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Antipredator Behavior Promotes Diversification of Feeding Strategies

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Synopsis Animals often facultatively engage in less risky behavior when predators are present. Few studies, however, have investigated whether, or how, such predator-mediated behavior promotes diversification. Here, we ask whether tadpoles of the spadefoot toad *Scaphiopus couchii* have a diminished ability to utilize a potentially valuable resource—anostracan fairy shrimp—because of behavioral responses to predation risk imposed by carnivorous tadpoles of the genus *Spea*. Observations of a congener of *Sc. couchii* that occurs in allopatry with *Spea*, coupled with an ancestral character state reconstruction, revealed that *Sc. couchii*'s ancestors likely consumed shrimp. By experimentally manipulating the presence of *Spea* carnivore-morph tadpoles in microcosms, we found that *Sc. couchii* reduce feeding and avoid areas where both *Spea* carnivores and shrimp occur. We hypothesize that the recurrent expression of such behaviors in sympatric populations of *Sc. couchii* led to the evolutionary fixation of a detritivorous feeding strategy, which is associated with a reduced risk of predation from *Spea* carnivores. Generally, predator-mediated behavior might play a key role in promoting diversification of feeding strategies.

Introduction

Predation is a ubiquitous—and potentially potent—agent of natural selection. Thus, most species have experienced prolonged and intense selection for adaptations that reduce the risk of being eaten (reviewed by Endler 1991). A common antipredation strategy among animals is to facultatively engage in less risky behavior when a predator is present (Skelly 1994; Peacor and Werner 2001). Although predator-mediated behavior has traditionally been regarded as having no long-term consequences, it can profoundly impact the population dynamics of prey (Werner and Peacor 2003; Schmitz et al. 2004; Miner et al. 2005; Preisser et al. 2005; Agrawal et al. 2007; Kishida et al. 2010) and even promote divergence between populations of prey (Edgell et al. 2009; Scoville and Pfrender 2010; Ingram et al. 2011).

Specifically, traits associated with an antipredator behavior might diverge between populations experiencing different regimes of predators. In a population recurrently experiencing predation, traits associated

with an antipredator response would be continually expressed and subject to the selective pressures of the predator environment. Consequently, traits associated with a predator-free environment would be subject to relaxed selection, which might result in the evolutionary loss of the ability to express such traits (reviewed by Lahti et al. 2009; Pfennig et al. 2010). Once this occurs, the formerly induced response is expressed constitutively and becomes “fixed” in the population (Edgell et al. 2009; Scoville and Pfrender 2010). Although populations experiencing high levels of predation might be expected to undergo such fixation, populations experiencing low levels would not (Scoville and Pfrender 2010). In this way, behavioral responses to predators (or any environmental cue) may actually precede, and even facilitate, genetically canalized change (Price et al. 2003; West-Eberhard 2003).

Predator-mediated behavior might be especially important in promoting the diversification of feeding strategies, particularly when both predators and prey share common resources; i.e., when they belong

to the same ecological guild (Polis et al. 1989; Holt and Polis 1997). With intraguild predation, prey individuals most similar to the predator in resource use run the greatest risk of predation, because of their close spatial and temporal proximity to the predator. Such selective predation can cause the prey population to diverge from the predator population in traits associated with the acquisition of resources, thereby generating a pattern that might be mistakenly construed as having arisen from ecological character displacement (i.e., trait-evolution stemming from selection that lessens competition for resources) (Schluter 2000). Although the primary agent of this divergent selection between populations would be predation—not competition—ecological character displacement between predators and prey might accentuate these differences. Such divergence might transpire especially rapidly if a behavioral response that is adaptive in the presence of predators is expressed concurrently with a resource-use phenotype that differs from that produced by heterospecific competitors (Pfennig and Murphy 2000, 2002).

Despite the above arguments that predator-mediated behavior facilitates diversification, this possibility remains relatively unexplored empirically (although see Ingram et al. 2011), especially in regard to the effects of such behavior on the evolution of novel feeding strategies. We, therefore, sought to investigate the role of antipredator behavior in promoting diversification of feeding strategies in spadefoot toad tadpoles.

Tadpoles of the genus *Spea* express environmentally triggered alternative phenotypes showing differential resource use; i.e., resource polyphenism (*sensu* Mayr 1963). Depending on their diet, the tadpoles can develop into either a small-headed omnivore morph, which feeds mostly on detritus, or a large-headed carnivore morph, which specializes on, and is induced by, anostracan fairy shrimp (Pomeroy 1981; Pfennig 1990). Production of this carnivore morph is presumably an adaptation for escaping intraspecific competition for a limited resource—detritus—by switching to a more protein-rich, but often underutilized resource: fairy shrimp (Pfennig 1992; Martin and Pfennig 2009). In addition, the carnivore's morphological features that enhance predation on shrimp (e.g., a large serrated beak, a wide mouth, and large jaw muscles) (Martin and Pfennig 2009) also render carnivores highly effective at preying on tadpoles (Pfennig and Frankino 1997).

Although this resource polyphenism is derived within spadefoot toads (Ledón-Rettig et al. 2008), the ability to opportunistically consume macroinvertebrates, such as fairy shrimp, is widespread among other closely related tadpoles (Appendix A) (Schiesari et al. 2009). Yet,

tadpoles of Couch's spadefoot toad, *Scaphiopus couchii*, which often co-occur with *Spea* tadpoles, generally avoid consuming shrimp, even if offered no alternative prey (Ledón-Rettig et al. 2008, 2009). Moreover, most *Sc. couchii* tadpoles grow poorly if limited to shrimp or to an otherwise highly proteinaceous diet (Buchholz and Hayes 2000; Ledón-Rettig et al. 2008, 2009). By contrast, as described below, another species of *Scaphiopus* that does not co-occur with *Spea*, *Scaphiopus holbrookii*, readily preys on shrimp and actually grows as well on shrimp as on detritus.

