generalist does with other trophic generalists. We found that, as predicted, specialists face increased intramorph competition. Such heightened competition could represent an important cost for the evolution of resource polymorphism and may prevent these polymorphisms from evolving in the first place.

Materials and methods

Study system

Mexican spadefoot toads (*Spea multiplicata*) express a striking resource polymorphism, the extremes of which are represented by two distinct ecomorphs: an “omnivore” morph—a round-

bodied tadpole with a long intestine, small jaw muscles, numerous labial teeth, and smooth mouthparts that feeds primarily on the pond bottom, and a “carnivore” morph—a narrow-bodied tadpole with a short intestine, greatly enlarged jaw muscles, few labial teeth, and notched mouthparts that feeds mostly in the water column (for pictures of the two morphs, and a review of this system, see Martin & Pfennig 2009; Ledón-Rettig & Pfennig 2011). Analyses of gut contents revealed that these morphs utilize different resources. Specifically, Pomeroy (1981) found that guts of omnivores were largely filled with microscopic detritus and algae, whereas those of carnivores contained mostly anostracan fairy shrimp.

Spea tadpoles develop into the omnivore by default, unless they ingest a sufficient quantity of anostracan fairy shrimp, at which point they may become a carnivore (species, populations, and sibships vary in their propensity to respond to the shrimp cue; reviewed in Ledón-Rettig & Pfennig 2011). Moreover, ancestral character state reconstruction has revealed that the carnivore morph is a novel morph that is present only within the genus *Spea* (Ledón-Rettig, Pfennig & Nascone-Yoder 2008). Thus, the carnivore morph is the derived morph, both developmentally and evolutionarily.

Competitively mediated disruptive selection has likely promoted the evolution of this resource polymorphism (Pfennig, Rice & Martin 2007; Martin & Pfennig 2009). Such selection disfavors intermediate individuals for two reasons. First, these individuals feed less effectively than extreme forms on the main alternative resource types: fairy shrimp and detritus (Martin & Pfennig 2009). Second, intermediate individuals also suffer greater intraspecific competition for resources. Intermediates are often the most common phenotype among young tadpoles (D. Pfennig, unpubl. data). Because competition is strongest between phenotypically similar individuals (Martin & Pfennig 2009), intermediate individuals face greater competition than more extreme forms.

Using this system, we combined stable isotope analysis of diet with a competition experiment to characterize dietary differences between carnivore and omnivore morphs and to assess the

potential ecological consequence of any such differences. We describe our specific methods below.

Tadpole collections

Tadpoles were collected from four ponds near Portal AZ approximately 16 days after each pond filled (and about 14 days posthatching). In all the ponds, *S. multiplicata* was the only species of *Spea* present (i.e., none of the ponds sampled contained tadpoles of the closely related species, *S. bombifrons*). In each pond, we collected tadpoles from randomly selected sites throughout the pond using a handheld dip net. Immediately after collection, we euthanized the tadpoles by immersion in a 0.1% aqueous solution of tricane methanesulfonate (MS 222). Tadpoles were then frozen on dry ice and shipped to the University of North Carolina, where they were prepared for morphological and stable isotope analyses as described below.

Morphological Analyses

To characterize morphological differences among the tadpoles, we followed the methods of Pfennig, Rice, & Martin (2007). Briefly, we began by measuring each tadpole's mass and snout-vent length (SVL). We then measured the width of the orbitohyoideus (OH) muscle and characterized the shape of each tadpole's keratinized mouthparts (MP). In addition we counted the number of rows of labial teeth (LT; Martin & Pfennig 2009) and approximated the length of each tadpole's intestines by counting the number of gut coils (GC). We standardized OH for body size (SVL) by regressing \ln (i.e., natural log) OH on \ln SVL and used the resulting residuals for the subsequent analyses. We then combined the MP, the LT, the GC and the residuals of \ln OH regressed on \ln SVL into a single multivariate shape variable (the "morphological index"; see Pfennig, Rice & Martin 2007) by calculating a principal-component score. We pooled tadpoles from all four ponds together for this principal components analysis and scaled our variables to unit variance, and centered to zero mean. From this analysis, we used PC1 (the first principal

component) as our morphological index, where PC1 explained 70% of the variance in tadpole morphology. Tadpoles with greater values of PC1 tend to possess more carnivore-like morphology, with larger OH muscles, more keratinized MP, fewer LT, and fewer GC, while tadpoles with lesser values tend to possess more omnivore-like morphology.

We then ascertained whether the morphological indices in each pond were distributed bimodally. To do so, we followed the methods presented in Martin & Pfennig (2010) by using the framework and software described in Brewer (2003). Within this Bayesian framework, we tested the hypothesis that the mixture of two normal distributions fit the data better than a single normal distribution. To evaluate this hypothesis, we used Akaike's information criteria (AIC) corrected for sample size (Burnham & Anderson 2002). Specifically, we calculated ΔAICc by taking the difference between AICc of the fitted single normal distribution minus AICc of the mixture of two normal distributions. We interpreted $\Delta\text{AICc} > 4$ as more support for the fit of a mixture model than the fit of a single normal distribution, while ΔAICc between -4 and 4 as equivalent support for the fit of the mixture model and single normal distribution, and ΔAICc less than -4 as more support for the fit of a single distribution (Burnham & Anderson 2002). The distribution of morphological indices was significantly bimodal in all four ponds (Supplementary Table S1). Therefore, to classify each tadpole as a carnivore or omnivore, we determined the morphological index value that corresponded to the minimum inflection point between the two modes of the overall distribution. We then categorized each tadpole as either an omnivore or a carnivore based on which side of this minimum point that particular tadpole fell. If a tadpole's morphological index fell below this minimum value, it was categorized as an omnivore; if it fell above this minimum value, it was categorized as a carnivore. Finally, we evaluated the prediction that carnivores and omnivores differ in morphology across all ponds with a linear mixed model with the morphological index as our response variable, morphotype classification as our predictor variable, and pond origin as a random intercept fit by restricted maximum likelihood (REML).

Stable isotope analysis

As noted above, an unpublished study had analyzed the gut content results of omnivores and carnivores (Pomeroy 1981). Gut contents alone can be problematic, however, when trying to infer diet structure of a population, for at least two reasons. First, because gut contents only reveal what individuals ate recently, they are subject to stochastic sampling error and also do not measure potential lifetime dietary differences between individuals. Additionally, and perhaps more importantly, gut contents tell us only what individuals consume and not what they actually assimilate. For instance, an individual with a carnivore phenotype might consume plant material occasionally, but it may be unable to assimilate this material if it lacks the long gut needed to process such material. In order to overcome these limitations, we also inferred the diets of the two morphs by using stable isotope analysis.

Stable isotopes analysis of muscle tissue records longterm dietary information for individual tadpoles (Dalerum & Angerbjorn 2005; McIntyre & Flecker 2006), which reduces the potential for stochastic effects. Such analyses also allowed us to measure dietary differences between morphs as well as between individual tadpoles. Additionally, stable isotope analysis offers a continuous measure of diet variation between individuals, allowing us to describe the correlation between diet and morphology. This last point is important, because previous studies of *Spea* tadpoles have relied solely on morphology as a proxy for diet (e.g., see Martin & Pfennig 2009 and references therein; Martin & Pfennig 2010). Yet, to fully understand how competitively mediated selection acts on both morphology and diet to drive divergence in resource use, it is critical to investigate how diet varies with morphology.

We examined nitrogen stable isotopes ($\delta^{15}\text{N}$). Measures of $\delta^{15}\text{N}$ indicate an organism's trophic positions in a food web (Post 2002), which appears to be the primary dietary axis along which carnivores and omnivores differ (see Study System). To perform these analyses, we used the tadpoles from the collections described above (35.75 ± 3.8 tadpoles from each of 4 ponds or 143 tadpoles in total; 60 carnivores and 83 omnivores). We then obtained tissue by removing the

entire tail of each tadpole. Tail tissue samples were dried in a 60°C oven for 48 hrs. A $1.0 \pm .2$ mg sample of dried tissue was placed into a 5 x 9 mm silver capsule and submitted to the University of California at Davis Stable Isotope facility.

From the stable isotope data, we were able to infer the trophic structure of the *Spea* resource polymorphism. Specifically, we used these data to determine if the alternative morphs in this system: (1) differed in diet (as previous gut content analyses had suggested; see above); and (2) were similar or different in trophic breadth (Fig. 1).

