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Primary Research Paper

Behavioural response of bullfrog tadpoles to chemical cues of predation risk are affected by cue age and water source

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Abstract

When confronted by signals of predators presence, many aquatic organisms modify their phenotype (e.g., behaviour or morphology) to reduce their risk of predation. A principal means by which organisms assess predation risk is through chemical cues produced by the predators and/or prey during predation events. Such responses to predation risk can directly affect prey fitness and indirectly affect the fitness of species with which the prey interacts. Accurate assessment of the cue will affect the adaptive nature, and hence evolution, of the phenotypic response. It is therefore, important to understand factors affecting the assessment of chemical cues. Here I examined the effect of the age of chemical cues arising from an invertebrate predator, a larval dragonfly (*Anax junius*), which was fed bullfrog tadpoles, on the behavioural response (activity level and position) of bullfrog tadpoles. The bullfrog response to chemical cues declined as a function of chemical cue age, indicating the degradation of the chemical cue was on the order of 2–4 days. Further, the decay occurred more rapidly when the chemical cue was placed in pond water rather than well water. These results indicate a limitation of the tadpoles to interpret factors that affect the magnitude of the chemical cue and hence accurately assess predation risk. These findings also have implications for experimental design and the adaptation of phenotypic responses to chemical cues of predation risk.

Introduction

Ecologists are becoming increasingly aware of the dramatic phenotypic responses that predators induce in their prey. Many prey taxa are known to modify their phenotype, including behavioural, physiological, and life historical traits, in order to reduce predation risk (reviewed in Chivers & Smith, 1998; Kats & Dill, 1998; Lima, 1998; Tollrian & Harvell, 1999). Such responses can have profound effects on the fitness of the responding prey (Stearns, 1989; West-Eberhard, 1989; Agrawal, 2001). Further, because modifying a trait to reduce predation risk typically is associated with a trade-off, such trait changes may affect interactions of the prey with other species in the community leading to indirect effects of the predator (denoted trait-mediated indirect interactions, reviewed in Werner & Peacor, 2003; Schmitz et al., 2004). To understand a predator's effect on prey, and the consequent indirect effects, it is therefore, important to understand the mechanisms by which prey assess and respond to predation risk.

In aquatic systems, prey commonly respond to predators through kairomones or other chemical cues released from the predator and/or prey during or after predation events (reviewed in Chivers & Smith, 1998; Kats & Dill, 1998; Lima, 1998; Tollrian & Harvell, 1999; Lass & Spaak, 2003). The nature of such chemical cues is complex, as prey perceive and differentiate many different chemicals associated with predators and predation events (Chivers & Smith, 1998; Schoeppner & Relyea, 2005). For example, prey may respond differently to the same predator that has fed on different prey, or prey may respond differentially to different predators that have fed on the same conspecific prey (Chivers & Smith, 1998; Persons et al., 2001; Schoeppner & Relyea, 2005). Further, a number of studies have reported graded responses of prey to different dosages of chemical cues (Loose & Dawidowicz, 1994; Peacor & Werner, 2001; Van Buskirk & Arioli, 2002; Relyea 2003).

In order to respond adaptively, it is important that prey can accurately assess the predation risk that produced the perceived chemical cues. Otherwise, their response will underestimate or overestimate the predation risk, leading to potentially maladaptive behaviour that does not effectively balance the trade-off between predation risk and foraging gains. Given the chemical nature of predation risk assessment in aquatic systems, it is likely that the concentration of the chemical cues, and hence the magnitude of the signal, is related closely to the density of predators and predation events.

We know little, however, about the persistence of such chemical cues nor how prey respond to aged chemical cues. As organic compounds, chemical cues likely degrade over time as has been found in studies of snails (Turner & Montgomery, 2003) and daphnids (Loose et al., 1993) responding to fish kairomones (see also Persons et al., 2001 for a terrestrial example). Indeed, some researchers have used antibacterial agents to remove bacteria from experiments and so reduce the breakdown of chemical cues (Tollrian et al., unpublished manuscript), and others have fed predators throughout long experiments to enhance signals from predators (Werner & Anholt, 1996).

In this study, the behavioural response of bullfrog (*Rana catesbeiana*) tadpoles to a gradient of differently aged chemical cues from larval dragonfly (*Anax junius*) predators, which were fed bullfrog tadpoles, was measured. The responses of tadpoles to the cue declined as the age of the cue increased, with the response disappearing to cue that was 2–4 days old. Further, the decline in the response was much stronger when the cue was placed in pond as opposed to well water. I discuss implications of these findings for experimental design, and for the evolution of adaptive traits in variable environments.

