

LETTER

Damage, digestion, and defence: the roles of alarm cues and kairomones for inducing prey defences

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Abstract

Inducible defences are widely used for studying phenotypic plasticity, yet frequently we know little about the cues that induce these defences. For aquatic prey, defences are induced by chemical cues from predators (kairomones) and injured prey (alarm cues). Rarely has anyone determined the separate and combined effects of these cues, particularly across phylogenetically diverse prey types. We examined how tadpoles (*Hyla versicolor*) altered their defences when 10 different prey were either crushed by hand or consumed by predators. Across all prey types, crushing induced only a subset of the defences induced by consumption. Consuming vs. crushing produced additive responses for behaviour but synergistic responses for morphology and growth. Moreover, we discovered the first extensive evidence that prey responses to different alarm cues depends on prey phylogeny. These results suggest that the amount of information available to the prey affects both the quantitative and qualitative nature of the defended phenotype.

Keywords

Alarm cue, amphibian, chemical communication, phylogeny, tadpole.

Ecology Letters (2005) 8: 505–512

INTRODUCTION

From simple single-celled organisms to plants and animals, most individuals can alter their phenotype in response to changes in biotic and abiotic factors (i.e. phenotypic plasticity; Pigliucci 2001). Many phenotypic changes appear to be adaptive, resulting in higher fitness in the inducing environment than alternative phenotypes (e.g. Dudley & Schmitt 1996; Van Buskirk & Relyea 1998). However, for organisms to properly adjust their phenotype, there must be reliable environmental cues that indicate the current or future environmental conditions (Moran 1992). In many systems, identifying the source and function of these cues poses a tremendous challenge (Burks & Lodge 2002).

Numerous plants and animals exhibit plastic defences against herbivores and predators (Karban & Baldwin 1997; Tollrian & Harvell 1999) and in many animals the defensive traits are induced by chemical cues that are produced during predation events (Petranka *et al.* 1987; Chivers & Smith 1998). These chemicals contain components from predators (termed 'kairomones') and components from injured prey (termed 'alarm cues'). As a result, the environmental information available to prey is potentially quite complex,

including information about the species and density of predator present and the species of prey being consumed (Larsson & Dodson 1993). A major question in the field of inducible defences asks how prey interpret this information when making their phenotypic decisions (Chivers & Smith 1998; Kats & Dill 1998; Chivers & Mirza 2001).

Because the chemical cues produced during predation contain both kairomones and alarm cues, prey may require both types of information when making their defensive decisions (the identity of the predator and the identity of the killed prey). Alarm cues (from damaged or crushed prey) have frequently been used as surrogates of predation, with the implicit assumption that the cues from damaged prey induce the complete suite of predator-induced defences. However, prey that do respond to predation cues often do not respond to damaged conspecifics alone (Alexander & Covich 1991; Brönmark & Pettersson 1994; Summey & Mathis 1998; Slusarczyk 1999; but see Pijanowska 1997; Stabell & Lwin 1997; Chivers *et al.* 2001). The lack of consistent responses to alarm cues may occur because prey responses to alarm cues alone are small (and thus difficult to detect) or because some prey only alter their traits when they obtain information from both alarm cues and kairomones.

To discriminate between these two possibilities and determine how prey use alarm cues, we must directly compare prey responses to damaged vs. consumed prey.

Prey should use the information contained in alarm cues to estimate their predation risk and develop their defences. Previous investigators have hypothesized that prey responses to alarm cues from heterospecifics should be related to either the frequency of coexistence between species that share a common predator (i.e. alarm cues from prey that frequently coexist should induce stronger responses than non-coexisting prey) or the phylogenetic relatedness between the responding prey and the prey that released the alarm cues (i.e. closely related prey should produce similar alarm cues and, thus, induce stronger responses than distantly related prey; Chivers & Smith 1998). While a number of behavioural experiments have examined the impacts of different alarm cues, support for either hypothesis has been equivocal because the majority of these studies have not been specifically designed to distinguish between the hypotheses. Given that these studies have primarily used only two diets or three diets, the results often support both hypotheses. More definitive tests require a large number of prey types that span across a wide range of prey phylogeny while controlling for coexistence.

