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Interpreting the smells of predation: how alarm cues and kairomones induce different prey defences

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Summary

1. For phenotypically plastic organisms to produce phenotypes that are well matched to their environment, they must acquire information about their environment. For inducible defences, cues from damaged prey and cues from predators both have the potential to provide important information, yet we know little about the relative importance of these separate sources of information for behavioural and morphological defences. We also do not know the point during a predation event at which kairomones are produced, i.e. whether they are produced constitutively, during prey attack or during prey digestion.

2. We exposed leopard frog tadpoles (*Rana pipiens*) to nine predator cue treatments involving several combinations of cues from damaged conspecifics or heterospecifics, starved predators, predators only chewing prey, predators only digesting prey or predators chewing and digesting prey.

3. We quantified two behavioural defences. Tadpole hiding behaviour was induced only by cues from crushed tadpoles. Reduced tadpole activity was induced only by cues from predators digesting tadpoles or predators chewing + digesting tadpoles.

4. We also quantified tadpole mass and two size-adjusted morphological traits that are known to be phenotypically plastic. Mass was unaffected by the cue treatments. Relative body length was affected (i.e. there were differences among some treatments), but none of the treatments significantly differed from the no-predator control. Relative tail depth was affected by the treatments and deeper tails were induced only when tadpoles were exposed to cues from predators digesting tadpoles or cues from predators chewing + digesting tadpoles.

5. These results demonstrate that some prey species can discriminate among a diverse set of potential cues from heterospecific prey, conspecific prey and predators. Moreover, the results illustrate that the cues responsible for the full suite of behavioural and morphological defences are not induced by tadpole crushing nor can they be induced by generalized digestive chemicals produced when predators digest their prey. Instead, both prey damage and predator digestion of conspecific tissues appear to be important for communicating predatory risk to phenotypically plastic anuran prey. Importantly, the production of chemical cues by predators may be unavoidable and prey have evolved the ability to eavesdrop on these signals.

Key-words: eavesdropping, inducible defence, phenotypic plasticity, predation cues, predator labelling

Introduction

Inducible defences are a well-studied phenomenon that has proved quite useful in understanding the ecology and evolution of phenotypically plastic traits (Tollrian & Harvell 1999). For individuals that employ changes in their defensive behaviour, morphology and life history, the magnitude and specificity of the response should depend on per capita food availability (Relyea 2004), how quickly traits can change relative to the speed of the environmental change (Padilla & Adolph 1996) and other information that the organism can collect about its environment (Burks & Lodge 2002). When the environment does not change frequently and resources are not limiting, the magnitude of the induction and the specificity of the plastic response (i.e. the specific traits induced) should be determined by the information available from the environment.

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In aquatic systems, chemical cues have been identified as an important source of information about predation risk for a wide range of taxa (Tollrian & Harvell 1999). When predators capture and consume prey, chemicals are released that have the potential to provide detailed information about predation risk. For example, prey can distinguish among different species of predators and produce predator-specific defences that can be linked to the riskiness of the predator and the predator's hunting strategy, suggesting that different predator species release predator-specific cues (i.e. kairomones; Turner *et al.* 1999; McCarthy & Fisher 2000; Relyea 2001b, 2003; Iyengar & Harvell 2002). In addition, prey can distinguish among different predator diets and produce diet-specific defences (Wilson & Lefcort 1993; Chivers *et al.* 1996; Laurila *et al.* 1997; Pettersson *et al.* 2000; Schoeppner & Relyea 2005). The magnitude of diet-specific defences can be correlated with the phylogenetic relatedness of the predator's diet, with closely related diets inducing stronger responses than distantly related diets (Mathis & Smith 1993; Schoeppner & Relyea 2005). This suggests that different prey species release prey-specific cues (i.e. alarm cues). Collectively, these studies indicate that kairomones and alarm cues both provide essential information for prey when inducing their anti-predator defences.

While alarm cues from different predator diets are important to prey defensive decisions, alarm cues by themselves often do not induce prey defences. For example, behavioural responses to alarm cues are not consistent among species within a taxonomic group or even among populations within a species (Walls & Ketola 1989; Pijanowska 1997; Summey & Mathis 1998; Stabell *et al.* 2003; Jacobsen & Stabell 2004). Moreover, in comparisons of traits induced by predators eating prey to traits induced by alarm cues alone, alarm cues alone do not induce the same suite of traits and magnitude of prey defences (Turner *et al.* 1999; Hagen *et al.* 2002; Schoeppner & Relyea 2005).