Two types of observations from natural populations suggest that diminished shrimp-eating ability in *Sc. couchii* stems from selection imposed by *Spea*. First, *Sc. couchii* generally avoid breeding in the same shrimp-rich ponds inhabited by *Spea* (Cornejo 1985). Second, when they do breed in the same pond, *Sc. couchii* tadpoles generally remain in shallow water on the pond's margin (D. Pfennig, personal observation). By contrast, the highest densities of shrimp and most carnivorous *Spea* tadpoles occur in deeper water at the pond's center (Fig. 1) (Pomeroy 1981, 23; D. Pfennig, personal observation).

These observations suggest that *Spea* might have actively excluded *Sc. couchii* from the shrimp resource. *Spea* tadpoles represent a real threat of predation to *Sc. couchii* (Pomeroy 1981; Cornejo 1985) and actually prefer *Sc. couchii* as prey over the tadpoles of other species (Pfennig 2000). We, therefore, specifically sought to test the hypothesis that a recurrent threat of predation by *Spea* caused *Sc. couchii* tadpoles to facultatively alter their behavior such that they indirectly avoided the shrimp resource and subsequently lost the ability to capitalize on this diet.

We evaluated this hypothesis through an ancestral character state reconstruction and through a series of experiments. We began by using the reconstruction to determine whether *Sc. couchii*'s poor performance on shrimp (i.e., their avoidance of shrimp and their inability to assimilate or grow well on such a diet relative to one of detritus) (Ledón-Rettig et al. 2008, 2009; Buchholz and Hayes 2000) is evolutionarily derived. Next, using *Sc. holbrookii* (a congener of *Sc. couchii* that does not face predation from *Spea*), we experimentally evaluated whether avoidance of a diet of shrimp is derived in *Scaphiopus*. Finally, we performed an additional experiment to test whether predatory *Spea* influence *Sc. couchii*'s foraging decisions, such that they would likely not be able to access the shrimp resource.

Our results suggest that avoidance of the shrimp diet is indeed derived in *Scaphiopus* and that the presence of *Spea* carnivores causes *Sc. couchii* tadpoles to avoid areas where both carnivores and shrimp are