When testing the impact of alarm cues and kairomones on prey defences, we also need to take an integrated approach that recognizes the full suite of defences that prey employ because damaged and consumed prey may not induce all traits in the same way (i.e. behaviour vs. morphology; Van Buskirk & Arioli 2002). To date, the focus has been on behavioural traits, yet biologists are becoming increasingly aware that many prey also defend themselves with inducible morphology and life history (Crowl & Covich 1990; Brönmark & Pettersson 1994; Relyea 2001; Laurila *et al.* 2002). To understand how alarm cues and kairomones affect prey defences, we need to simultaneously examine behaviour, morphology, and life history.

We addressed these challenges using larval anurans (tadpoles), which are well known for their ability to alter their behaviour, morphology, and life history in response to predators (Relyea 2001, 2002; Van Buskirk 2002). We exposed grey tree frog tadpoles (*Hyla versicolor*) to a wide range of coexisting prey types that were either crushed by hand or consumed by a caged dragonfly predator (*Anax junius*) and then observed how the tadpoles altered their behaviour, morphology, and growth. We used prey types that all commonly coexist so that any differences among prey types could not be explained by the coexistence hypothesis. Further, by using predator-naïve tadpoles, we prevented any potentially confounding effects of learning. We tested the following hypotheses: (1) different alarm cues

should induce different phenotypes; (2) crushed and consumed prey induce different suites and magnitudes of defences; and (3) alarm cues from closely related prey should induce stronger defences than alarm cues from distantly related prey.

METHODS

We exposed grey tree frog tadpoles to chemical cues emitted from a factorial combination of 10 prey types experiencing two modes of prey death (crushed by hand or consumed by *Anax*) in a randomized block design. The 20 treatments were replicated five times (five spatial blocks) for a total of 100 experimental units. The 10 prey types spanned a wide range of phylogeny: no prey, grey tree frog tadpoles, spring peeper tadpoles (*Pseudacris crucifer*), wood frog tadpoles (*Rana sylvatica*), leopard frog tadpoles (*R. pipiens*), spotted salamander larvae (*Ambystoma maculatum*), damselfly nymphs (*Lestes* spp.), dragonfly nymphs [*Sympetrum* spp.; a small dragonfly species that is quite small and induces few changes as a predator (Relyea 2003a)], and two snail species (*Physa acuta* and *Stagnicola elodes*). Crossing these 10 prey types with the two modes of prey death (crushed or consumed) produced two types of controls. The first control was an empty predator cage to quantify tadpole phenotypes when no predation cues were present. The second control was a starved dragonfly nymph to quantify tadpole phenotypes when only predator kairomones were present. Although this experiment did not include a treatment of starved predators plus crushed conspecifics, subsequent experiments have confirmed that this treatment induces changes similar to starved predators alone (N.M. Schoeppner & R.A. Relyea unpublished data).

We conducted the experiment in outdoor pond mesocosms (wading pools). Each mesocosm contained 80 L of well water, 100 g of leaf litter (*Quercus* spp.), 5 g of rabbit chow, and an aliquot of pond water containing algae and zooplankton. These mesocosms have been used in previous studies with great success (Relyea 2001, 2002). Each pool contained one predator cage (a 500 mL plastic cup covered with 1 × 2 mm mesh screen that prevented predators and prey types from escaping) that was either empty or held a single larval dragonfly. All pools were covered with 60% shade cloth lids to prevent colonization by amphibians and invertebrates during the experiment. On 30 June 2002, we added 20 predator-naïve hatchlings to each pool (haphazardly selected from a mixture of hatchlings from 32 clutches of eggs). These 32 clutches of eggs were laid in the laboratory by amplexing pairs of tree frogs that were collected on 16 May 2002, and then reared as tadpole in wading pools prior to the experiment. In short, the tadpoles had not been exposed to predator cues as either eggs or hatchling tadpoles.