Numerous studies have demonstrated the existence of kairomones, but the actual source of kairomones from the predator is not well characterized. One possibility is that predators always produce kairomones and therefore prey would always be aware of the predator's presence. An alternative is that kairomones are chemicals produced only when predators chew or digest prey (e.g. digestive enzymes or digestive by-products; Crowl & Covich 1990; Covich *et al.* 1994; Pettersson *et al.* 2000). This latter scenario is supported by the observation that fed predators induce prey defences whereas starved predators frequently do not induce prey defences (Crowl & Covich 1990; Stirling 1995; McCollum & Leimberger 1997; Slusarczk 1999; Schoeppner & Relyea 2005). If kairomones are produced during digestion and the cues are generalized digestive enzymes, then the digestion of any diet would induce prey defences (although these enzymes might have to be combined with prey-specific alarm cues to induce prey defences). In contrast, if kairomones are digestive by-products, prey defences would only be observed when the predator digests the target prey. Such by-products could either be modified prey tissues or chemicals emitted by the bacterial flora of the predator's digestive system when digesting a particular species of prey (termed 'predator labelling'; Crowl & Covich 1990; Mathis & Smith 1993; Pettersson *et al.* 2000; Stabell *et al.* 2003; Jacobsen & Stabell 2004). While these questions are clearly critical for understanding how prey have evolved to detect their predators and induce adaptive defences, few studies have simultaneously evaluated all of these alternatives.

Our goal was to determine the source and effectiveness of alarm cues and kairomones for inducing behavioural and morphological defences in prey. Using larval anurans, organisms well known for their plasticity (Miner *et al.* 2005), we examined the separate and combined effects of alarm cues from crushed conspecifics and kairomones from either starved predators, predators fed heterospecific prey, predators fed conspecific prey, predators that chew but do not digest conspecific prey and predators that digest but do no chew conspecific prey. Using these treatments, we tested the following predictions (Table 1): (i) If alarm cues alone can induce prey defences, then crushed prey should induce the same traits as predators fed conspecific prey; (ii) If kairomones alone can induce prey defences and they are always produced by the predator, then starved predators should induce the same traits as predators fed conspecific prey; (iii) If kairomones are always produced but must be detected in combination with alarm cues, then starved predators plus crushed prey should induce the same traits as predators fed conspecific prey; (iv) If kairomones are only produced after prey are eaten and the kairomones are generalized digestive enzymes, then predators fed heterospecific prey should induce the same traits as predators fed conspecific prey; (v) If kairomones are generalized digestive enzymes, but must be detected in combination with alarm cues, then predators fed heterospecific prey plus crushed prey should induce the same traits as predators fed conspecific prey; (vi) If kairomones are not generalized digestive enzymes but are digestive, prey-specific by-products, then predators fed heterospecific prey plus crushed prey should induce weaker defences than predators fed conspecific prey; (vii) If kairomones are digestive, prey-specific by-products, then predators that only chew conspecific prey should induce weaker defences than predators that chew and digest conspecific prey; and (viii) If kairomones are digestive, prey-specific by-products, then predators that have been fed conspecific prey, but chew heterospecific prey, should induce similar defences as predators that chew and digest conspecific prey.

Materials and methods

We used a completely randomized design consisting of nine treatments replicated five times for a total of 45 experimental units. The nine treatments were as follows: (i) a no-predator control; (ii) crushed tadpoles (i.e. alarm cues alone); (iii) a caged predator that was starved (i.e. kairomones alone); (iv) crushed tadpoles plus a caged predator that was starved; (v) a caged predator that chewed and digested snails (*Physa integra* Haldeman); (vi) crushed tadpoles plus a caged predator that chewed and digested snails; (vii) a caged predator that only

Table 1. A comparison between the potential sources of cues associated with predation and the predictions about how different cue manipulations should impact the magnitude of a prey's inducible defences