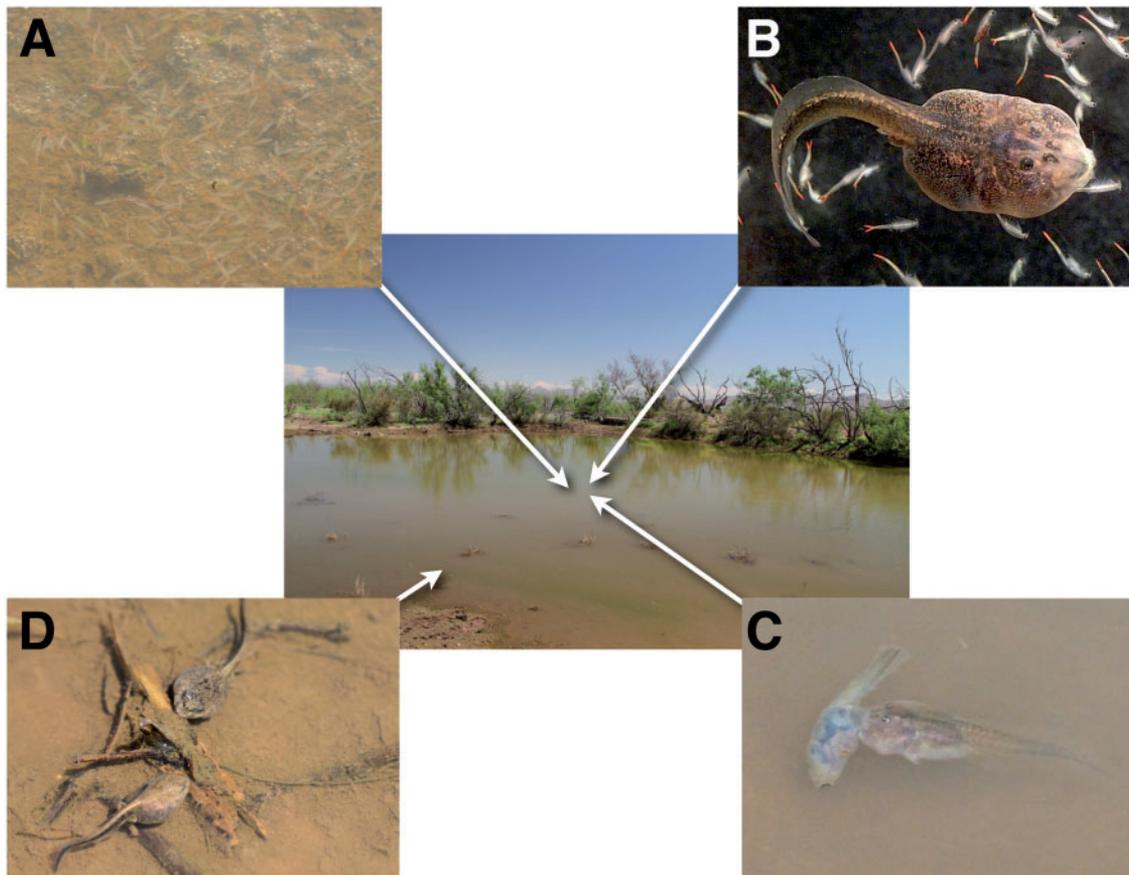


Fig. 1 Tadpoles of Couch's spadefoot toads (*Sc. couchii*) typically occur in different parts of the same pond than do tadpoles of plains spadefoot toads (*Sp. bombifrons*) and Mexican spadefoot toads (*Sp. multiplicata*), and consequently, have limited access to a nutritious shrimp resource. (A) Anostracan fairy shrimp typically school in the center of ponds. (B) *Spea* tadpoles produce a distinctive carnivore morph, which specializes on shrimp and which therefore occurs mostly in the pond center. (C) However, carnivores also frequently prey on other tadpoles. (D) Possibly as an adaptive response to minimize predation from *Spea* carnivores, *Sc. couchii* remain on the pond margin, where they specialize on organic detritus and plants. Thus, predation pressure imposed by *Spea* may have led to an evolutionary reduction in shrimp-eating ability in *Sc. couchii*. This process may have been mediated by predator-induced behavior.

found. We, therefore, hypothesize that the recurrent expression of this behavior may have caused *Sc. couchii* tadpoles to lose the ability to utilize the shrimp diet. In this way, predator-mediated behavior may have preceded and even facilitated divergence between ancestral and present-day *Sc. couchii* feeding strategies. Generally, predator-mediated behavior may play an underappreciated role in promoting diversification of feeding strategies.

Methods

Ancestral character state reconstruction

To determine whether *Sc. couchii*'s poor performance on shrimp is evolutionarily derived, we created an ancestral character state reconstruction of larval diets in spadefoots and related species using the maximum likelihood (ML) model Mk1 in Mesquite 2.73 (Maddison and Maddison 2010). Species whose

larvae do, and those whose larvae do not, consume macroinvertebrates were given the character states "1" and "0", respectively. We designated larvae as macroinvertebrate-consumers if they had been observed consuming macroinvertebrates in a natural or laboratory setting, or if field-caught specimens had macroinvertebrates in their guts (species and references are cataloged in Appendix A). All transition rates were assumed equal, and all branch lengths were set to one. The best estimate of the character state at the node that gave rise to *Sc. couchii* was determined using a likelihood ratio test (Maddison and Maddison 2010). A likelihood ratio of at least 7:1 for characters at that node was considered to be significant (Schluter et al. 1997). This reconstruction was compared to a previously published reconstruction of resource polyphenism (this study, Fig. 2) (Ledón-Rettig et al. 2008).

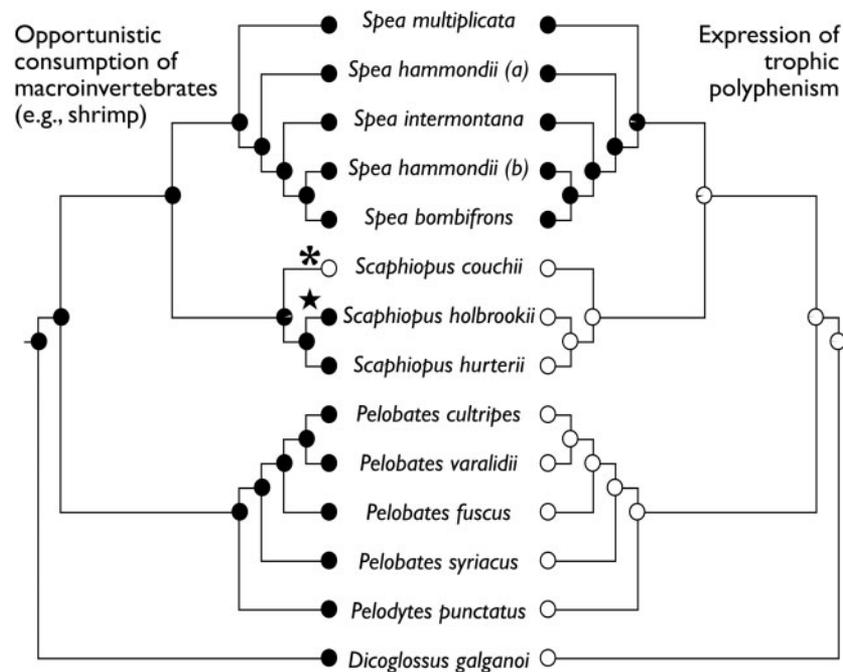


Fig. 2 Occurrence of opportunistic shrimp consumption and shrimp-induced polyphenism in spadefoots (outgroup *Dicoglossus*). Relative support for ability to consume shrimp (left tree) or to express a carnivorous morph (right tree) (from Ledón-Rettig et al. 2008) is indicated in black. Unlike most spadefoot larvae, *Sc. couchii* grow poorly on the shrimp (asterisk). The larvae of congener *Sc. holbrookii* (star) are capable of consuming shrimp although they do not express trophic polyphenism (assessed in this study).