Materials and methods

A laboratory experiment was conducted at the University of Michigan's E. S. George Reserve in Southeastern Michigan. A factorial experimental design was used to examine the effect of chemical cue age (six "cue age" treatments) and biological activity in pond water (two "water type" treatments) on the strength of chemical cues as measured by behavioural responses of bullfrog tadpoles. Note that the experiment was designed to examine the degradation of the chemical cue over time, which is distinct from acclimation (or habituation) by prey to predation risk cues that are also predicted to decrease over time (Lima & Bednekoff, 1999). The experimental design allows the determination of how differently aged chemical cues affect the behaviour of tadpoles with the same history of exposure to chemical cue, and thus it was not confounded by potential acclimation to the chemical cue. Examining the interaction between acclimation to cue and the degradation of cue is beyond the scope of this study.

Creation of differently aged chemical cues from a predator was as follows. Four days before behavioural measurements were made, 7.5 l of water was placed in each of 35 cm × 25 cm × 14 cm plastic containers. The 12 treatments were grouped into 4 spatial blocks each on one of 4 shelves (therefore yielding 48 containers total). Environmental conditions (e.g., light level and temperature) of spatial blocks are nearly equivalent, and therefore, no differences are expected. Half of the 48 containers received aged well water, and half received water from a nearby pond. Water from both sources was poured through a 53 μm nitex mesh to remove larger debris or zooplankton, but retain microbes that may affect chemical cues. Cue age treatments were created by adding chemical cues to the containers 100, 50, 15, 5, and 0 h *before* tadpoles were added to the containers. That is, chemical cues were created on 5 separate occasions, over a wide time range, in order to create chemical cues of different age. In the sixth cue treatment, the no cue control, water without cue was added. The chemical cues added to the containers were created by combining 0.4 l aged pond water, 1 final instar *Anax junius*, and 1~300 mg bullfrog tadpole, in each of 12 plastic cups. After 2 h, water from nine cups in which *Anax* had eaten the tadpole was combined in a bucket and mixed, and then 0.4 l was removed and added to each appropriate container (one container for both water type treatments replicated four times). This procedure was designed to equalize the amount of cue that went into each container across different cue ages, with the cue arising from 1 ''averaged'' *Anax*.

The choice of the duration of cue age treatments extending onto the order of days is likely order of magnitudes longer than typical encounter times between tadpoles that survive close encounters with *Anax*. However, as with other aquatic organisms (Tollrian & Harvell, 1999), tadpoles respond to predators without direct encounters via chemical cues (Van Buskirk & Arioli, 2002). The chemical cue concentration will result from the production and degradation of chemical cues within a given area, and is therefore, related to the degree of predation risk. Indeed studies have shown that tadpoles respond more strongly to cues arising from higher numbers of predators (Peacor & Werner, 2001) or arising from predators eating higher numbers of prey (Van Buskirk & Arioli, 2002).

Each container received nine bullfrog tadpoles that ranged in live mass from 120 to 500 mg. Tadpoles were collected from the Michigan DNR ponds near Saline, Michigan, placed in a wading pool with well water, and fed Purina rabbit chow. Several hours before the experiment, tadpoles were divided into three size classes, and then three tadpoles from each size class were combined in a small holding container and added to the experimental containers. This procedure helped to equalize the mean size and size variation of tadpoles, which may be important given that response to predator cue may decrease with size. To induce feeding by tadpoles, 0.8 g of food (representing approximately 3% of tadpole mass) was added to the experimental containers prior to adding tadpoles. A 3:1 mixture (by mass) of finely ground rabbit chow and Tetramin Fish Flakes was used. Observational measurements were initiated 1 h after tadpoles were added to the experimental containers. Bullfrog tadpoles are known to reduce activity, and increase the use of the substrate of experimental containers in mesocosm studies (Peacor, 2002), presumably to reduce predation risk. I, therefore, examined the effect of the predator cue on tadpole behaviour by recording (1) the number of tadpoles active (feeding or swimming), and (2) tadpole position as measured by the number of tadpoles above the tank bottom (i.e., not touching the bottom), at 12 min intervals for 14 intervals. The time-average of both responses for each container were computed.

I used MANOVA to examine the effect of spatial block, cue age, and water type (i.e., pond or well water) on tadpole behaviour (i.e., number active and position). I then used multivariate simple effects to determine the effect of cue age in each water water type (alone), and the effect of water type at specific cue ages. Data were analyzed using SPSS 12.0 (SPSS, 2003). Data met the assumption of equal covariances and normality. Results were considered significant below an α of 0.05.