Potential source of cue	Prediction about the magnitude of induction
Alarm cues from damaged prey	Crushed conspecific prey = Predators consuming conspecific prey
Kairomones that are always produced	Starved predators = Predators consuming conspecific prey
Kairomones that are produced only when consuming prey + Alarm cues	Starved predators + Crushed conspecific prey = Predators consuming conspecific prey
Kairomones (generalized digestive enzymes)	Predators fed heterospecific prey = Predators consuming conspecific prey
Kairomones (generalized digestive enzymes) + Alarm cues	Predators fed heterospecific prey + Crushed conspecific prey = Predators consuming conspecific prey
Kairomones (digestive by-products)	Predators digesting heterospecific prey + Crushed conspecific prey < Predators consuming conspecific prey
Kairomones (digestive by-products)	Predators chewing conspecific prey < Predators chewing and digesting conspecific prey
Kairomones (digestive by-products)	Predators chewing heterospecific prey but digesting conspecific prey = Predators chewing conspecific prey and digesting conspecific prey

chewed tadpoles; (viii) a caged predator only digested tadpoles; and (ix) a caged predator that chewed and digested tadpoles. Collectively, these nine treatments allowed us to identify the sources of the chemical cues that induce anti-predator defences in tadpoles.

We performed the experiment in 100-L wading pool mesocosms that contained well water, 5 g rabbit chow, 100 g leaf litter (primarily *Quercus* spp.), and zooplankton and algae collected from three nearby ponds. These mesocosms were set up in an old field at the Aquatic Research Laboratory of the Pymatuning Laboratory of Ecology in north-western Pennsylvania on an array of benches that raised the pools 50 cm off the ground. The wading pools were filled with well water on 27 and 28 April 2004 and covered with 60% shade cloth lids to prevent colonization by insects and other amphibians during the experiment. We added one predator cage to each pool that was either empty or contained a single late-instar dragonfly nymph (*Anax junius* Drury) as dictated by the treatment. We used 450-mL plastic cups covered with fibreglass mesh screen as our predator cages, which allowed the cues from the predator and consumed prey to diffuse through the pools while preventing the predator from preying on the tadpoles in the experiment (Petranka *et al.* 1987; Relyea & Werner 2000; Relyea 2001a, 2003; Schoeppner & Relyea 2005). Using such cages, one cannot completely eliminate the possibility that visual or tactile cues might also play a role, but chemical cues seem to be of primary importance in larval anurans and the cage likely reduces the availability of alternative cues. Moreover, anuran responses to predators appear to be largely innate (Petranka *et al.* 1987).

We used leopard frog tadpoles (*Rana pipiens* Schreber) that were collected as 10 newly laid egg masses on 17 April 2004 and hatched and reared in wading pools to prevent their exposure to predator cues prior to the experiment. The tadpoles were fed rabbit chow *ad libitum* prior to the experiment. On 10 May 2004, we added 20 tadpoles to each pool, selected haphazardly from a mixture of tadpoles from the 10 egg masses. The tadpoles were still early in development (Gosner stage 25; Gosner 1960) and their initial mass was 25·1 ± 1·4 mg (mean ± SE). Twenty tadpoles were placed in a 7-L plastic tub to assess mortality caused by handling (24-h survival was 100%).

We added the chemical cue treatment to the pools three times per week. The first cue addition took place on 12 May, 2 days after the tadpoles were added to the mesocosms. All of the treatments employing prey consumption by predators received 300 mg of prey (snails or tadpoles). All of the treatments employing prey crushing received 300 mg of tadpoles (in 100 mL of water) that had been killed (via cranial concussion) and then macerated in a blender for 1 min. The starved predators were not fed for 5 days prior to being used in the experiment and were kept in the pools for no more than 5 days before being replaced with new starved predators. To create the treatments that employed only chewing predators or only digesting predators, predators from the digestion pools were removed, placed into the chewing pools and fed 300 mg of tadpoles. Once the predators had consumed the tadpoles (which generally only required a few minutes), they were immediately returned to digestion pools. To equalize disturbance among pools, all empty predator cages were lifted each time the chemical cue treatments were applied and 100 mL of water was added to all treatments that did not receive crushed tadpoles. Any predators that died were replaced with either starved predators or predators that had previously been fed leopard frog tadpoles in the laboratory (depending upon the treatment). All of the prey added to predator cages had been consumed by the end of the experiment.