We used box plots to infer the median, minimum, and maximum $\delta^{15}\text{N}$ values, as well as 25th and 75th percentiles for each morph classification. This also allowed us to determine the relative trophic levels that each group occupied and to examine the range of $\delta^{15}\text{N}$ values within each morph, which we interpreted as each morph's trophic breadth.

We first scaled each tadpole's $\delta^{15}\text{N}$ value to the shrimp $\delta^{15}\text{N}$ value from its pond. We did so by subtracting each tadpole's $\delta^{15}\text{N}$ value from that of the $\delta^{15}\text{N}$ value for shrimp that were collected from that pond (shrimp were sampled at the same time as the tadpoles from each pond). We performed this correction to control for any environmental variation that might cause $\delta^{15}\text{N}$ values to differ between ponds (i.e., shrimp might differ from pond to pond, which would cause tadpoles from different ponds to also differ even though their diets might actually be similar). We combined all the tadpoles from each pond into one analysis and then used the box plots to compare trophic breadth between morphs.

We statistically tested the hypothesis that omnivores and carnivores differed across all ponds in diet with a linear mixed model with the corrected $\delta^{15}\text{N}$ as our response variable, morphotype classification as our predictor variable, and pond origin as a random intercept fit by REML, and assuming unequal variances. We also statistically tested the hypothesis that omnivores and carnivores differed across all ponds in trophic breadth. To do so, we compared the variances of the corrected $\delta^{15}\text{N}$ for each morph.

Finally, for each morph and in each pond, we evaluated whether a tadpole's morphology reliably predicted its resource use, as measured by its corrected $\delta^{15}\text{N}$ value. These data were used to infer the possible existence of individual specialization (e.g., see Fig. 1d).

Comparing intra-morph competition for each morph

We found that carnivores utilize a narrow range of resources than omnivores (see Results) and could therefore be regarded as more of a dietary specialist. Thus, to determine if intramorph competition is a potential cost of the evolution of novel resource-use specialists (see Introduction), we compared the intensity of intramorph competition for both omnivores and carnivores.

In order to do so, we analyzed data from a previously published experiment (see Testing Prediction 3 in Martin & Pfennig 2009). This experiment was originally designed to test whether intraspecific competition is more intense the more similar any two conspecific competitors are to each other in resource use. However, because both carnivores and omnivores were used in this earlier experiment, we were able to re-analyze the data to determine if intramorph competition is less intense for omnivores than for carnivores.

In this earlier experiment, pairs of tadpoles were reared together in laboratory microcosms and given limited amounts of the two main resources: detritus and shrimp. We measured the growth of tadpoles and determined if tadpoles grew less the more similar they were to their tankmate in resource use.

To summarize the experimental design, randomly selected two-week old *S. multiplicata* tadpoles were weighed and assigned to one of two treatment groups: (1) an experimental group, in which two unrelated tadpoles were placed together in a tank, and (2) a control group, in which one tadpole was placed alone in a tank. Similar-sized siblings were placed in adjacent experimental and control tanks, thereby allowing for a comparison of growth of siblings whose rearing environments were similar in all respects except for the presence or absence of a potential

competitor. Before starting the experiment, the time it took for each tadpole to eat three fairy shrimp was measured. Shrimp-eating time is a highly repeatable measure, and tadpoles that are most similar in time to eat shrimp are most likely to compete for food (Pfennig, Rice & Martin 2007).

We analyzed a subset of data from this experiment that included the experimental tankmates that were most similar in predilection to consume shrimp; i.e., tankmates that were < 60 min different in shrimp eating time (31 experimental tanks). We then asked whether the intensity of competition differs within each morph class. More specifically, we measured how the intensity of competition varies between the more carnivore-like experimental pairs (those that consumed all three shrimp in a shorter period of time) and the more omnivore like experimental pairs (those that consumed all three shrimp in a longer period of time). Our response measure was each experimental tadpole's percent growth during the course of the experiment (final mass/initial mass), controlling for that of its matched control sibling. In particular, we subtracted from each experimental tadpole's percent change in mass the percent change in mass of its matched sibling in a neighboring control tank. Thus, values <0 indicated that the focal tadpole reared in competition grew less than did its sibling reared alone. By contrast, values >0 indicated that the focal tadpole reared in competition grew more than did its sibling reared alone.

If competition is less intense for more omnivore-like pairs of tadpoles than for more carnivore-like pairs of tadpoles, then the growth (adjusted percent change in mass) of experimental tadpoles should be higher the more omnivore-like (i.e., longer mean shrimp eating times) the experimental tankmates were. To test this prediction, we fit a linear model with the adjusted percent change in mass for all experimental tadpoles within the 60 min subset as the response, our measure of morphotype (mean time to eat three shrimp for each experimental pair) as a predictor, and the absolute value of the difference in mean shrimp eating time for each experimental pair as a covariate to account for the variation in shrimp eating time within each tank.

Results

Stable isotope analyses

Carnivores and omnivores (as expected) differed in morphological index: the overall (i.e., across all ponds) mean (\pm s.e.m.) morphological index for carnivores (1.62 ± 0.10 , $N = 60$ individuals) was significantly greater than that for omnivores (-1.18 ± 0.09 , $N = 83$ individuals; $F_{1,138} = 484.76$, $P < 0.0001$; Fig. 2a). Also as expected, we found two discrete dietary groups in every pond (see Supplementary Fig. S1). Overall, omnivores and carnivores differed significantly in mean $\delta^{15}\text{N}$ values, with carnivores consuming resources from a higher trophic level than omnivores (Fig. 2b). Indeed, the overall (i.e., across all ponds) mean (\pm s.e.m.) $\delta^{15}\text{N}$ value for carnivores (2.05 ± 0.09) was significantly greater than that for omnivores (-0.47 ± 0.07 ; $F_{1,138} = 106.45$, $P < 0.0001$). Moreover, omnivores and carnivores differed significantly in trophic breadth: carnivores possessed a narrower range of $\delta^{15}\text{N}$ values (standard deviation for carnivores: 0.44) than did omnivores (standard deviation for omnivores: 0.84; $F_{82,59} = 3.56$, $P < 0.0001$). Overall, the range of $\delta^{15}\text{N}$ values for carnivores (1.81 units) was less than half that of omnivores (3.95 units; Fig. 2b).

Additionally, we found evidence of a fine-scale relationship between resource use and morphology for omnivores, but not for carnivores. In particular, in two of the four ponds, an omnivore's morphological index significantly predicted its $\delta^{15}\text{N}$ value (Fig. 3). By contrast, in none of the ponds did a carnivore's morphological index predict its $\delta^{15}\text{N}$ value (data for all ponds combined; $F_{1,58} = 0.70$; $P = 0.40$; data for individual ponds are presented in Supplementary Fig. S2).

Comparing intra-morph competition for each morph

Competition was more intense among carnivores than among omnivores. Specifically we found a significant linear relationship between our measure of morphotype (mean time to each

shrimp) for each experimental pair and focal tadpole growth ($F_{1,28} = 6.16$; $P = 0.019$) The focal tadpoles that performed best in the experimental tanks were those that were the most omnivore-like in foraging behavior (i.e., those with the longest shrimp handling time). In contrast, the focal competitors that were the most carnivore-like performed the worst (Fig. 4).

Discussion

We used stable isotopes to infer the trophic breadth utilized by alternative carnivore and omnivore morphs of spadefoot toad tadpoles. We also analyzed the results of an experiment to assess the potential ecological consequence of any such differences. We found that these two morphs do indeed differ in trophic breadth, with carnivores exhibiting narrower trophic breadth—and occupying a higher trophic level—than omnivores (Fig. 2b). These data are consistent with earlier findings suggesting that carnivores consume mostly shrimp, whereas omnivores (as a group) harvest resources from more diverse (and generally lower) trophic levels (Pomeroy 1981). Thus, carnivores appear to be trophic specialists and omnivores trophic generalists (e.g., see Fig. 1c).

These morph-specific differences in trophic breadth (Fig. 2b) appear to reflect underlying morph-specific differences in morphology. In contrast to carnivores, omnivores possess an elongate intestine (Pomeroy 1981; Ledón-Rettig, Pfennig & Nascone-Yoder 2008). An omnivore's long intestine enables it to process and assimilate plant and bacterial material more effectively than a short-gutted carnivore could. Additionally, omnivores possess numerous labial teeth for rasping biofilm and algae from hard surfaces (Martin & Pfennig 2009; Martin & Pfennig 2011). Presumably, these features explain why the more morphologically omnivore-like a tadpole is, the better that individual grows when fed exclusively detritus (Martin & Pfennig 2009). By contrast, carnivores have morphological features (e.g., keratinized mouthparts and large jaw musculature) that render them more capable of subduing the large, active shrimp prey (Frankino & Pfennig 2001; Martin & Pfennig 2009; Martin & Pfennig 2011).