We added the crushed or consumed prey to the pools three times per week. Equal masses of each prey type (350 mg) were either crushed by hand or fed to the larval dragonflies. Because the diets differed in individual size, the number of prey could not be held constant, but differences in prey number do not affect anti-predator responses (N.M. Schoeppner & R.A. Relyea unpublished data). At each feeding, the consumed prey were added to the predator cages and we checked that each predator had consumed its diet. If the predator had not eaten, the uneaten prey were left in the cage and the predator was replaced. At the end of the experiment, only a few of the treatments had any uneaten prey. Because this was a small fraction of the total amount of prey fed to the predator during the experiment, these pools were not excluded. The prey used for the crushed cue treatments were first euthanized and then macerated in a blender for 30 s. The crushed prey were then distributed evenly to the appropriate pools. To equalize disturbance during feeding, we lifted all empty cages and then returned them to the pools.

After 17 days, we observed tadpole behaviour (24 h after cue addition). For each pool, the number of tadpoles visible and the number of visible tadpoles that were active (moving) was recorded, permitting us to quantify the proportion of tadpoles observed (i.e. not hiding) and the proportion of tadpoles active. Each pool was observed 10 times and we used the mean behaviours of each pool as our behavioural response variables.

After 20 days, all tadpoles were removed and preserved in 10% formalin for subsequent morphological measurement. Survival was excellent across all treatments ($98.23 \pm 0.03\%$) and there was no pattern among the treatments. Tadpole morphology was measured using an image analysis system (Optimas Bioscan, Bothell, WA, USA). We weighed each tadpole and then measured seven morphological dimensions: tail length and depth; tail muscle depth and width; and body depth, length, and width (see Fig. 1 in Relyea 2000). Because the tadpole's body is round, we placed a glass plate under the tadpole's tail in the lateral view. For simplicity, we