We observed tadpole behaviour on six different days over the course of the experiment. We recorded the number of tadpoles that could be seen (i.e. not hiding in the leaf litter) and the number of those tadpoles that were active (i.e. moving). We then divided the latter by the former to determine the proportion of active tadpoles. This approach assumes that the activity level of tadpoles that are observable is representative of the activity of all tadpoles in the pool. On three of the observation days, the predators had been fed the previous day: 13 May (six observations); 27 May (six observations); 1 June (10 observations). On the other three observation days, the predators had been fed earlier the same day: 17 May (12 observations); 19 May (9 observations); 21 May (10 observations). Multiple observers took the observations and all observations on a given day were completed within 2 h. We ended the experiment on 2 June 2004, 24 days after tadpoles were added to the mesocosms. All tadpoles in the experiment were counted, euthanized, and preserved in 10% formalin for subsequent morphological analysis. Preserved tadpoles (up to 20 per replicate) were measured using an image analysis system (BioScan; Optimas Corp., Bothell, WA) in which we measured two morphological traits (tail depth and body length) that have been documented as inducible by predators (Relyea & Werner 2000; Relyea 2003, 2004; Schoeppner & Relyea 2005). All tadpoles were positioned with a glass plate under their tail during measuring to provide an undistorted lateral image.

STATISTICAL ANALYSIS

Because behavioural observations were taken on multiple days, the behavioural data were analysed using repeated-measures analyses of variance to test for the effect of treatments, time, and their interaction. For each observation day, our response variables were the number of observed tadpoles and the proportion of observed tadpoles that were active (i.e. moving), averaged across all observations. Pairwise comparisons between treatments were conducted using Tukey's Honestly Significant Difference (HSD) test ($\alpha = 0{\cdot}05$).

To determine how the different chemical cue treatments affected tadpole shape independent of differences in size, we first conducted a multivariate analysis of covariance (MANCOVA) using cue treatment as a categorical variable, tadpole mass as the covariate and the two morphological traits measured for all of the tadpoles as the response variables. Prior to the analysis, tadpole mass was cube-root transformed to improve the linearity of the relationship between mass and the morphological traits. We then confirmed that within-group regression lines were parallel (a critical assumption of the MANCOVA approach). To produce mass-independent estimates of the morphological traits, we saved the residuals from the MANCOVA analysis and then added the residuals for each tadpole to the estimated marginal means for the appropriate treatment. This approach is a common solution for examining size-independent morphology (Dahl & Peckarsky 2002; Schoeppner & Relyea 2005; McCoy *et al.* 2006). Using tank means for all traits as our response variables, we then used a multivariate analysis of variance (MANOVA) to determine how the treatments affected mass and the mass-adjusted morphological traits. We used Tukey's HSD tests to make pairwise comparisons between treatments ($\alpha = 0{\cdot}05$).

Results

The first analyses were on tadpole behaviour (Fig. 1). When we analysed the number of animals observed (i.e. not hiding), we found significant effects of treatment ($F_{8,36} = 4{\cdot}6$, $P = 0{\cdot}001$) and time (Greenhouse-Geisser $F_{2{\cdot}3,81} = 81{\cdot}3$, $P < 0{\cdot}001$), but no significant time-by-treatment interaction (Greenhouse-Geisser $F_{18,81} = 1{\cdot}7$, $P = 0{\cdot}07$). The time effect simply reflected an increasing number of observed tadpoles on each observation day during the experiment (from a mean of 6·0 tadpoles on the first observation day to 11·4 tadpoles on the last observation day) that could be attributed both to increased activity over ontogeny and increased apparency with greater size. Compared with the no-predator treatment, the only cue treatment that significantly reduced the number of animals observed was the addition of crushed tadpoles ($P = 0{\cdot}024$). The remaining cue treatments did not affect the number of observed tadpoles compared with the no-predator control ($P > 0{\cdot}14$; Fig. 1A). Moreover, none of the eight treatments differed from the treatment containing dragonflies that were chewing and digesting tadpoles ($P > 0{\cdot}09$). Thus, this behavioural response did not distinguish among our hypotheses (Table 1).

When we analysed the activity of the observed animals, we found that tadpole activity was affected by treatment ($F_{8,36} = 5{\cdot}1$, $P < 0{\cdot}001$) and time (Greenhouse-Geisser $F_{3{\cdot}7,134} = 13{\cdot}8$, $P < 0{\cdot}001$) but showed no time-by-treatment interaction (Greenhouse-Geisser $F_{30,134} = 1{\cdot}5$, $P = 0{\cdot}053$). There was no pattern to the time effect, suggesting that difference in activity level with time may have simply been a reflection of the environmental conditions on the observation day (e.g. amount of sunlight, temperature, predator feeding time). Compared with the no-predator control, tadpoles exhibited significantly reduced activity when exposed to cues from dragonflies digesting tadpoles ($P = 0{\cdot}018$) or dragonflies chewing and digesting tadpoles ($P = 0{\cdot}001$; Fig. 1B); the remaining cue treatments were not different ($P > 0{\cdot}3$). Compared with cues from dragonflies chewing and digesting tadpoles, adding cues from starved predators, crushed tadpoles,