Although their distinctive features enable carnivores to take advantage of the ecological opportunity presented by the highly nutritious shrimp resource, these same features may resign carnivores to a life of trophic specialization. Such specialization can become problematic when the carnivore's shrimp resource becomes scarce (shrimp decline in abundance rapidly in natural ponds and are nearly always a limiting resource; Pfennig 1992). In such circumstances, carnivores may be unable to switch to alternative resources (e.g., detritus, plant material, and bacteria), because they are poorly equipped morphologically (and perhaps also physiologically and behaviorally) to process resources other than shrimp (Martin & Pfennig 2009). By contrast, omnivores can consume and assimilate a wide range of food items, including small crustaceans and even the occasional moderate-sized shrimp, as long as they do not have to compete for the latter food items (Frankino & Pfennig 2001). Thus, for functional morphological reasons, carnivores may have no recourse other than to remain as trophic specialists, whereas omnivores retain the capacity to be either generalists opportunistically or (as described below) specialize individually to minimize competition.

These functional trade-offs, combined with the carnivore's dependence on an ephemeral, scarcer resource (because shrimp occur at a higher trophic level than the alternative food resources of plants, bacteria, and organic detritus, shrimp are also scarcer to begin with), presumably explain our experimental results: that carnivores face more intense intramorph competition than omnivores (Fig. 4). Indeed, whereas all carnivores are forced to compete for shrimp, omnivores utilize prey from diverse trophic levels (Fig. 2b), which should reduce dietary overlap and thereby competition among omnivores. More generally, functional limitations associated with increased specialization might often lead to increased intramorph competition among specialist. Thus, whether or not a population actually evolves alternative resource-use specialists—and, hence, resource polymorphism—should depend on whether or not the benefits of increased resource specialization (i.e., increased access to profitable prey) exceed the costs of increased intramorph competition.

Although competition among specialized morphs could represent an important factor that precludes the evolution of resource polymorphism, it is not the only such factor. The absence of ecological opportunity may also prevent a resource polymorphism from evolving in a population (Martin & Pfennig 2010). For competitively mediated selection to promote the evolution of a resource polymorphism, ecological opportunity (in the form of alternative resource types underutilized by other species) must also be present (Smith & Skúlason 1996; Martin & Pfennig 2010). In the absence of such resources, a resource polymorphism is unlikely to evolve. By contrast, when underutilized resources are present, a population experiencing intense intraspecific competition can expand the range of resources it uses as an adaptive response to competition. Consistent with this theory, resource polymorphisms are found most often in environments where intraspecific competition is intense and ecological opportunity (e.g., underutilized resources) is present (Collins 1981; Walls, Belanger & Blaustein 1993; Robinson & Wilson 1994; Wimberger 1994; Smith & Skúlason 1996; Robinson & Wilson 1998; Skúlason, Snorrason & Jónsson 1999; Svanback et al. 2008; Martin & Pfennig 2010). In the case of *Spea* tadpoles, variation in shrimp abundance is crucial in explaining variation in carnivore abundance, and hence, the presence/absence of resource polymorphism (Pfennig 1990; Martin & Pfennig 2010). Thus, the evolution of resource polymorphism may depend on there being ample ecological opportunity to increase the chances that the benefits of specialization will outweigh its costs.

Our results help explain how a generalist can coexist with a specialist that utilizes higher-quality resources. In resource polymorphisms with a generalist and specialist, the specialist often monopolizes the higher-quality (i.e., more profitable) resource type (Smith & Skúlason 1996). For example, in spadefoot tadpoles, the specialist (i.e., the carnivore morph) outcompetes the generalist (i.e., the omnivore morph) for the shrimp resource (Frankino & Pfennig 2001), which is the resource on which spadefoot toad tadpoles grow best (Pfennig 2000; Pfennig & Murphy 2000). Fitness trade-offs may explain how specialists and generalists coexist under such circumstances. Specifically, although specialists may benefit by gaining access to the more

profitable resource, they may concomitantly experience greater intramorph competition (Fig. 4). Negative frequency-dependent selection may therefore maintain both morphs within the same population, such that both the specialist and generalist have, on average, equal fitness.

Interestingly, in two of the four ponds examined, we found a significant positive relationship between our morphological index and $\delta^{15}\text{N}$ among omnivores (Fig. 3), but not among carnivores (Supplementary Fig. S2). Indeed, in the two ponds where there was a significant relationship (Fig. 3a, d), those omnivores that differed the most from carnivores morphologically also differed the most from carnivores in diet; omnivores that were intermediate in morphology were also intermediate in diet; and omnivores that were the most similar to carnivores morphologically were also the most similar in diet. In other words, although omnivores as a group are dietary generalists, individually they may specialize in what food items (or combinations of food items) they eat. Thus, dietary generalization among omnivores may be achieved when different omnivores specialize on slightly different trophic levels. Such individual specialization reduces pair-wise dietary overlap between individuals, which can lessen the intensity of competition (Bolnick et al. 2003; see also below). In fact, in the two ponds where the significant relationship was detected, qualitative estimates of resource levels and tadpole densities (e.g., see Martin & Pfennig 2010) indicated that the per capita resource abundance was low (J. Paull, unpubl. data), suggesting that intraspecific competition may have indeed been highest in those ponds where individual specialization was detected.

Our data may therefore clarify how competitively mediated selection drives divergence in both diet and morphology among specialists versus among generalist. Compared to omnivores, carnivores show similar variation in morphology (Fig. 2a), but low variation in diet (Fig. 2b). Moreover, no (obvious) fine-scale relationship exists between diet and morphology among carnivores (see Results). Therefore, rather than favoring carnivores that are increasingly more divergent from their fellow carnivores in diet, competitively mediated selection may favor competitively superior carnivores. Indeed, carnivores with larger morphological index values

subdue shrimp more easily and therefore outcompete fellow carnivores with lower morphological index values (Martin & Pfennig 2009). By contrast, omnivores show high variation in diet (Fig. 2b), and (in two of the four ponds) they exhibited a fine-scale relationship between diet and morphology (Fig. 3a, d). Thus, selection for more extreme omnivores may reflect selection for dietary divergence, which allows individuals to avoid competition with other omnivores by consuming slightly different resources. Competitively mediated selection may therefore reduce intramorph competition differently among specialists than among generalists.

In sum, our results reveal that, in a population containing alternative resource-use morphs—one of which is a generalist and the other of which is a novel specialist morph—the latter may often experience more severe intramorph competition. If these costs outweigh the benefits of specializing on a more nutritious resource, they may preclude the evolution of alternative resource use specialists, even in a population experiencing strong intraspecific competition for resources. Instead, such populations would be expected to evolve trophic generalists only. Generally, the evolution of resource polymorphism likely depends on the presence of sufficient ecological opportunity to increase the chances that specialization's benefits will outweigh its costs.

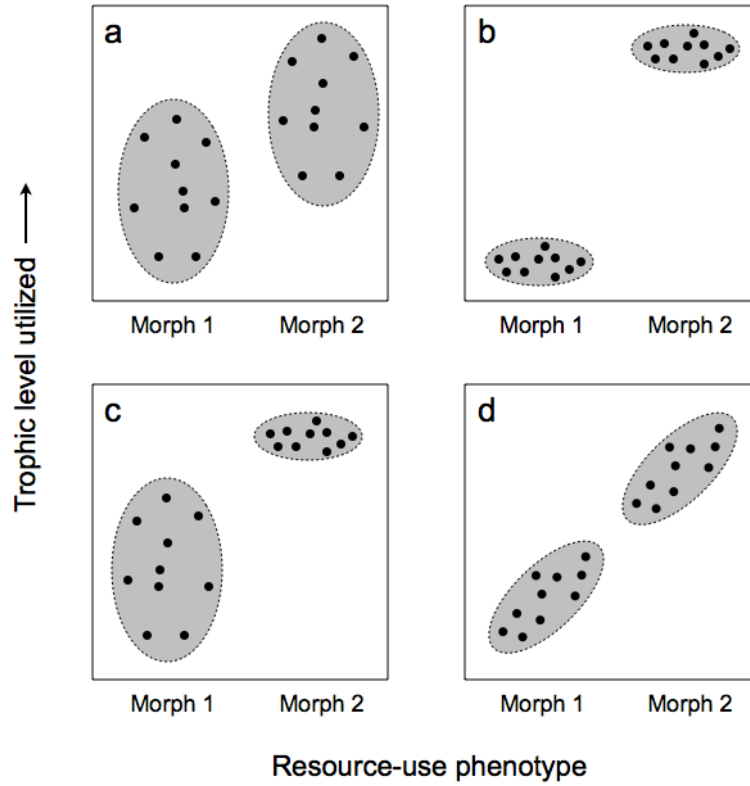


Fig. 1. Possible combinations of alternative morphs. Resource polymorphisms may consist of: (a) two generalists; (b) two specialists; (c) a generalist and a specialist; or (d) two generalists, with individual specialization. In all panels, dots are meant to signify different individuals, whereas the shaded ovals demarcate distinct morphotypes.