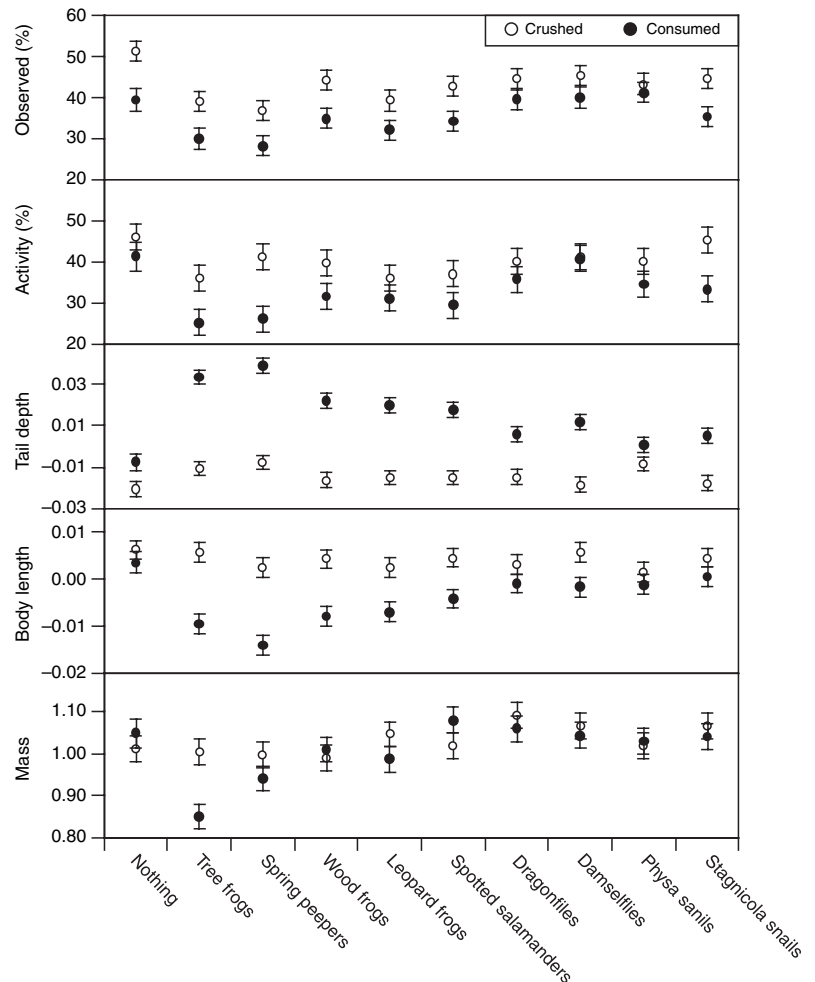


Figure 1 Behaviour, relative morphology, and mass of larval grey tree frogs (mean residuals \pm 1 SE) exposed to chemical cues from crushed (open symbols) or consumed (filled symbols) diets from a wide range of phylogeny. For crushed diets, the treatment termed 'nothing' indicates a cue-free environment. For consumed diets, the treatment termed 'nothing' indicates a starved-predator environment. Relative morphology was calculated by regressing the log-transformed dimensions of all individuals against their log-transformed mass and then saving the mean residuals from each pool.

only report on the two tadpole dimensions that most consistently respond to predators (tail depth and body length).

Statistical analysis

Because we were interested in differences in tadpole shape, we had to first correct for differences in overall size. To make the morphological dimensions size-independent, we regressed the two morphological measurements (log-transformed to improve the linearity of the relationship) against the log-transformed mass of each individual and then saved the residuals. We calculated the mean residuals from each pool and used these mean residuals as our morphological response variables. This approach has been widely applied in past studies of morphological plasticity (Relyea 2000, 2001, 2002).

We analysed all of the data in a single multivariate analysis of variance (MANOVA) that examined the effects of block, cue type (crushed or consumed), prey type, and their interactions on grey tree frog behaviour, mass, and the two size-independent morphological dimensions. Block interactions were never significant; thus, we pooled the block interaction degrees of freedom with the error term. For significant univariate effects, we conducted mean comparisons using Fisher's LSD test.

To test the relationship between the grey tree frog's phenotypic responses and the phylogenetic relatedness of the different crushed and consumed prey, we used phylogenetic divergence times. For example, invertebrates diverged from chordates 990 million years ago (mya) and salamanders diverged from anurans 250 mya (Feller & Hedges 1998; Kumar & Hedges 1998). Within the anurans, ranids (wood frogs and leopard frogs) and hylids (grey tree frogs and spring peepers) diverged 100 mya (Wallace *et al.* 1971). Within the hylids, *Pseudacris* and *Hyla* diverged *c.* 50 mya (Hedges 1986). Because some of the taxa are not phylogenetic independent (e.g. the four invertebrates, the two ranids), we averaged the values for each taxonomic group (within a block) to represent invertebrates and ranids, respectively. In short, the nine taxa were reduced to five independent taxa: grey tree frogs, peepers, ranids, salamanders, and invertebrates. Using these dates, we conducted a multivariate analysis of covariance (MANCOVA) using blocks, cue type (crushed vs. consumed), and divergence date as a covariate [using log (divergence date + 10 mya)] and the tadpole activity, hiding, mass, and mean residuals for the two morphological traits as the response variables.