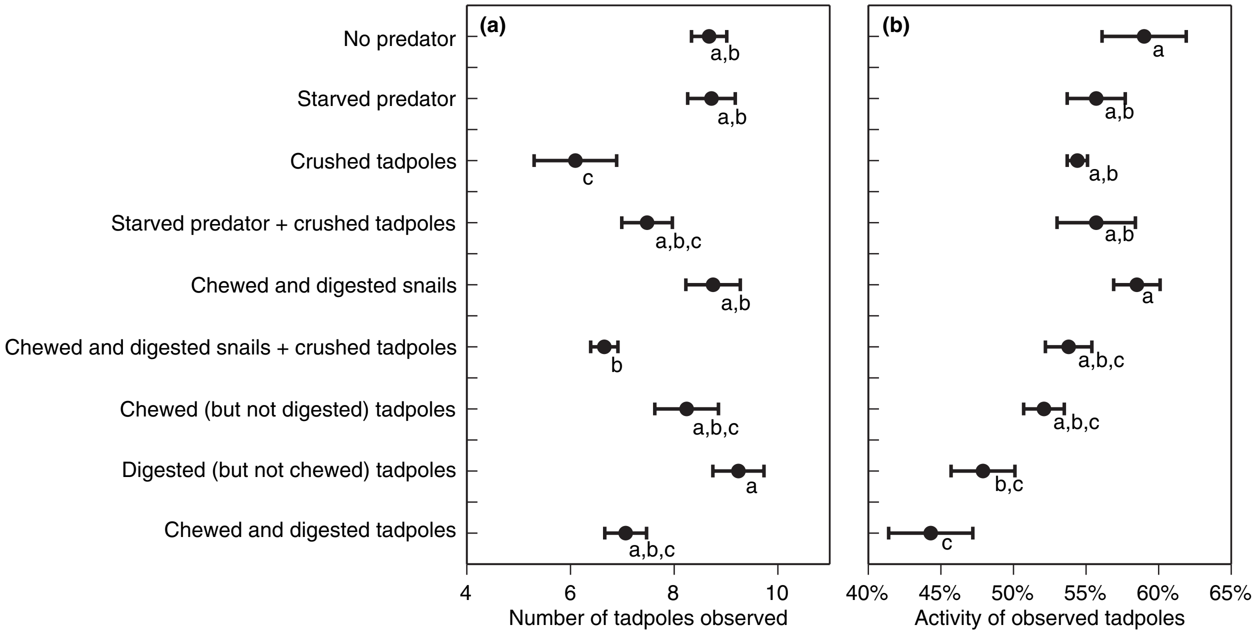

Fig. 1. The effects of different combinations of chemical cues on (a) the number of leopard frog tadpoles observed and (b) the proportion of time that observed tadpoles were active. Behavioural observations were taken on six different days. The data presented are means across all 6 days (± 1 SE) because there was not a significant treatment-by-time interaction. Different letters indicate significantly different means.

starved predators + crushed tadpoles, and chewed and digested snails all induced weaker responses ($P < 0{\cdot}05$); the remaining cue treatments were not different ($P > 0{\cdot}07$). These results refute the hypotheses that cues associated with dragonflies consuming tadpoles come from crushed tadpoles, starved predators, consumed heterospecifics or most combinations of these cues. The data support the hypotheses that the cues could be emitted from consumed snails + crushed tadpoles, from chewed tadpoles or from digested tadpoles.

We found a multivariate effect of the treatments on tadpole mass and morphology (Wilks' $F_{22,99} = 5{\cdot}1$, $P \leq 0{\cdot}001$). Univariate tests indicated significant effects of the cue treatments on tail depth and body length (both univariate tests, $P < 0{\cdot}001$) but no effects on mass (univariate test, $P = 0{\cdot}175$). When we examined tail depth (Fig. 2A), we found that only tadpoles exposed to cues of dragonflies digesting tadpoles ($P = 0{\cdot}008$) or dragonflies chewing and digesting tadpoles ($P < 0{\cdot}001$) induced a relatively deeper tail compared with the no-predator control; all other treatments had no effect ($P > 0{\cdot}7$). Compared with cues from dragonflies chewing and digesting tadpoles, only the treatment containing digested (but not chewed) tadpoles induced a similarly deep tail ($P = 0{\cdot}313$). These results support the hypothesis that the cues associated with dragonflies consuming tadpoles are emitted during the process of digesting tadpoles and refute the alternative hypotheses (Table 1).