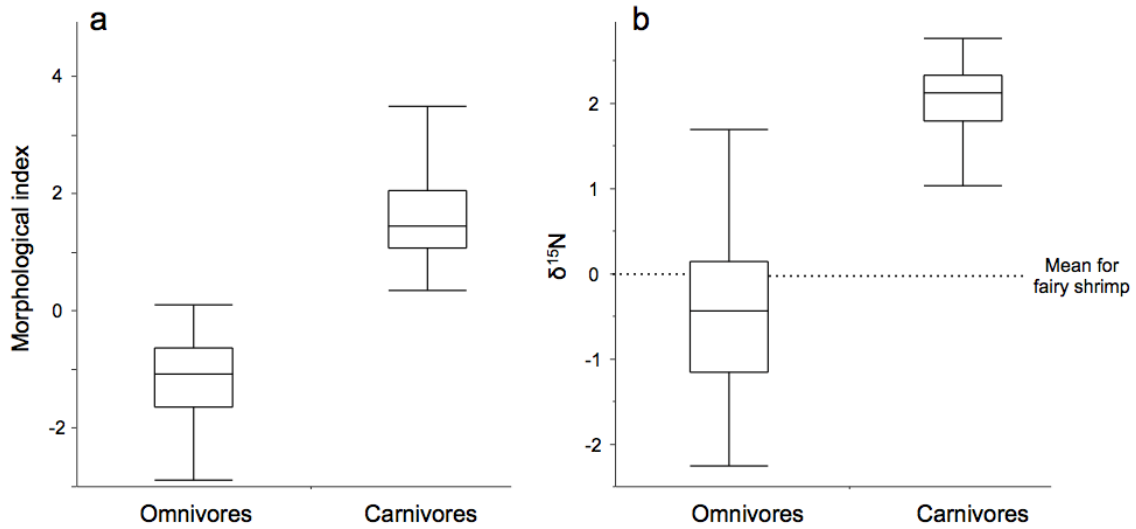


Fig. 2. Comparison of (a) morphological index and (b) stable isotope ($\delta^{15}\text{N}$) values (a measure of trophic levels utilized) of omnivores ($N = 83$ individuals) versus carnivores ($N = 60$ individuals) from four different ponds. Box plots show median (horizontal lines), 25th and 75th percentiles (top and bottom of box), and range (whiskers, excluding outliers).

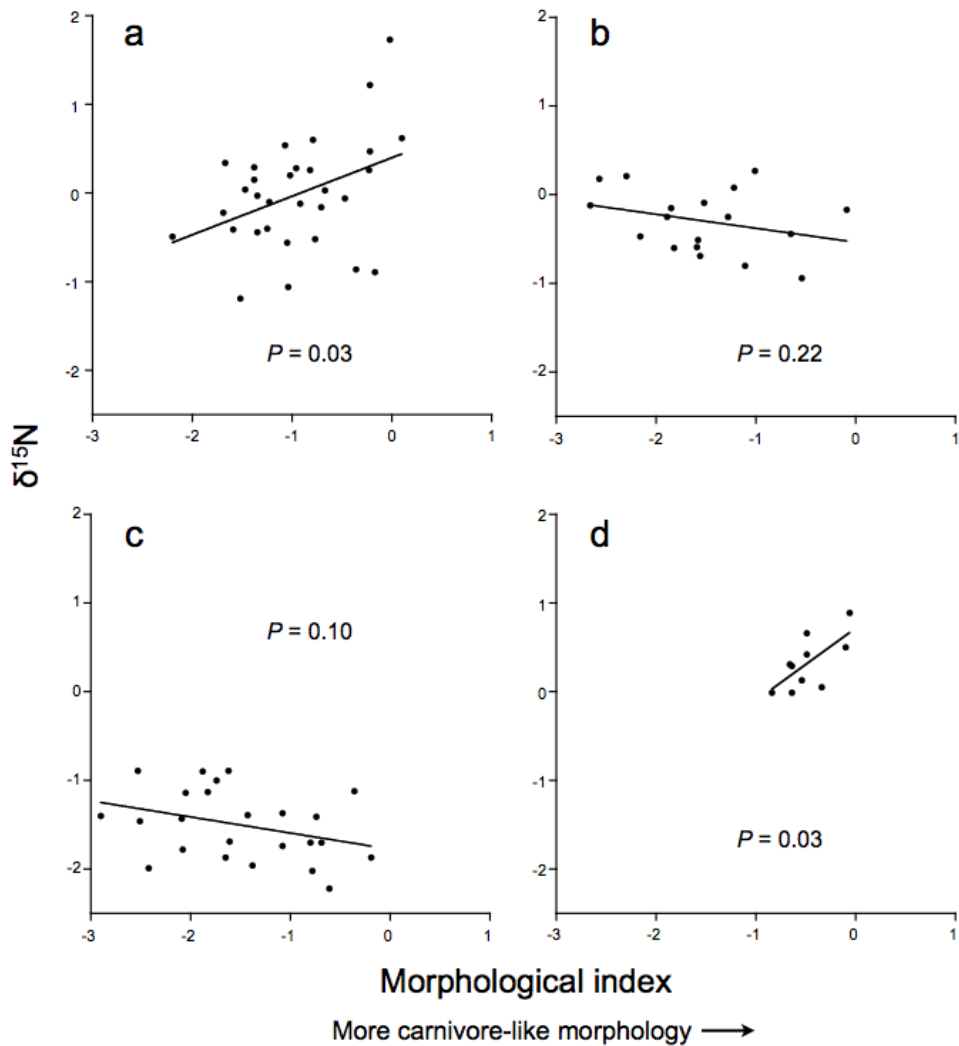


Fig. 3. Relationship between an omnivore's morphological index and its $\delta^{15}\text{N}$ values in four ponds: (a) Ava Ranch; (b) Crissal; (c) P.O.N.; and (d) Price Canyon. Each dot represents an individual omnivore; least-squares regression lines are shown for illustrative purposes only. Note that a significant relationship exists between trophic morphology and resource use in Ava Ranch and Price Canyon ponds, possibly suggesting individual specialization in these ponds.

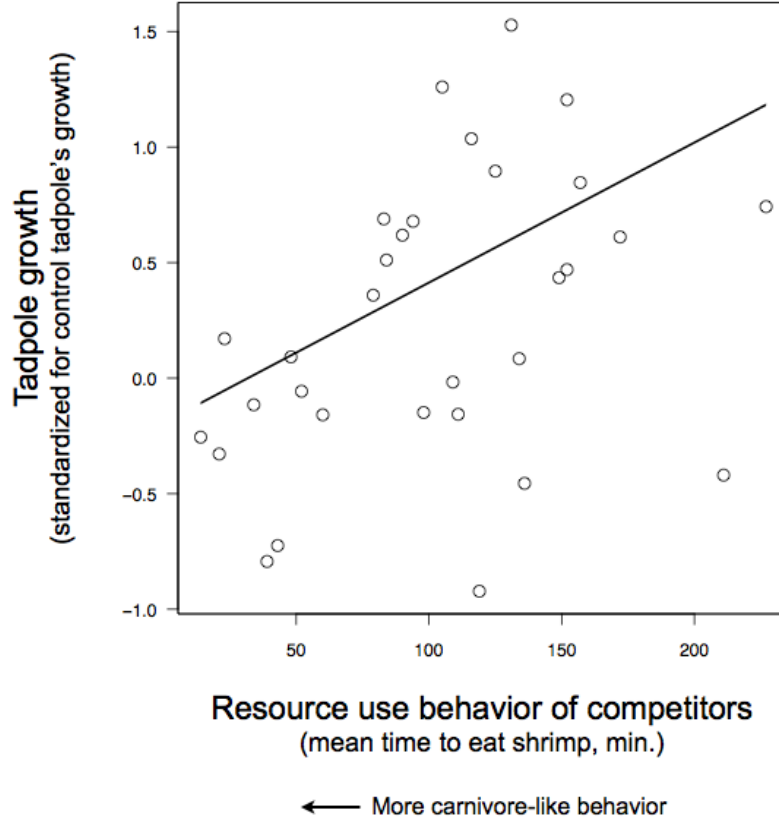


Fig. 4. Experimental evidence that the trophic specialist (carnivores) suffer more from competition with each other than the trophic generalists (omnivores) do with each other. Shown is the relationship between resource-use behavior of two competitors (mean time to eat shrimp, where more carnivore-like tadpoles eat shrimp faster) and tadpole growth (specifically, growth of the focal tadpole compared to a control sibling that did not experience competition).