RESULTS

There were significant multivariate effects of block (Wilks' $F_{20,236} = 5.3$, $P < 0.001$), prey type (Wilks' $F_{45,321} = 2.9$,

$P < 0.001$), cue type (Wilks' $F_{5,71} = 65.1$, $P < 0.001$), and the prey type-by-cue type interaction (Wilks' $F_{45,321} = 1.8$, $P = 0.003$). Block effects occurred for all traits (univariate tests, $P < 0.02$), likely because of block position in the field. Blocks closer to the forest edge experienced more shade, likely producing differences in periphyton which can affect the magnitude of predator-induced phenotypes (Relyea 2002). Importantly, the lack of a prey type-by-cue type interaction confirms that the pattern of response to the different treatments was consistent across all blocks.

The percentage of tadpoles observed in the pools was affected by prey species ($F_{9,75} = 5.2$, $P < 0.001$) and cue type ($F_{1,75} = 48.4$, $P < 0.001$) but not their interaction ($F_{9,75} = 0.9$, $P = 0.527$; Fig. 1). Across all prey treatments, consumed prey caused 8% more hiding than crushed prey. Across both cue types, there was strong hiding when the treatments used grey tree frogs or spring peepers ($P < 0.001$), moderate hiding with the other amphibian species ($P < 0.01$), and little hiding with the invertebrate prey ($0.15 > P > 0.01$). Compared with grey tree frogs reared with no cues, there was a 12% increase in hiding with crushed conspecifics ($P = 0.001$), a 12% increase in hiding with starved predators ($P = 0.004$), and a 21% increase in hiding with consumed conspecifics ($P < 0.001$).

Tadpole activity was affected by prey species ($F_{9,75} = 3.1$, $P = 0.003$) and cue type ($F_{1,75} = 26.8$, $P < 0.001$) but not their interaction ($F_{9,75} = 0.9$, $P = 0.495$; Fig. 1). Across all prey species, consumed prey induced 17% lower activity than crushed prey. Compared with the control treatment, consumed amphibians induced the largest activity reductions ($P < 0.008$) while invertebrate prey induced the smallest activity reductions ($P > 0.03$). Compared with grey tree frogs reared with no cues, we found a 10% reduction in activity with crushed conspecifics ($P = 0.027$), a non-significant 3% reduction in activity with starved predators ($P = 0.460$), and a 20% reduction in activity when conspecifics were fed to predators ($P < 0.001$).

Tail depth was affected by prey species ($F_{9,75} = 11.2$, $P < 0.001$), cue type ($F_{1,75} = 328.3$, $P < 0.001$) and their interaction ($F_{9,75} = 6.0$, $P < 0.001$; Fig. 1). The interaction occurred because there were no differences among the crushed prey (univariate $P = 0.109$), but there were substantial differences among the consumed prey (univariate $P < 0.0001$). Compared with dragonflies consuming no prey, increases in tail depth were large when dragonflies consumed grey tree frogs and peepers ($P < 0.001$), moderate when dragonflies consumed wood frogs, leopard frogs, and salamanders ($P < 0.001$), and small when dragonflies consumed invertebrates (damselfly larvae, $P = 0.002$; dragonfly larvae, $P = 0.022$; *Stagnicola* snails, $P = 0.034$; *Physa* snails, $P = 0.153$). Compared with grey tree frogs reared with no cues, crushed conspecifics and starved predators each caused small effects on tail depth ($P = 0.055$

and $P = 0.022$, respectively) while predators consuming conspecifics caused a fivefold larger increase in tail depth ($P < 0.001$).

Body length was affected by prey species ($F_{9,75} = 4.3$, $P < 0.001$), cue type ($F_{1,75} = 83.6$, $P < 0.001$) and their interaction ($F_{9,75} = 3.1$, $P = 0.003$; Fig. 1). The interaction occurred because crushed prey had no effect on body length (univariate $P = 0.789$) while consumed prey had significant effects (univariate $P < 0.001$). Compared with starved dragonflies, all consumed prey induced relatively shorter bodies ($P < 0.05$) except the invertebrate prey ($P \geq 0.05$). Compared with grey tree frogs reared with no cues, we found no effect of crushed conspecifics ($P = 0.867$) or starved predators ($P = 0.481$) but a large decrease in body length when predators consumed conspecifics ($P < 0.001$).