The treatments also affected body length (Fig. 2B). Compared with the no-predator treatment, none of the cue treatments induced a significant change in body length ($P \geq 0{\cdot}1$). However, cues from digested tadpoles induced significantly shorter bodies than cues from crushed tadpoles, starved predators + crushed tadpoles or chewed and digested snails + crushed tadpoles ($P \leq 0{\cdot}01$). Compared with cues from dragonflies chewing and digesting tadpoles, none of the other treatments differed ($P > 0{\cdot}07$). Thus, this morphological response did not distinguish among our hypotheses (Table 1).

Discussion

The results of this study make it clear that the phenotypes expressed by leopard frog tadpoles depend on the information available to the tadpoles. When only alarm cues were available from crushed conspecifics, the tadpoles tended to hide more, but did not alter their activity or relative morphology. As more information was available from the predator (from predators digesting tadpoles or from predators chewing and digesting tadpoles), the tadpoles induced additional traits including reduced activity and deeper tail fins. Hence, the tadpoles' phenotypic decisions were contingent on the information that was in the environment.

The phenotypes expressed in this study were generally consistent with previous studies. Reduced activity increases prey survival by making the prey less conspicuous to the predator (Skelly 1994). Cues from consumed prey induce many species of tadpoles to form relatively deeper tail fins compared with tadpoles not exposed to predator cues (McCollum & Van Buskirk 1996; Relyea 2001a; Laurila *et al.* 2004). Tadpoles with this suite of predator-induced phenotypes survive predation better than non-induced phenotypes; the behavioural defences allow reduced detection by predators and the morphological defences allow an improved ability to escape an attack (Van Buskirk & Relyea 1998). However,