Diet and feeding performance in *Sc. holbrookii*

Although evidence from the literature (Appendix A) revealed that the consumption of macroinvertebrates is widespread among spadefoot larvae, we conducted an experiment with *Sc. holbrookii* to corroborate this conclusion. Parts of six *Sc. holbrookii* egg masses were collected from a natural pond near Hoffman, NC. At hatching, 300 randomly selected tadpoles were distributed among 100 replicate tanks ($21 \times 10.8 \times 35$ cm³ clear plastic tubs, filled with 800 mL of dechlorinated water), which were arranged on racks in the same room maintained at 26°C and a natural light cycle. One day after hatching, the larvae were fed either brine shrimp nauplii (*Artemia salina*) or ground fish food (Wardley cichlid floating pellets; hereafter detritus) *ad libitum*: Brine shrimp resemble the fairy shrimp upon which *Spea* larvae feed in nature, whereas ground fish food resembles detritus in form and nutrition. These treatments were randomized and interspersed among replicate microcosms. Seven days after hatching, tadpoles in the shrimp treatment were switched to adult brine shrimp, simulating the development of this resource in nature. Throughout the experiment, excess food and tadpoles' waste were removed from the microcosms with a disposable pipette. Ten days after hatching, tadpoles were over-anesthetized and fixed in buffered formalin. Ultimately, 13 detritus and 13 shrimp

replicates (containing 3 tadpoles each) were randomly chosen and used for these analyses.

Each tadpole's snout-vent-length (SVL) was measured using NIH ImageJ software (Rasband 1997–2009) on a digital image captured with a Lecia (Wetzlar, Germany) DFC480 R2 camera. We used a one-way ANOVA to determine if the relative performance (average SVL per microcosm) of *Sc. holbrookii* larvae differed on a diet of shrimp versus detritus. These and all further statistical analyses were conducted using R statistical software (R Core Development Team).

Predator-mediated behavior and survival in *Sc. couchii*

As noted in the Introduction section, observations of natural ponds have revealed that, when they occur in the same ponds as *Spea* tadpoles, *Sc. couchii* tadpoles remain in the shallow water, where both carnivore-morph *Spea* tadpoles and shrimp are scarce (Fig. 1). Consequently, *Sc. couchii* tadpoles are largely excluded from access to shrimp. We, therefore, tested whether *Spea* influence not only *Sc. couchii*'s survival, but also their spatial and foraging decisions. To do so, we housed *Sc. couchii* tadpoles in conspecific (*Sc. couchii* only) or heterospecific (*Sc. couchii* and *Spea bombifrons*) microcosms. We fed these tadpoles either shrimp or detritus to determine whether the type of diet could modify the outcome of the interaction between

Spea and *Sc. couchii*. Thus, there were four different treatment groups, under which focal *Sc. couchii* tadpoles were reared: (1) conspecifics only and detritus, (2) conspecifics only and shrimp, (3) heterospecifics and detritus, and (4) heterospecifics and shrimp.

Two families each of *Sp. bombifrons* and *Sc. couchii* were bred for this experiment. Adults of both species were collected from Portal, AZ, where they occur in sympatry. All animals had been housed in a colony at the University of North Carolina, Chapel Hill for 2–3 years. To induce breeding, adult males and females were injected 0.07 mL luteinizing hormone-releasing hormone (Sigma 7134, St Louis, MO, USA) and left for 12 h in nursery tanks.

For each treatment, there were 25 replicate tanks ($21 \times 10.8 \times 35$ cm³ clear plastic tubs, filled with 3 L of dechlorinated water), which were randomized and interspersed in the same room. Three days post-hatching, tadpoles were distributed among treatments. Conspecific treatments contained 24 *Sc. couchii* tadpoles, and heterospecific treatments contained 12 *Sc. couchii* and 3 *Sp. bombifrons* tadpoles. These ratios were chosen in order to keep the level of competition for detritus and shrimp relatively equal between conspecific and heterospecific treatments; *Sp. bombifrons* tadpoles are $\sim \times 4$ the size of *Sc. couchii* tadpoles by weight at 9 days after hatching (C. C. Ledón-Rettig, unpublished data). These tadpoles were chosen randomly and in equal proportions from each of the four families. Tadpoles were fed either brine shrimp or detritus. One end of each tub was propped up against its clear plastic lid, such that there was a “shallow” and “deep” end of each replicate (3.5 and 8.5 cm of water, respectively, which are typical depths experienced by *Sc. couchii* larvae in their natural ponds) (Newman 1987). The outside bottom of each tub was bifurcated by a strip of white tape to delimit the shallow and the deep ends. In all tanks, shrimp or detritus had been completely consumed before the tadpoles were fed again.

Beginning the day after larvae were distributed, replicates were scan sampled (Altmann 1974) thrice a day (at 0800, 1200, and 1400 h), for a total of 5–10 s per replicate. Three measures were taken: the proportion of *Sc. couchii* at the shallow end, the proportion of *Sc. couchii* feeding, and survival of *Sc. couchii*. In total, 10 observations were made over the course of 4 days. After the last observation was completed, tadpoles were over-anesthetized with MS-222 and preserved in buffered formalin.

To assess the effects of diet and predators on behavior and survival, we performed logistic regression using a binomial error structure and a logit link function, a preferred method for analyzing proportional data (Warton

and Hui 2011). We measured preference for depth as the number of tadpoles at the shallow end of a microcosm divided by the total number of surviving tadpoles (during a given time point); foraging behavior as the number of feeding tadpoles divided by the total number of surviving tadpoles (during a given time point); and survival as the number of tadpoles remaining at the end of the experiment divided by the initial number of tadpoles in that microcosm. For all models we included the fixed independent variables of community (heterospecific or conspecific) and diet (detritus or shrimp), and for the two behavioral responses we included the random variable of microcosm (to account for the nonindependence of repeated measurements on each replicate over time) (Gueorguieva and Krystal 2004; Bolker et al. 2009; Fieberg et al. 2009). We used likelihood ratio tests to determine which community and dietary factors should be retained in the models and to evaluate the significance of their contributions. Interactions between community and diet were removed for the depth-preference model, but retained for the feeding-behavior and survival models.