CHAPTER III

THE EFFECTS OF COMPETITION ON DIET AND MORPHOLOGY

Introduction

Resource competition plays an important role in driving the evolution of resource-use diversity both within and between species. Divergence arises as natural selection favors individuals that are phenotypically dissimilar from their competitors, which reduces the effects of competition. Spadefoot toad tadpoles are quickly becoming a model system for investigating resource competition and the evolution of phenotypic diversity both within species and between species (reviewed in Martin & Pfennig 2009, 2010; Pfennig & Martin 2010). In such investigations it is critical to understand the relationship between diet and the functional morphological traits associated with diet. This allows researchers to understand how selection acts on both diet and morphology to drive phenotypic divergence. Until now, investigations in spadefoot toads have used morphology as a proxy for diet, and the relationship between diet and morphology was based on an unpublished Ph.D. thesis (Pomeroy 1981). In my thesis, I utilize stable isotope analysis to investigate the relationship between diet and morphology in spadefoot toad tadpoles. This technique promises to clarify the effects of competition on resource use, independent of morphology

Clarifying the effects of intraspecific competition on diet and morphology

Using stable isotope analysis, I clarified how natural selection might act on both morphology and diet to drive resource polymorphism. For instance, in chapter 2 of this thesis I demonstrated that, while, competitively mediated selection might drive divergence between

carnivores and omnivores in morphology (Martin & Pfennig 2009), such selection may be acting differently within each morph. In particular, carnivores showed high variation in morphology but relatively low variation in diet (Fig. 2), and there is no (obvious) relationship between diet and morphology among carnivores (Fig. 3). Therefore, if competitively mediated selection is driving the evolution of more extreme carnivores, it might do so by favoring better competitors rather than individuals that are more divergent in diet. In contrast, omnivores show a correlation between diet and morphology. Therefore, selection for more extreme omnivores might reflect selection for dietary divergence allowing individuals to avoid competition (with other omnivores) by consuming slightly different resources.

Clarifying the effects of interspecific competition on diet and morphology

In addition to clarifying how selection acts between the alternative morphs that are part of a resource polymorphism, stable isotope analysis can potentially be used to clarify how selection acts between different species undergoing character displacement. In character displacement, coexisting species diverge in resource-use due to selection acting to reduce their competitive interactions (reviewed in Dayan & Simberloff 2005; Pfennig, D. W. & Pfennig, K. S. 2010). Such selection may act differently within each species. Two species of spadefoot toads, *Spea multiplicata* and *Spea bombifrons*, undergo ecological character displacement where they co-occur. Specifically, they undergo character displacement in trophic morphology (Pfennig & Murphy 2000, 2003; Pfennig et al. 2006, 2007). When each species occurs alone, they produce similar frequencies of both morphs (Pfennig & Murphy 2003; Pfennig et al. 2006). By contrast, when they occur together, *S. bombifrons* produce mostly carnivores, whereas *S. multiplicata* produce mostly omnivores (Pfennig & Murphy 2003; Pfennig et al. 2006).

Previous research found that each species experiences different modes of selection during character displacement. They found that *S. multiplicata* tadpoles experience stabilizing selection on trophic morphology, while *S. bombifrons* tadpoles experience directional selection on trophic

morphology. Directional selection in *S. bombifrons* favors more carnivore-like tadpoles and this likely reduces the amount of resource competition with *S. multiplicata*. Stabilizing selection in *S. multiplicata* favors more intermediate phenotypes. It remains unclear why selection should favor more extreme phenotypes in *S. bombifrons* but not in *S. multiplicata*. It seems that competition would be further reduced if more omnivore-like phenotypes were favored in *S. multiplicata*. Using stable isotope analysis to investigate the dietary structure within each of these populations may help to clarify this apparent paradox.

It is possible that morphological variation does not actually reflect dietary variation. If this is the case, the divergent directional selection between species in trophic morphology may not act to reduce diet overlap between species. Selection might actually be acting on some other aspect of foraging behavior besides diet. For instance, directional selection on trophic morphology in *S. bombifrons* might favor better competitors for shrimp rather than individuals that are more divergent in diet. Indeed, individuals that are morphologically more carnivore-like can subdue shrimp more easily (Martin & Pfennig 2009). If this is the case, stable isotope analysis might show low diet variation within *S. bombifrons* tadpoles such that there is low potential for diet to respond to selection. In contrast, stabilizing selection on trophic morphology in *S. multiplicata* might favor a dietary generalist phenotype that can take advantage of a wider resource spectrum opportunistically. If this is the case, stable isotope analysis might show higher diet variation within *S. multiplicata* tadpoles in comparison to *S. bombifrons* tadpoles.

Additionally, by examining the mode of selection on $\delta^{15}\text{N}$ (a proxy for trophic level) within each species, stable isotope analysis, can be used to investigate how selection acts directly on diet rather than treating morphology as a proxy for diet. This would also clarify whether diet and trophic morphology both respond to competitively mediated selection similarly or whether diet and morphology respond to this type of selection differently. For example, we might find that competitively mediated selection favors more extreme carnivore morphology in *S. bombifrons*,

but we might find no selection on carnivore diet. Such data should help to clarify why selection does not always act similarly between species undergoing character displacement.

Clarifying the effects of community-wide competition on diet and morphology

Stable isotope analysis might also be used to clarify how spadefoot toad communities respond to community-wide competition. This technique can be used, in general, to address how species in a community partition resources and avoid competition. Community coexistence of closely related species is facilitated by resource partitioning, where each species uses the available resources in different ways (Schoener 1974). Resources can be partitioned along a continuous spectrum in two ways (fig. 5). If the resource spectrum is broad (i.e., if there is ample ecological opportunity), then species may diverge from each other in their use of resources along the resource spectrum to minimize overlap. If however, the resource spectrum is narrow (i.e., if there is restricted ecological opportunity), then species might avoid competition by specializing on a narrower set of resources and minimize overlap in this way. In the first scenario, sympatric populations would have significantly different average isotopic signatures from each other, but with isotopic variances equal to that of their allopatric counterparts. In the second scenario, sympatric populations would have a smaller isotopic variance than their allopatric counterparts while the average compared with allopatry may or may not be different.

Spadefoot toads often occur as three species communities. In some of the ephemeral ponds where tadpoles develop, three species can be found; *Spea multiplicata*, *Spea bombifrons* and *Scaphiopus couchii*. As noted above Pfennig et. al. (2007) show that in sympatry, the selection function, for *S. multiplicata*, is stabilizing. However, in allopatry, selection on their morphology is disruptive, and these tadpole populations may often exhibit resource polymorphism. Since *S. multiplicata* tadpoles produce mostly omnivores in sympatry, it is expected that they should experience directional selection for more extreme omnivores. Instead, they experience stabilizing selection.

This suggests that when it occurs in sympatry with other community members, *S. multiplicata* may have become more specialized trophically (i.e. they might have a narrower trophic niche), rather than becoming more trophically divergent. *S. multiplicata* may suffer from increased competition from *S. bombifrons* (a superior competitor for shrimp (Pfennig & Murphy 2000) on one end of the shared resource spectrum and *S. couchii* (an obligate omnivore; Rettig et.al. 2008) on the other end. Thus *S. multiplicata* may become more trophically specialized on a diet intermediate between *S. bombifrons* and *S. couchii* to avoid competition with these two species.

This hypothesis could be tested using stable isotope analysis to compare differences in the means and standard deviations of the isotopic signatures between species where they co-occur in sympatry compared to where they occur alone in allopatry. If *S. multiplicata* avoids community-wide competition by utilizing a much smaller niche than it does in allopatry, then we would expect its isotopic variance in sympatry, with the other two species, to be narrower than its isotopic variance in allopatry. These data might then explain why *S. multiplicata* undergoes stabilizing selection in sympatry with *S. bombifrons* and *S. couchii*; because it may suffer from resource competition imposed by both species, but on opposite sides of their shared resource spectrum.