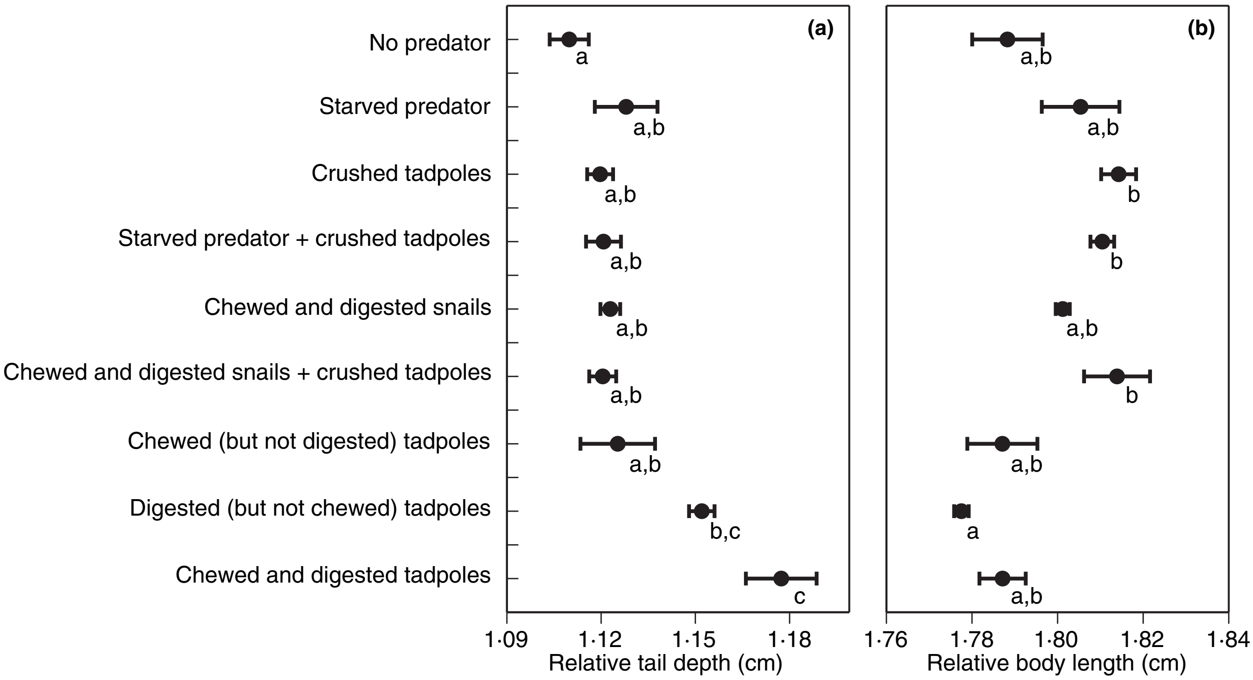

Fig. 2. The effects of different combinations of chemical cues on the (a) relative tail depth and (b) relative body length of leopard frog tadpoles. All dimensions are mass-independent and the data are means (+1 SE). Different letters indicate significantly different means.

predator-induced tadpoles also typically grow more slowly (Van Buskirk 2000). In this study, we did not find a significant effect of exposure to predation cues on leopard frog mass but this may be because tadpole mass was measured later in ontogeny. Previous studies have shown that mass differences often only occur early in ontogeny (Relyea & Werner 2000; Van Buskirk 2001).

Cues from crushed tadpoles induced hiding but did not induce lower activity or a change in morphology. A multitude of studies spanning a wide range of aquatic species has shown that alarm cues from crushed conspecifics can induce behavioural responses (reviewed in Chivers & Smith 1998), but the extent of the changes is wide ranging. Across a diversity of prey taxa, alarm cues alone either do not induce behavioural responses (Crowl & Covich 1990; Wilson & Lefcort 1993; Stirling 1995; Magurran *et al.* 1996; Lefcort 1998; Summey & Mathis 1998), alarm cues induce weaker responses compared with predators consuming prey (Hazlett & Schoolmaster 1998; McCarthy & Fisher 2000; Hagen *et al.* 2002) or alarm cues induce fewer traits compared with predators consuming prey (Hazlett & Schoolmaster 1998; Turner *et al.* 1999; McCarthy & Fisher 2000; Hagen *et al.* 2002). For morphological defences, most studies have shown that alarm cues alone do not induce morphological changes (Walls & Ketola 1989; Brönmark & Pettersson 1994; Schoeppner & Relyea 2005; but see Stabell & Lwin 1997; Stabell *et al.* 2003). All of this suggests that alarm cues alone do contain the information necessary for making some behavioural adjustments but not the information necessary for making the full suite of behavioural changes or the longer-term investment in morphological defences.

Cues from starved predators alone were not sufficient to induce changes in leopard frog traits. Several previous experiments using starved predators have reported no induction of defences in a range of species (Crowl & Covich 1990; Stirling 1995; McCollum & Leimberger 1997), but a few studies have found that starved predators can induce defensive behaviour (Hazlett & Schoolmaster 1998; McCarthy & Fisher 2000; Pettersson *et al.* 2000) and morphology (Walls & Ketola 1989; Iyengar & Harvell 2002; Van Buskirk & Arioli 2002) compared with a no-predator control. When investigators have compared induction by starved predators vs. induction by predators fed prey, responses to starved predators have been relatively weak or intermediate to those induced by predators consuming prey (Walls & Ketola 1989; McCollum & Leimberger 1997; Vilhunen & Hirvonen 2003; but see Pettersson *et al.* 2000). These results suggest that starved predators generally emit little or no kairomone.

Because prey do not respond the same way to cues from crushed prey or cues from starved predators as they respond to predators consuming prey, it may be that prey need to encounter the two cues simultaneously to obtain information about the predator species and the predator diet before committing to some defensive decisions (e.g. morphological defences). In our experiment, we found that the combination of starved predators + crushed tadpoles induced no significant phenotypic changes. Few studies have examined the combination of starved predators + crushed prey and no study has examined this combination in tadpoles. In a study using predatory crabs (*Callinectes bellicosus* Stimpson), Jacobsen & Stabell (2004) found that crabs that had consumed snails (*Tegula funebralis* Adams) induced a crawl out response in the snails, but cues from starved crabs plus crushed snails induced an intermediate response. Interestingly, this intermediate response to the combination of starved predators and crushed prey was also observed in our study. Both studies suggest that the lack of strong responses to crushed prey across a range of prey defences is not because the two cues have to be detected simultaneously. Rather, it appears that something unique occurs when predators consume their prey.