To further test whether the presence of a predator *per se* influenced the behavior of *Sc. couchii* tadpoles, we evaluated whether the degree to which *Sp. bombifrons* expressed the carnivore phenotype (and, hence, the degree to which they represented a predatory threat to *Sc. couchii*) influenced the foraging behavior of their *Sc. couchii* tankmates. To do so, for each *Sp. bombifrons* tadpole we measured three morphological traits that were diagnostic of the carnivore type of morphology (OH, GL, and MP; see above). Following the methods of Martin and Pfennig (2009), we used principal component analysis to combine these three traits into a single multivariate shape variable (the “morphological index”). The larger this index (i.e., PC1, which explained 69% of the variance in our data), the greater the degree to which individual *Sp. bombifrons* tadpoles expressed the distinctive carnivore type of morphology. To confirm that diet influenced the expression of *Sp. bombifrons*’ carnivory in this experiment (*sensu* Pfennig 1990), we took the maximum morphological indices among microcosms and used an ANOVA to determine whether diet predicted variation in these maximum values. Maximum values were used because the presence of a *Spea* carnivore will often suppress the expression of carnivory in other *Spea* individuals (Pfennig 1999; Frankino and Pfennig 2001). We then modeled the effect of *Sp. bombifrons*’ carnivory on *Sc. couchii*’s behavior (occupying the shallow end or foraging) using logistic regression with a binomial error structure and logit link function. In each model, proportional behavioral data were the binary response variable and the explanatory variables were diet, the maximum

morphological index of the predators within that microcosm, and microcosm as a random repeated factor. We used likelihood ratio tests to determine which factors should be retained in the models and to evaluate the significance of their contributions.

Results

Ancestral character state reconstruction

The ML reconstruction suggested that *Sc. couchii*'s ancestors typically consumed shrimp (Fig. 2); and the ancestral node that gave rise to *Sc. couchii* favored the consumption of macroinvertebrates as the best character state (relative likelihoods for macroinvertebrate consumption: lack of macroinvertebrate consumption were 18.7:1).

Shrimp-induced plasticity and feeding performance in *Sc. holbrookii*

In contrast to *Sc. couchii* tadpoles, which generally avoid consuming shrimp and grow poorly on shrimp when it is the only available resource (see Introduction section), we found that *Sc. holbrookii* tadpoles grew as well on shrimp as on detritus (SVL: $F_{1,28} = 0.03$, $P = 0.87$). This result, combined with the findings from the ancestral character state reconstruction described above, suggests that ancestral *Sc. couchii* likely utilized shrimp as a resource.

Predator-mediated behavior and survival in *Sc. couchii*

In the presence of *Sp. bombifrons*, *Sc. couchii* were more likely to associate with the shallow end of their tank ($\chi^2_{(1)} = 31.60$, $P < 0.0001$) (Fig. 3A) and less likely to be feeding ($\chi^2_{(1)} = 64.88$, $P < 0.0001$) (Fig. 3B). However, the effect of *Spea*'s presence on *Sc. couchii*'s

feeding behavior was diet-dependent; *Spea* had a more drastic impact on *Sc. couchii*'s feeding behavior when tadpoles were given a shrimp diet ($\chi^2_{(1)} = 12.91$, $P = 0.0003$). Furthermore, *Sc. couchii*, housed with *Spea*, had lower survival ($\chi^2_{(1)} = 37.00$, $P < 0.0001$) (Fig. 3C), and this effect was more severe in the presence of shrimp ($\chi^2_{(1)} = 11.01$, $P = 0.0009$).

Sp. bombifrons had greater expression of carnivory when fed shrimp than when fed detritus ($F_{1,48} = 5.86$, $P = 0.02$). *Sp. bombifrons* morphology was inversely correlated with the amount of time *Sc. couchii* spent feeding: the more carnivore-like *Spea* were, the less likely were their *Sc. couchii* tankmates to be feeding ($\chi^2_{(1)} = 5.29$, $P = 0.02$) (Fig. 4A), independently from the effects of diet ($\chi^2_{(1)} = 1.14$, $P = 0.29$). In contrast, *Sp. bombifrons* morphology was not related to the proportion of *Sc. couchii* occupying the shallow ends of microcosms ($\chi^2_{(1)} = 0.81$, $P = 0.37$) (Fig. 4B). In no instance was diet a significant factor in determining *Sc. couchii* behavior once *Sp. bombifrons* morphology was accounted for (in each case, $P > 0.08$).