Clarifying the effects of interspecific competition on the ontogeny of diet and morphology

Stable isotope analysis might help to clarify the ontogeny of character displacement. Spadefoot toads exhibit phenotypic plasticity in trophic morphology such that character displacement could occur facultatively. Though, it is unclear whether character displacement does indeed proceed facultatively, or rather, via hard selection on intermediate phenotypes. In facultative character displacement, individuals within each species would adjust their diets in response to competition imposed by the competing species. In selective character displacement, individuals that develop intermediate phenotypes (phenotypes that overlap between each species)

would suffer higher mortality than individuals that develop more extreme phenotypes. Each of these two scenarios would produce similar distributions of phenotypes late in ontogeny (when character displacement is generally measured and detected in this system).

To determine how character displacement unfolds during ontogeny, stable isotope analysis could be used to investigate the diet structure within each species of spadefoot toad, both early and late in ontogeny. If character displacement is facultative, then I would expect each species to show similar isotopic means and ranges early in ontogeny. I would then expect the means to differ late in ontogeny (in accordance with the predictions of character displacement) but with the late occurring isotopic ranges of both species, being similar to the early occurring isotopic ranges of both species. This would reflect a facultative shift in diet.

If character displacement is selective (i.e. phenotypically intermediate individuals suffer higher mortality than individuals that develop more extreme phenotypes), then I would expect each species to, once again, show similar isotopic means and ranges early in ontogeny. I would also expect the means to differ late in ontogeny, however in this case, the isotopic ranges should decrease in the later ontogenetic stage. If character displacement is selective, then the overlapping portion of the isotopic range, that was present between species early in ontogeny, should be missing late in ontogeny.

In general, stable isotope analysis may be used in this way to study the effects of competition on ontogenetic changes in population diet structure. Additionally, stable isotope analysis may be used in this way to address how ontogenetic changes in morphology relate to ontogenetic changes in diet.

Summary

Although morphology is often used as a proxy for understanding diet variation, it may not always reflect diet in a straightforward manner. Because spadefoot toad tadpoles are quickly becoming a model system for investigations into the effects of resource competition, it is essential that we understand the relationship between diet and morphology in this system.

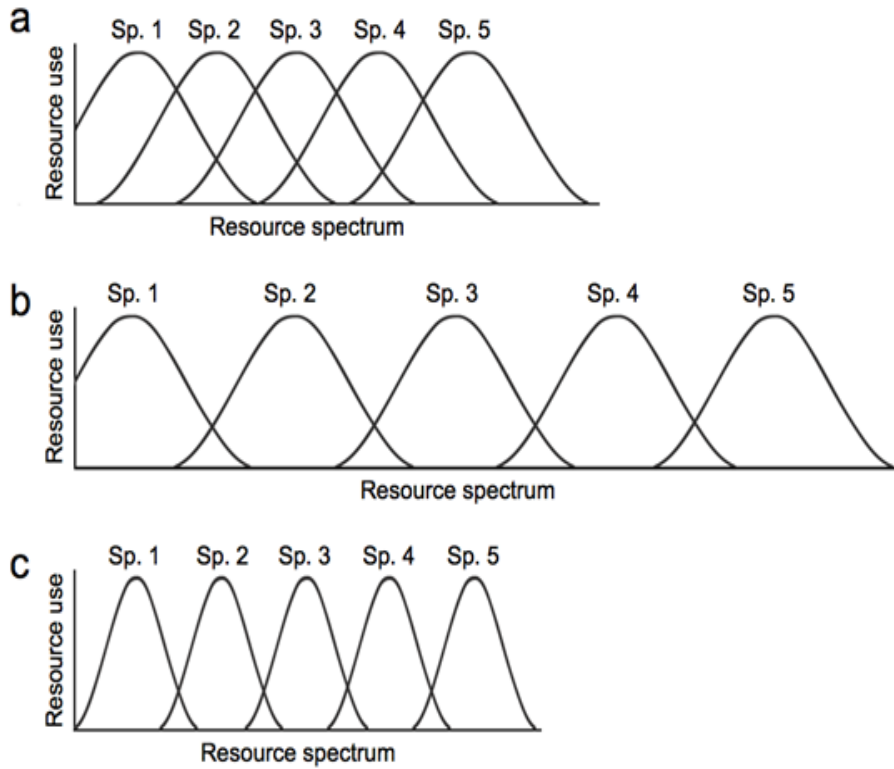


Fig. 5. How species within a community may partition resources. (A) Here 5 species share space in a resource spectrum (e.g. Seed size or proportional use of different resources). (B) Given ample ecological opportunity these species can diverge in resource use to minimize overlap. (C) Without ecological opportunity, species can specialize on a narrower set of resources to minimize overlap.

APPENDIX

CHAPTER II Supplemental Table

Table S1. Estimates of bimodality of tadpole morphological indices in natural ponds. ΔAICc refers to the difference between a single normal distribution and a mixture of two normal distributions fitted using Bayesian methods. $\Delta\text{AICc} > 4$ suggest more support for the fit of a mixture model (i.e., two normal distributions) than the fit of a single normal distribution, while ΔAICc between -4 and 4 suggest equivalent support for the fit of the mixture models and single normal distribution and ΔAICc below -4 suggest more support for the fit of a single normal distribution.

Pond	Sample size	ΔAICc
Ava Ranch	41	33.87
Crissal	33	12.33
P.O.N.	36	21.94
Price Canyon	32	9.15

CHAPTER II Supplemental Figures

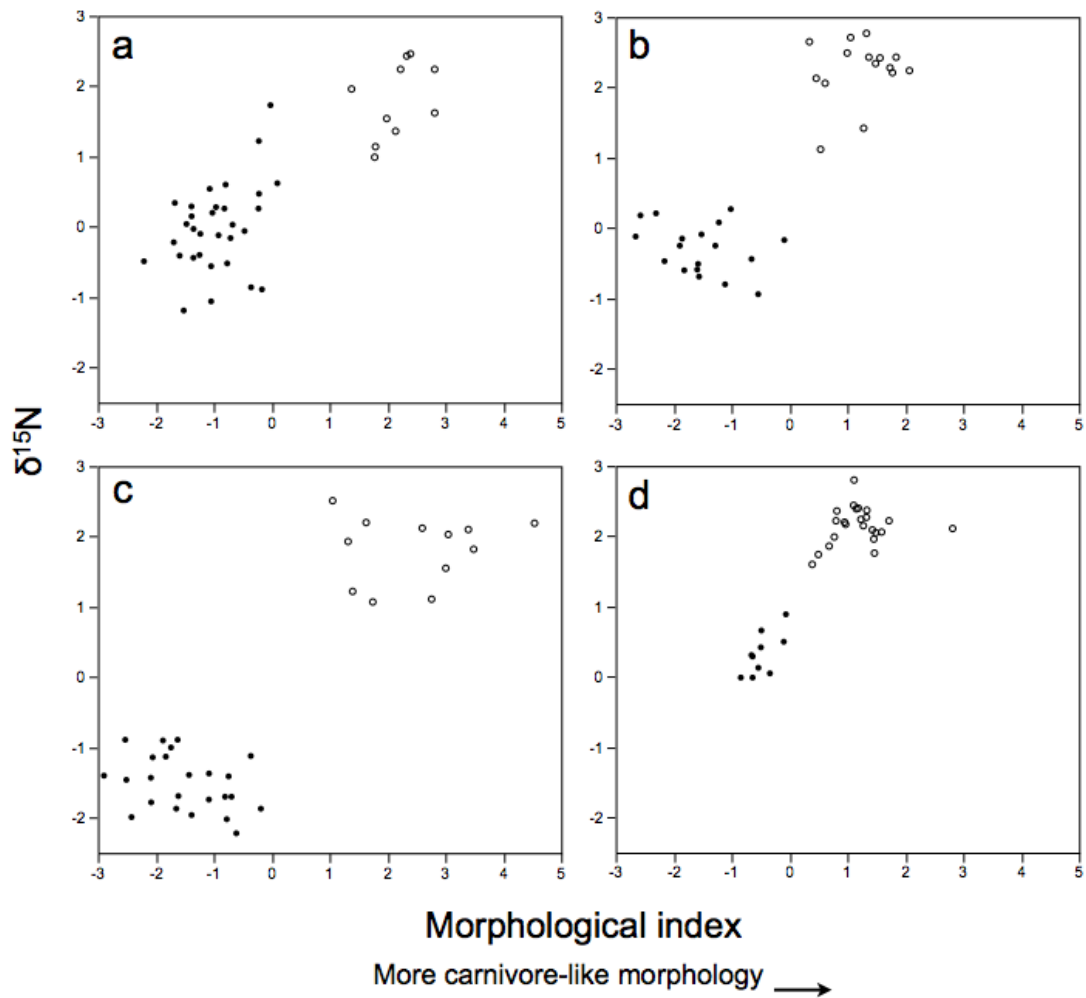


Fig. S1. Resource use, as inferred by $\delta^{15}\text{N}$, plotted against morphological index for individual carnivores (open circles) and omnivores (closed circles) in four ponds: (a) Ava Ranch; (b) Crissal; (c) P.O.N.; and (d) Price Canyon.