When prey are consumed, the cues that induce anti-predator defences may be released when the predator chews the prey, digests the prey or both. Our behavioural assay found that the predators chewing tadpoles induced no significant reduction in activity, predators digesting tadpoles induced a significant reduction in activity and predators chewing and digesting tadpoles induced the greatest reduction in activity. Although the latter two treatments did not differ statistically, the tendency for a stronger response to chewing and digesting may reflect a greater total concentration of cues in this treatment or a combination of cues that tend to induce stronger responses. The lack of activity response to tadpoles being chewed is consistent with the lack of activity response to tadpoles being crushed. However, it contrasts with Fraker *et al.*'s (2009) recent finding (using wood frogs, *R. sylvatica* LeConte) that while crushed tadpoles do not induce reduced activity, live tadpoles poked with a needle do induce reduced activity, suggesting that alarm cues are not released from killed and crushed prey but are actively released when tadpoles are attacked. Our leopard frogs did not reduce activity or respond morphologically to conspecifics being crushed or conspecifics being chewed. Instead, both traits require cues that are produced when predators digest their prey. In short, the prey required information from a predator that is digesting prey before they induce most of their defences.

Only two previous studies have addressed the effects of cues from digestion cues alone on tadpole morphology. Using tree frog tadpoles (*Hyla chrysoscelis* Cope), Richardson (2006) found that cues from digested prey induced a smaller increase in tail depth compared to cues from chewed and digested prey. Using pinewood tree frogs (*Hyla femoralis* Bosc) LaFiandra & Babbitt (2004) found no difference in tail depth between cues from digestion alone and cues from chewing and digestion but tail colour was more strongly affected by the latter treatment. While more studies are needed to arrive at generalities about the relative importance of cues released when prey are chewed or digested, these studies concur with our finding that cues from digesting prey are critical for inducing many anti-predator traits. Therefore, an effective counter strategy for predators might be to consume their prey at locations where they hunt but digest their prey elsewhere to limit the information available for the induction of defences in other potential prey (e.g. localized defecation in pike; Brown *et al.* 1995).

If prey require cues from digestion before producing their full suite of defences, then these cues could be digestive by-products including modified alarm cues (Crowl & Covich 1990; Hagen *et al.* 2002; Stabell *et al.* 2003). The cues from predators consuming snails did not induce phenotypic changes, indicating that the cues of digestion cannot simply be digestive by-products produced when consuming any species of prey. Studies demonstrating diet-specific anti-predator responses lend further support to the hypothesis that digestive by-products are prey-specific (Wilson & Lefcort 1993; Chivers *et al.* 1996; Laurila *et al.* 1997; Pettersson *et al.* 2000; Schoeppner & Relyea 2005). Because responses to digestive by-products could potentially be further influenced by the presence of alarm cues, we addressed this possibility by combining the cues of predators consuming snails + cues from crushed tadpoles. We found that the behavioural phenotype was not different from the crushed tadpoles alone (i.e. digesting snails had no additional effect) and the tail depth response was weaker than that induced by predators chewing and digesting tadpoles. Therefore, it appears that the cues that induce the complete anti-predator response in tadpoles must be alarm cues that are from conspecifics (or, in some cases, closely related heterospecifics) that are modified by different predators during digestion.

Conclusions

Our results indicate that the chemical information available to prey is a complex mixture of cues emitted by both predators and prey. Tadpoles appear to rely on information from damaged conspecifics when making some behavioural defence decisions but require information from predator digestion when making a range of additional behavioural and morphological defence decisions. This difference in required information may reflect the relative costs of rapid and reversible behavioural decisions vs. slower and less reversible morphological decisions. Our results also suggest that predators do not always produce these inducing chemicals. Indeed, kairomones should not be constantly produced because selection should act to eliminate the production of any constitutive chemical that allowed prey to detect their predators (Crowl & Covich 1990). Interestingly, digestion plays a critical role in the induction of prey behaviour and morphology and digestion appears to produce effective cues via the production of digestive by-products. This suggests that predators do not intentionally warn their prey with the cues they emit during a predation event, but appear to have no choice because the cues are related to prey digestion. Hence, prey have evolved to 'eavesdrop' on chemical cues emitted by predators that indicate the presence of predation risk. This finding also implies that the predators might be able to make themselves 'chemically invisible' to the prey when they consume diets that contain alarm cues that particular species of prey cannot recognize (Stabell *et al.* 2003) and that predator behaviour (i.e. where predators choose to chew vs. digest their prey) should play a pivotal role in determining whether prey can detect predatory risk (Brown *et al.* 1995; Lima 2002).

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