Discussion

Although ecologists have long recognized that many species facultatively engage in less risky behavior when predators are present (reviewed by Werner and Peacor 2003), few studies have investigated the evolutionary consequences of these induced defenses. For example, little is known about whether, or how, induced defenses promote evolutionary transitions to novel feeding strategies. In the present study, we asked whether Couch's spadefoot toad tadpoles, *Sc. couchii*, have a diminished ability to utilize a valuable food resource—anostracan fairy shrimp—because of induced behaviors resulting from intraguild predation-risk imposed by

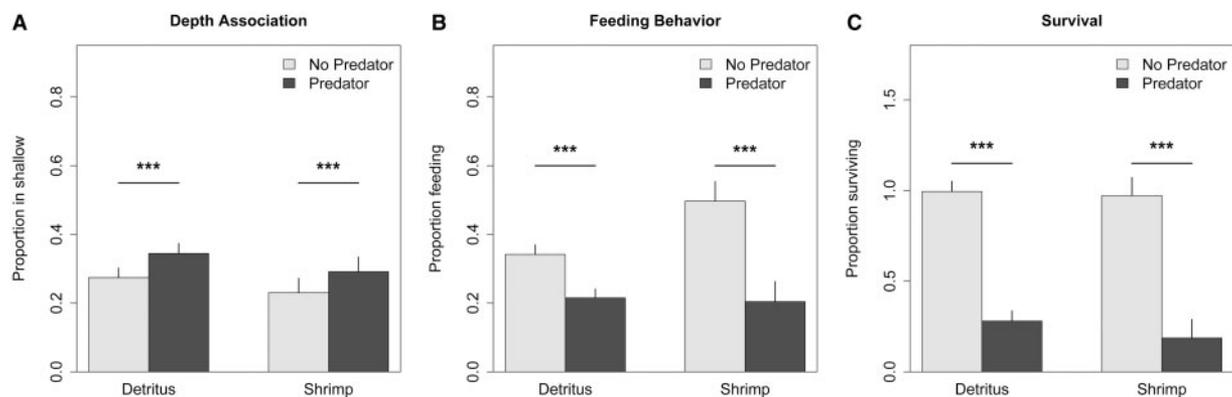


Fig. 3 Compared with when they are housed with conspecifics only, when *Sc. couchii* are housed with predatory *Sp. bombifrons* tadpoles, they are (A) more likely to associate with shallow water, (B) less likely to be feeding, and (C) less likely to survive. Asterisks denote significant differences between predator and no predator environments ($P < 0.0001$) and bars indicate \pm SE of model coefficients for treatment groups. Significant interaction effects between diet and predator environments were found when measuring tadpole foraging behavior ($P = 0.0003$) and survival ($P = 0.0009$).

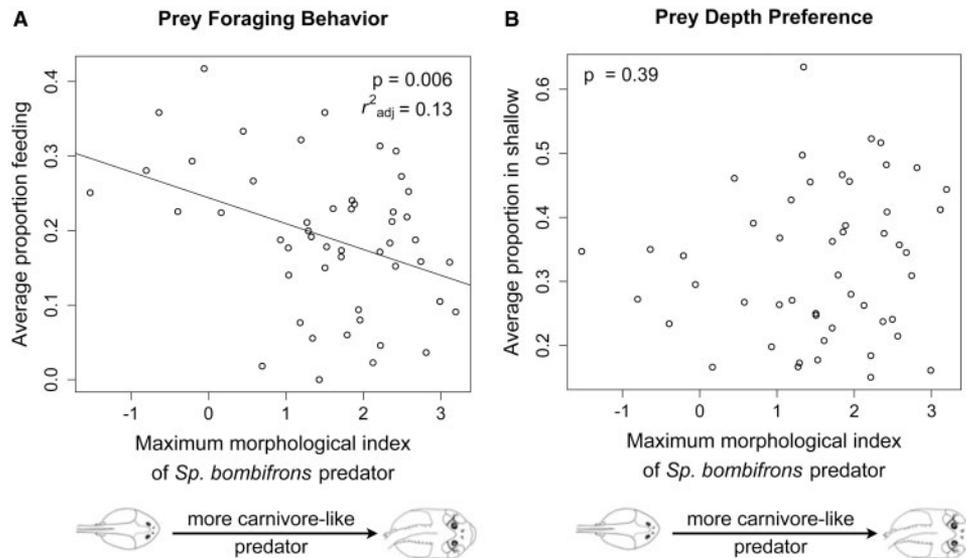


Fig. 4 (A) The proportion of *Sc. couchii* tadpoles observed foraging decreased with increasing expression of carnivory among their *Sp. bombifrons* tankmates. (B) In contrast, there was no relationship detected between *Spea*'s expression of carnivory and *Sc. couchii*'s depth preference. The morphological index is a multivariate shape variable that describes the degree to which a *Spea* tadpole expresses the distinctive carnivore phenotype. The statistics portrayed here are derived from the mean proportion (over all time points) of *Sc. couchii* occupying the shallow end or foraging in a microcosm regressed on the maximum morphological index for the *Sp. bombifrons* in the same microcosm. These results are qualitatively identical to those derived from generalized linear mixed models (see Section "Results")

carnivore-morph tadpoles of spadefoot toads of the genus *Spea*.

Our results indicate that the risk of predation by *Spea* might have facilitated the apparent loss of shrimp-eating abilities and the acquisition of a novel feeding strategy (specializing solely on detritus) in *Sc. couchii*. Specifically, an ancestral character state reconstruction, together with observations of *Sc. couchii*'s congener (*Sc. holbrookii*), revealed that ancestral *Sc. couchii* likely consumed shrimp and that this trait was subsequently lost (Fig. 2). Moreover, our experimental results suggested that this loss of a trait may have stemmed from antipredator behavior that is induced only in *Spea*'s presence (Fig. 3); when *Sp. bombifrons* were present, *Sc. couchii* were less likely to be feeding and more likely to prefer shallow water (in natural ponds, carnivore-morph *Spea* and shrimp congregate in deeper water) (Fig. 1). Finally, time spent feeding was negatively correlated with the degree to which the *Spea* tankmates expressed the carnivore-type morphology (Fig. 4). Thus, a recurrent threat of predation by carnivorous *Spea* might have caused *Sc. couchii* tadpoles to facultatively alter their behavior such that they avoided the shrimp, or areas of ponds where shrimp are found, until they eventually evolved a new feeding strategy that was associated with a lower risk of predation from *Spea*.