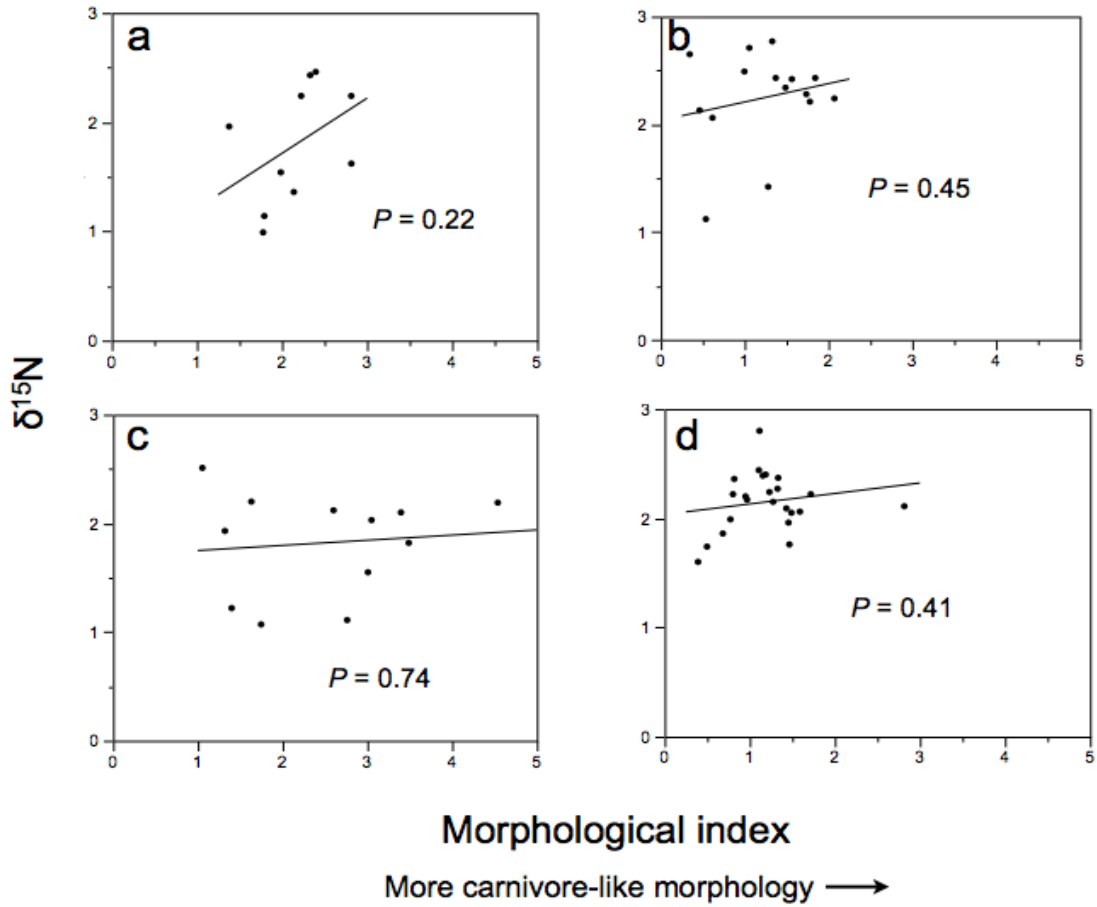


Fig. S2. Relationship between a carnivore's morphological index and its $\delta^{15}\text{N}$ values in four ponds: (a) Ava Ranch; (b) Crissal; (c) P.O.N.; and (d) Price Canyon. Each dot represents an individual carnivore. Least-squares regression lines are shown for illustrative purposes only.

ADDITIONAL FIGURES

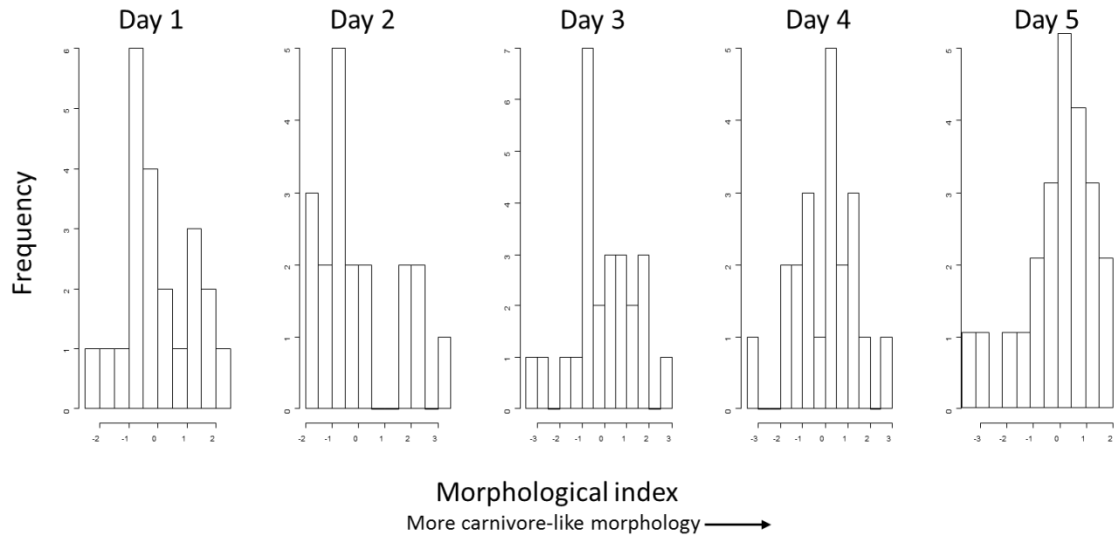


Fig. A1. The ontogeny of resource polymorphism. In 2009, I serially sampled tadpoles from a pure *S. multiplicata* pond (Crater pond). I sampled the pond every 3 days, for 5 days until metamorphosis. I began sampling a few days post hatching. To visualize when bimodality arises during the ontogeny of resource polymorphism, I developed histograms, along the morphological index, for each collection. It does not appear that bimodality was achieved in this pond (i.e. it did not exhibit resource polymorphism), therefore I was unable to describe the ontogeny of resource polymorphism.