Tadpole mass was affected by prey species ($F_{9,75} = 4.5$, $P < 0.001$) and cue type ($F_{1,75} = 2.5$, $P = 0.116$) with a nearly significant interaction ($F_{9,75} = 1.8$, $P = 0.080$; Fig. 1). The marginal interaction occurred because the crushed prey had no impact on tadpole mass (univariate $P = 0.086$) whereas consumed prey had a significant impact (univariate $P = 0.002$). Compared with starved dragonflies, consumed grey tree frogs and peepers caused reductions in mass ($P < 0.04$) while the remaining consumed prey had no effect ($P > 0.2$). Compared with grey tree frogs reared with no cues, we found no effect of crushed conspecifics or starved predators ($P > 0.35$), but predators consuming conspecifics caused a 15% reduction in mass ($P < 0.001$).

When we examined the relationships between the phylogenetic distance of each prey and the grey tree frog's response (Fig. 2), we found significant multivariate effects of block (Wilks' $F_{20,127} = 3.6$, $P < 0.001$), cue type (Wilks' $F_{5,38} = 13.9$, $P < 0.001$), divergence date (Wilks' $F_{5,38} = 9.1$, $P < 0.001$), and the cue type-by-divergence date interaction (Wilks' $F_{5,38} = 3.8$, $P = 0.007$). For the two behavioural traits (percent observed and percent activity), the traits were affected by cue type ($P \leq 0.014$) and divergence time ($P \leq 0.01$), but not by their interaction ($P > 0.22$). For mass and the two morphological traits (tail depth and body length), the traits were affected by cue type ($P \leq 0.003$), divergence time ($P < 0.02$), and their interaction ($P < 0.01$). For these latter three traits, we found significant effects of divergence date when the prey were consumed ($P \leq 0.002$) but not when they were crushed ($P > 0.3$).

DISCUSSION

The results of this study indicate that prey make use of the diverse information available from alarm cues and kairomones when making their defensive decisions. The phenotypic changes induced by the caged dragonfly larvae are likely adaptive. For example, increased hiding and decreased