Although our data are consistent with the notion that predator-mediated selection caused *Sc. couchii* to evolve a new feeding strategy, we cannot rule out the possibility that competitively mediated selection also played a role (as it has in promoting ecological character displacement among different species of *Spea*) (Pfennig and Murphy 2000, 2002; Pfennig et al. 2007). Indeed, the fact that *Sc. couchii* were more likely in the presence of *Sp. bombifrons* to associate with shallow water (where shrimp are rare) could have arisen, in part, from interference competition over access to shrimp. Such agonistic interactions can favor divergence between competitors (Adams 2004; Peiman and Robinson 2007).

Although both predation and competition could have served as agents of divergent selection in this system, predation likely played the more significant role for three reasons. First, *Spea* are known to be important predators of *Sc. couchii* (Pomeroy 1981; Cornejo 1985; Pfennig 2000) and likely contributed to the observed lower survival in heterospecific microcosms (Fig. 3C). Second, the shift to a detritivorous feeding strategy would not reduce competition with the "omnivore" morph of *Spea* tadpoles, which feed mostly on detritus on the pond margins and are, in fact, the more common morph in most natural ponds (Pomeroy 1981, 23). Third, *Sc. holbrookii*

(*Sc. couchii*'s congener) does not experience predation pressure from *Spea* but does experience competition with other species (Richmond 1947), and still maintains generalist feeding strategy and ability to consume shrimp. Yet, in general, we know surprisingly little about how different selective agents, such as predation and competition, interact in shape ecological and evolutionary processes (MacColl 2011).

We hypothesize that because of predation pressure (and possibly also competition) imposed by *Spea*, the ability to consume shrimp was diminished in *Sc. couchii* tadpoles that co-occur with *Spea*. We further hypothesize that this process was mediated by predator-induced behavior. In other words, dietary plasticity was diminished as this *Sc. couchii* population became "fixed" for a detritus-feeding strategy. How could such fixation occur?

The loss of plasticity and the subsequent fixation of a newly favored, canalized phenotype (also known as "genetic assimilation") (Waddington 1953) can proceed via two routes. First, when the maintenance or expression of plasticity is costly (Relyea 2002), selection should act to eliminate such facultative responses and instead favor alleles that regulate expression of the newly favored trait (West-Eberhard 2003). Second, plasticity might be lost through mutational degradation or genetic drift (Masel et al. 2007). Experiments have demonstrated the loss of plasticity (Suzuki and Nijhout 2006), and numerous studies have shown that traits experiencing relaxed selection can be lost in natural populations (reviewed by Lahti et al. 2009). Although we do not have enough evidence to test the above pathways to genetic assimilation, our preliminary data argue against the notion that selection promoted the loss of shrimp-eating ability. The population from which our *Sc. couchii* were derived exhibits substantial genetic variation in ability to capture and assimilate shrimp (Ledón-Rettig et al. 2010). If shrimp-eating abilities were being lost due to selection, we would expect to detect less variation in *Sc. couchii*'s performance on shrimp relative to that on detritus. Thus, while we speak of the shrimp-consuming strategy as being "lost" in *Sc. couchii*, this might be true only at a population level; variation in traits associated with consuming shrimp may be retained by a given family or individual.

A comparison of antipredator behaviors elicited by *Sc. couchii* populations that are sympatric and allopatric with *Spea* would corroborate our hypothesis that the presence of carnivorous *Spea* tadpoles caused *Sc. couchii* to lose the ability to consume shrimp (unfortunately, *Sc. couchii*'s range is almost entirely included within that of *Spea*). Such studies might also

help illuminate whether predator-induced plasticity was already present in *Sc. couchii* before they encountered *Spea*, and whether sympatric *Sc. couchii* therefore merely exploited a pre-existing behavior. It is also possible, however, that predator-induced plasticity evolved in sympatry. Indeed, because different populations of *Spea* vary in inherent tendency to produce carnivores (Pfennig and Murphy 2002), such variation might have promoted diversification in behavior and microhabitat use among different sympatric populations of *Sc. couchii*. Thus, heterogeneous predation pressure might explain the evolution of behavioral plasticity in *Sc. couchii*.

We suggest the following evolutionary scenario for how *Sc. couchii* evolved a new feeding strategy. Initially, both *Spea* and *Scaphiopus* tadpoles consumed anostracan shrimp, which is a valuable, but often underutilized, resource in the ephemeral ponds in which both species breed (Pfennig 1992, 2000). Over time, *Spea* tadpoles evolved or refined a resource-polyphenism that enhanced their ability to consume shrimp (Ledón-Rettig et al. 2008) and tadpoles (Pfennig 1999). Consequently, sympatric *Sc. couchii* adjusted to the presence of increasingly predaceous *Spea* by evolving the ability (or taking advantage of a pre-existing ability) to respond by reducing their activity and moving to the pond's margin, which was relatively devoid of carnivore-morph *Spea* (and also of shrimp). Because *Sc. couchii* experienced this microhabitat recurrently, and consumed detritus primarily, they eventually lost the behavioral, morphological, and physiological traits needed to prey upon shrimp. This reduction of dietary plasticity might even have promoted *Sc. couchii*'s specialization on detritus. Populations that experience constant (as opposed to variable) environments experience stronger positive selection on environment-specific traits (Snell-Rood et al. 2010). Indeed, a comparison of body length-adjusted gut lengths among 13 species of anuran larvae suggests that current *Sc. couchii* larvae are perhaps the most adept at consuming low-quality diets such as detritus (Altig and Kelly 1974).