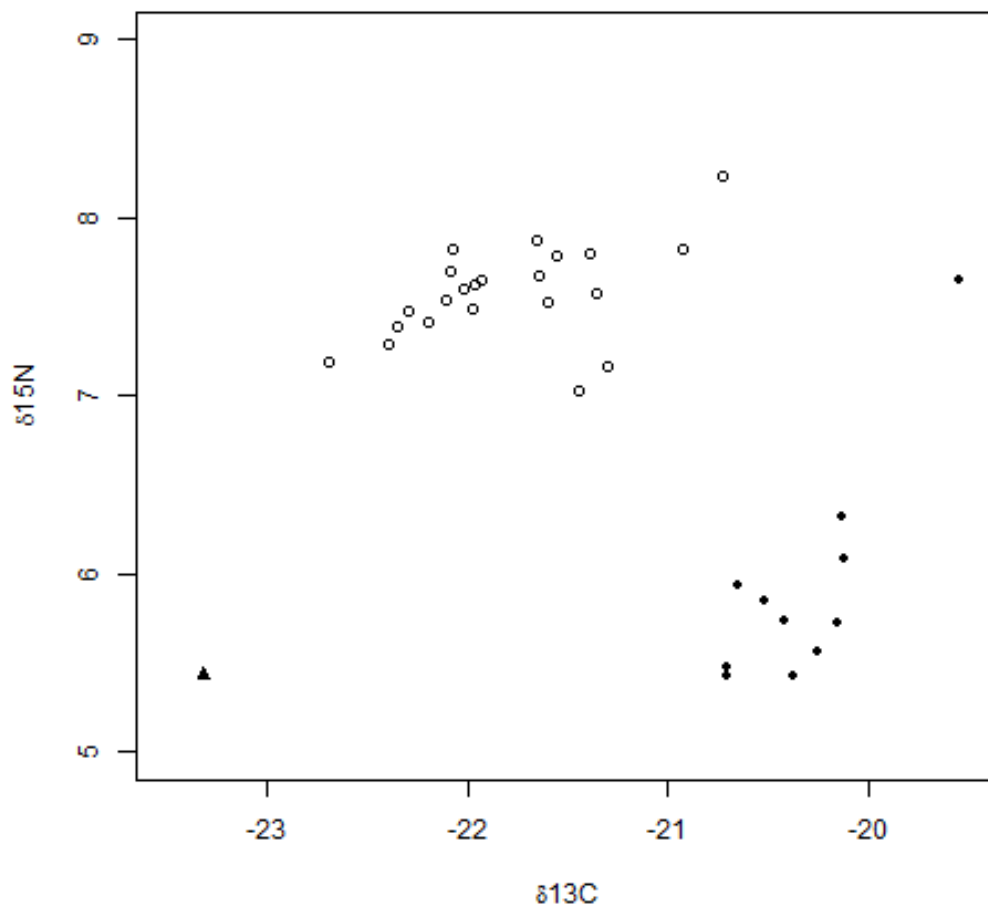


Fig. A2. Stable isotopic bi-plot part I. This figure shows a stable isotope bi-plot within a population of *S. multiplicata* (from Price Canyon pond) that exhibited resource polymorphism. This demonstrates how resources are utilized, within a resource polymorphic population, in isotopic niche space. Tadpoles fall within two major resource-use groups as inferred by two axes of isotopic niche, $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$. $\delta^{15}\text{N}$ is a proxy for trophic level with higher values representing higher trophic levels. $\delta^{13}\text{C}$ likely reflects differences in limnetic resources (lower values of $\delta^{13}\text{C}$) vs. littoral resource (higher values of $\delta^{13}\text{C}$). Individual carnivores are represented by (open circles), omnivores by (closed circles). The average isotope value for a limnetic resource, fairy shrimp (an important resource for carnivores) is represented by a (closed triangle).

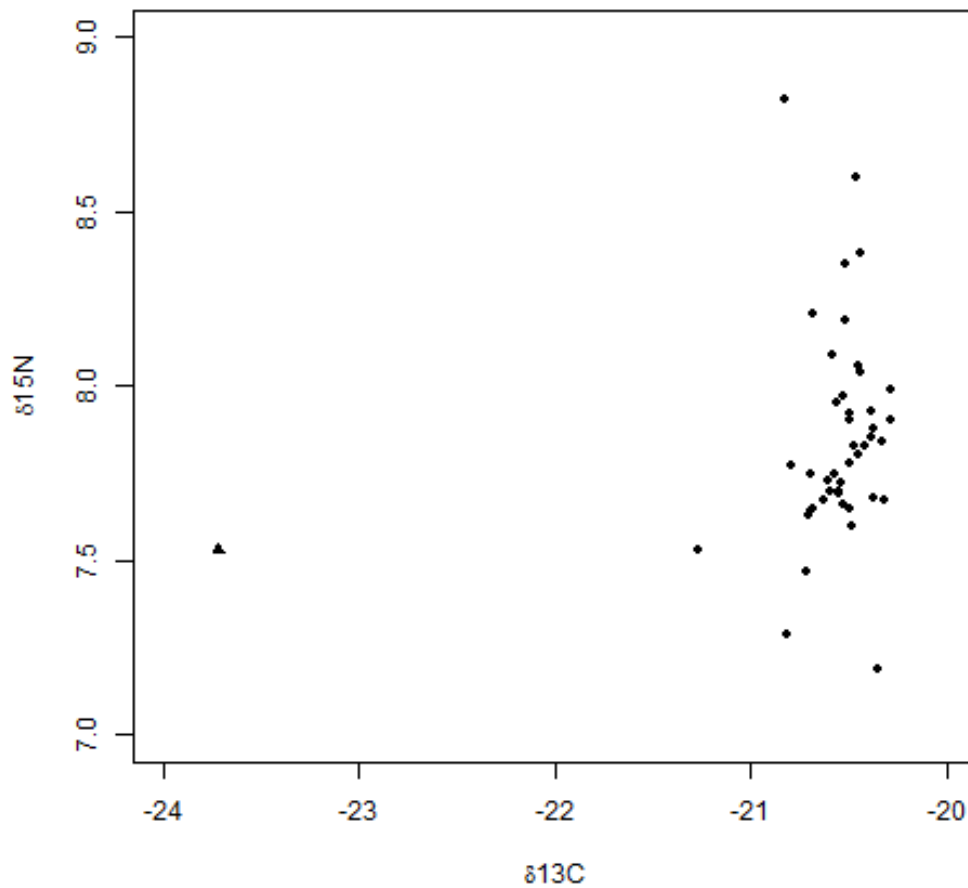


Fig. A3. Stable isotopic bi-plot part II. This figure shows a stable isotope bi-plot within a population of *S. multiplicata* (from Rock Tank pond) that did not appear to exhibit resource polymorphism. There appears to be one major dietary group that uses similar resources. All tadpoles are represented by (closed circles). The average isotope value for fairy shrimp is represented by a (closed triangle). These tadpoles appear to be omnivores, based on their relationship to shrimp in isotopic space. Carnivores appear to be absent from this pond.

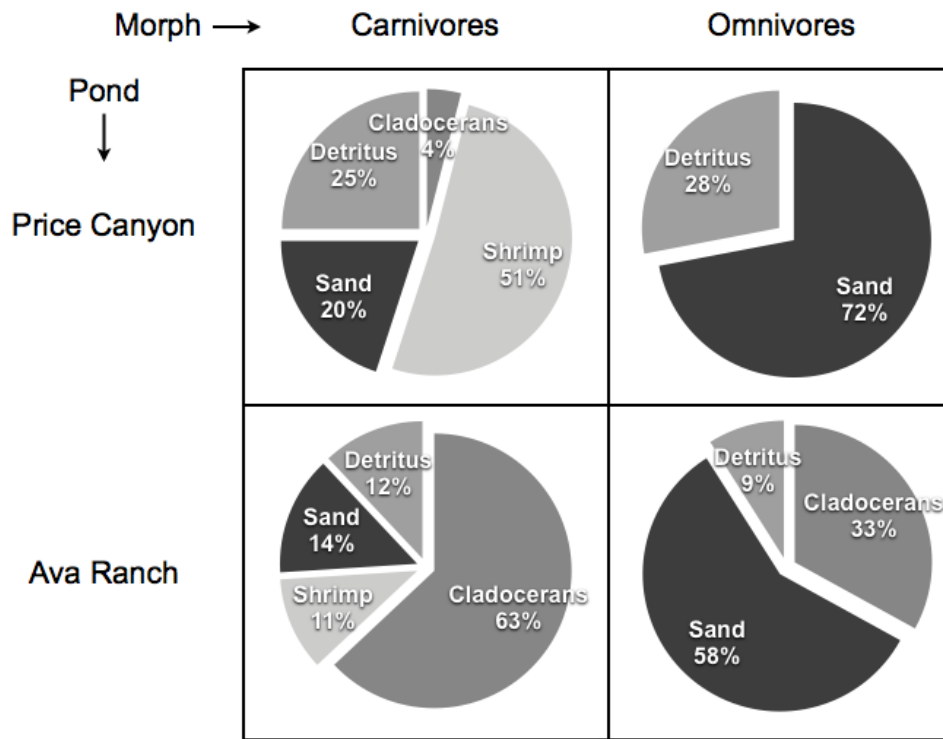


Fig. A4. Stomach content analysis in omnivores and carnivores. I randomly selected ten carnivores and ten omnivores, from each of AVA pond and Price Canyon pond for stomach content analysis. These tadpoles were also used as part of the stable isotope analysis described in chapter II. Stomach contents were classified according to the following four broad categories: “shrimp”, which included any anostracan fairy shrimp; “cladocerans”, which included any small crustaceans, mostly *Daphnia*; “detritus”, which included any small pieces of plant material and other small unidentifiable dark organic material; and “sand”, which included any crystalline material. I considered sand to be a possible food resource, since, before consumption, this material was likely coated with a complex aggregation of microorganisms; i.e., a biofilm. I then estimated the volumetric proportions of each of these food items by following the methods presented in (Genner et al. 1999), which utilized a modified version of the points method of Hynes (1950).

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