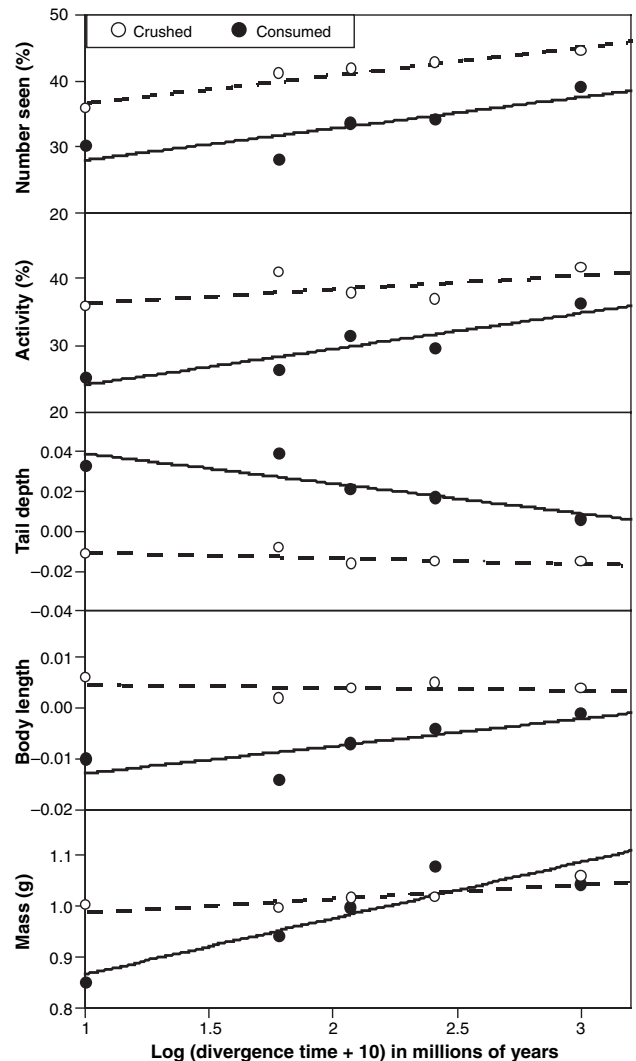


Figure 2 The relationship between phenotypic responses of grey tree frog tadpoles and the phylogenetic distance of either crushed prey (open symbols, dashed lines) or consumed prey (closed symbols, solid lines). The analysis was based upon 50 experimental units but only the 10 treatment means are plotted to provide graphical clarity.

activity in response to predators are consistent with a plethora of previous studies (Kats & Dill 1998). In general, less apparent prey have increased survival due to decreased detection by predators (Skelly 1994), but this behaviour comes at the cost of slower growth in predator-free environments (Harvell 1992; Skelly 1992). The increase in tail depth and decrease in body length is consistent with past studies of morphological defences in tadpoles (Van Buskirk 2002; Relyea 2003a). Tadpoles with relatively deeper tails and smaller bodies survive better in the presence of predators (Van Buskirk & Relyea 1998), but this phenotype experiences slower growth (Van Buskirk 2000). We

observed reduced growth in our experiment, with the largest growth reductions occurring in the treatments that induced the strongest defences. For amphibians, reduced growth is important to fitness because it results in delayed metamorphosis (which can be deadly in a drying pond), decreased size at maturity, and decreased future egg production (Berven & Gill 1983; Semlitsch *et al.* 1988).

Cues from crushed prey alone did not induce the same suite of defences as cues from consumed prey. Crushed and consumed prey both induced increased hiding and decreased activity, but only the consumed prey consistently induced deep tails and short bodies. This result supports the hypothesis that the additional information provided by the simultaneous exposure to both kairomones and alarm cues allow prey to mount more complete and effective anti-predator defences. This difference may exist because behavioural defences are typically more easily reversed than morphological defences (see Relyea 2003b). Thus, if alarm cues provide incomplete information about predation risk, perhaps prey use easily reversible behavioural defences so that their defensive decision can be quickly reversed if the information turns out to be incorrect. In contrast, prey may require more complete information (alarm cues plus kairomones) before investing in defences that are more difficult (or impossible) to reverse.

Within the subset of traits induced by both crushed and consumed prey (the two behavioural traits), crushed prey induced weaker defences. There has been equivocal support for the importance of alarm cues alone for inducing behavioural defences. For example, across 20 species of larval anuran, nearly half of the species did not respond behaviourally to crushed conspecifics (Wilson & Lefcort 1993; Summey & Mathis 1998). The species that did respond were distributed across three families, suggesting that the lack of response is not limited to the loss of alarm cues in one family. Moreover, the equivocal impact of crushed prey on prey behaviour is also found in other taxa including *Daphnia* (Stirling 1995; Pijanowska 1997), snails (Alexander & Covich 1991; Turner 1996), and sea urchins (Parker & Shulman 1986; Hagen *et al.* 2002). Collectively, these data suggest that while crushed prey can induce some phenotypic changes, the changes are often restricted to behavioural traits and the magnitude of the change is frequently small compared with the magnitude induced by consumed prey.

If prey simply detect and respond to kairomones and alarm cues, the response to the consumed cues should be equivalent to the additive combination of the responses to the crushed cues alone and the predator kairomones alone. Our data indicated that responses to consumed conspecifics are more than additive for morphology and growth. From these data, one cannot determine if the synergism is simply the result of encountering both cues simultaneously, or if

there is something about consuming the prey in and of itself that causes the synergy. For example, the latter scenario could occur if actual predation produces compounds that are not produced by starved predators (i.e. digestive enzymes or digested prey tissues; Stabell *et al.* 2003). Further studies are needed to identify the mechanism responsible for the synergistic responses.