A potentially important feature of this system is that not only do prey exhibit an inducible defense, but the predator also produces an inducible offense (*sensu* Padilla 2001): the distinctive carnivore morphology. Such plasticity among prey and predator can stabilize populations when these inducible responses are density-dependent (Vos et al. 2004; Verschoor et al. 2004; Mougi and Kishida 2009). That is, if the defensive traits of prey become more pronounced or widespread when predator densities are high, then the per capita consumption rate of the prey is expected to decrease (Miner et al. 2005). As a consequence of this decreased consumption rate, the frequency and/or magnitude of

inducible offenses among predators is reduced and become less threatening to prey, causing defenses of the prey to be less pronounced. However, such negative feedback between an induced offense and an induced defense does not appear to occur between *Spea* and *Sc. couchii*; the consumption of shrimp is the cue that induces and actually enhances *Spea*'s carnivorous attributes (Pfennig 1990). Thus, *Spea*'s shrimp-induced plasticity should engender a positive feedback cycle wherein the degree to which *Sc. couchii* are excluded from shrimp is directly proportional to the availability of this resource. The evolutionary consequence of this predator–prey interaction, and the modification of this interaction by a second prey species (shrimp), appears to be complete exclusion from a diet of shrimp.

Generally, we are only beginning to understand the effects of biotic and abiotic modifiers of predator-induced behavior on population dynamics (Miner et al. 2005; Kishida et al. 2010), let alone their repercussions for diversification. The interactions among *Spea*, *Sc. couchii*, and fairy shrimp, in concert with variation in the plasticity of both predator and prey, provide an excellent opportunity to investigate these issues. The present study suggests that predator-mediated behavioral plasticity might play a general and important role in promoting diversification of feeding strategies.

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Supplementary Data

Supplementary Data are available at *ICB* online.

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Appendix A

Taxa used for ancestral character state reconstruction for Pelobatoidea and outgroup, *Discoglossus galganoi*. Although we only indicated two species as consuming heterospecific eggs, it is likely that the larvae of many species listed here feed opportunistically on eggs.

Genus	Species	Common name	Diet	Analysis type	References
Spadefoot					
<i>Spea</i>	<i>bombifrons</i>	Great Plains spadefoot	<i>Omnivore</i> : Algae, plants, detritus and invertebrates <i>Carnivore</i> : invertebrates and tadpoles	Natural and laboratory observation	this study, Bragg 1965
	<i>multiplicata</i>	Mexican spadefoot	<i>Omnivore</i> : Algae, plants, detritus and invertebrates <i>Carnivore</i> : invertebrates and tadpoles	Natural and laboratory observation, Gut content	Bragg 1965, Pomeroy 1981, Pfennig 1990
	<i>hammondii</i>	Western spadefoot	<i>Omnivore</i> : Algae, plants, detritus and invertebrates <i>Carnivore</i> : invertebrates and tadpoles	Natural observation	Turner 1952, Bragg 1965
	<i>intermontanus</i>	Great Basin spadefoot	<i>Omnivore</i> : Algae, plants, detritus and invertebrates <i>Carnivore</i> : invertebrates and tadpoles	Natural observation	Bragg 1965
<i>Scaphiopus</i>	<i>couchii</i>	Desert spadefoot	Algae, plants and detritus	Natural and laboratory observation	Bragg 1965, Buchholz and Hayes 2002
	<i>holbrookii</i>	Eastern spadefoot toad	Algae, plants, detritus and invertebrates and other tadpoles	Natural and laboratory observation	this study, Bragg 1964
	<i>hurteri</i>	Hurter's spadefoot toad	Algae, plants, detritus and invertebrates and other tadpoles	Natural and laboratory observation	Bragg 1944, 1964
<i>Pelobates</i>	<i>fuscus</i>	Common spadefoot	Algae, plants, detritus and invertebrates	Gut content	Pavignano 1990
	<i>syriacus</i>	Eastern spadefoot toad	Algae, plants, detritus and invertebrates	Gut content	Degani 1986
	<i>varaldii</i>	Moroccan spadefoot toad	Algae, plants, detritus and invertebrates	Natural observation	S. D. Busack (personal communication)
	<i>cultripes</i>	Western spadefoot toad	Algae, plants, detritus, invertebrates and heterospecific eggs	Natural and laboratory observation, Gut content	Diaz-Paniagua 1989, Busack and Zug 1975, Tejedo 1991
Non-spadefoot					
<i>Pelodytes</i>	<i>punctatus</i>	Common parsley frog	Algae, plants, detritus, invertebrates and heterospecific eggs	Natural and laboratory observation, Gut content	Diaz-Paniagua 1985, Tejedo 1991
<i>Discoglossus</i>	<i>galganoi</i>	Iberian painted frog	Algae, plants, detritus, invertebrates and other tadpoles when nutrient-limited	Natural observation	Álvarez and Nicieza 2002, S. D. Busack (personal communication)