The fundamental difference between cues from crushed and consumed prey also can be found in our analysis of alarm cue phylogeny. The phylogenetic-relatedness hypothesis predicts that an organism's defensive responses will be strong when closely related prey are killed but weak when distantly related prey are killed (Chivers & Smith 1998; Chivers & Mirza 2001). The decrease in the magnitude of response with phylogenetic relatedness could arise from one of two mechanisms: (1) more distantly related prey do not release the same chemicals; or (2) predation on more distantly related prey communicates a decreased risk of predation (due to predator search images; Persons *et al.* 2001). This hypothesis appears to have never been tested across a wide range of prey relatedness. For the two behavioural traits, we found support for the hypothesis when the prey were either crushed or consumed. While a number of behavioural experiments have examined the impacts of different alarm cues, past experiments have not used both closely related (within the same order) and distantly related prey. In our study, all consumed amphibians induced strong responses while the insect and snail prey induced weak (or no) response. For mass and the two morphological traits, we also found support for the phylogenetic relatedness hypothesis, but only when prey were consumed (crushed prey never induced any morphological changes). There have been very few studies of predator diet on morphology and mass (Brönmark & Pettersson 1994; Stabell *et al.* 2003) and no previous tests of the phylogenetic-relatedness hypothesis. Our results provide the first extensive evidence that prey responses to different alarm cues (from a group of coexisting prey) can follow a strong phylogenetic pattern. More studies are needed to determine the generality of this pattern in other species. While several authors have stated that fish respond more strongly to alarm cues from closely related fish than from distantly related fish (Smith 1982; Mathis & Smith 1993; Stabell & Lwin 1997), no study to date has tested the hypothesis using a large number of coexisting diets that span a range of phylogenetic relatedness.

CONCLUSIONS

The use of environmental cues is critical for organisms to exhibit adaptive plasticity, yet for organisms with predator-induced defences we know relatively little about the

complexity of the chemical cues that are used. Our results suggest that the chemical cues associated with predation are complex, but not without pattern. Despite the fact that many researchers use crushed prey as surrogates of predation (reviewed in Chivers & Smith 1998), it appears that the cues emitted by damaged or crushed prey can be fundamentally different from the cues emitted by consumed prey; crushed prey frequently do not induce the full suite or magnitude of traits that are induced by consumed prey. In such cases, prey have apparently evolved a reliance on both alarm cues and kairomones. However, this is not to say that alarm cues are unimportant. When alarm cues are combined with the kairomones, they can have large impacts on the induced defence. This reliance may have evolved because alarm cues alone provide no information about which predator is present and kairomones alone (i.e. from starved predators) provide no information about which prey species are being killed by the predator (which may be critical information when predator preference changes over time). This research underscores the importance of simultaneously examining the impacts of crushed and consumed prey across a wide range of phylogeny and a diversity of traits. With this approach, we can better arrive at generalizable patterns as to how prey obtain information from their environment and make their phenotypically plastic decisions.

ACKNOWLEDGEMENTS

Our thanks to Josh Auld, Adam Marko, Playä Hoverman, and Laura Howell for assisting with the experiments. We also thank Christine Relyea for her invaluable expertise in digitizing the 2000 tadpoles necessary for this experiment. Josh Auld, Playä Hoverman, and three anonymous referees provided insightful comments that improved this manuscript. This research was funded by the University of Pittsburgh McKinley Fund (NMS) and the National Science Foundation (RAR). All applicable Occupational Health and Safety protocols were followed in conducting this experiment.

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Editor, Masakado Kawata

Manuscript received 7 December 2004

First decision made 12 January 2005

Manuscript accepted 27 January 2005