Results

The chemical cue treatment had a strong effect on tadpole behaviour (Fig. 1, MANOVA, $F_{10,64} = 6.4$, $p < 0.0001$). Multivariate simple effects tests indicated that chemical cue treatment affected tadpole behaviour in both pond and well water ($F_{10,64} > 3.7$, $p < 0.001$ for both). The percentage of tadpoles active was significantly lower for the 0 and 5 h chemical cue duration treatments than for the 100 h treatments and the control in both pond and well water (Pairwise comparisons, $p < 0.05$). Similarly, the percentage of tadpoles above the bottom was significantly lower for the 0 and 5 h chemical cue duration treatments than it was for both the 100 h treatments and the control in well water (Pairwise comparisons, $p < 0.05$). Whereas a similar trend was present, this response was not significant in pond water. The response of the tadpoles to cue was weaker in the pond water treatments than in the well water treatments

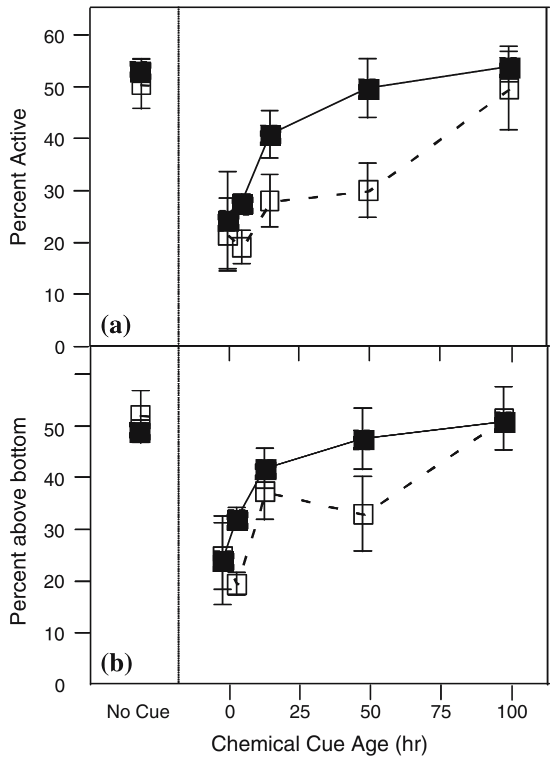

Figure 1. (a) Percent tadpoles active (mean ± standard error), and (b) percent tadpoles above the container bottoms, as a function of the age of chemical cues arising from the *Anax* predator. The chemical cue was placed in well water (empty squares – dashed lines) and filtered pond water (filled squares – solid lines).

(MANOVA, $F_{2,32} = 7.6$, $p = 0.002$). The cue age by water type interaction was not significant (MANOVA, $F_{10,64} = 1.4$, $p = 0.2$). While not critical to the interpretation to this study, note that the presence of an overall main effect of water type, and obvious lack of an effect of water type in the control (and at the lowest and highest cue duration) is suggestive of a cue age by water type interaction. This lack of a significant interaction may arise from the fact that ANOVA resolves interaction less efficiently than main effects (Wade,1992). Due to tadpoles being unusually lower and less active in one spatial block, there was also an effect of spatial block on tadpole behaviour (MANOVA, $F_{6,64} = 4.5$, $p = 0.0007$). I could not identify a reason for this block effect, and thus assume it was spurious.

These results support a general pattern of decreased behavioural response to chemical cue as a function of cue age in both the pond water and well water; tadpole behaviour in the older cue treatments was the same as in the control without cue, and as cue age decreased, the behavioural responses deviated more strongly from the control as the number of tadpoles that were inactive and on the container bottom increased. However, as cue age decreased, the divergence from behaviour in the control occurred sooner (for older cue) when in pond water than well water. Thus, tadpoles showed a behavioural response to cues of short duration, the response decreased as cue age increased, and the decrease was stronger if the cue was in pond than well water.

Discussion

The results indicate that the chemical cues used by bullfrog tadpoles to perceive the presence of the invertebrate predator *Anax* persisted for a time period on the order of days. Further, this duration was strongly dependent on the water source, lasting a shorter time if in pond water than in well water. This study suggests that the range of the chemical cue duration was approximately 2 days (pond water treatment) to 3 or 4 days (well water treatment). Note that it was not the intention to quantify these durations precisely, as the duration will likely be strongly dependent on the source of the water, as indicated by this experiment. Note also that the effect of water source was unlikely influenced by differences in resources in pond and well water, because the water was filtered, and any remaining differences in resource level would tend to promote stronger responses in the pond water treatment, not weaker responses.

The rate of breakdown of the chemical cues in natural settings is likely due to processes such as microbial degradation, adsorption onto organic matter, hydrolysis and photodegredation. The latter two processes would not be affected by water source in the experiment. In contrast, because the concentration of microbes and organic matter is higher in pond water, both microbial degradation and adsorption onto organic matter should be higher in pond than well water treatments, and thus lead to the observed faster attenuation of the chemical cue. Because the well water treatment likely had microbes that grew in concentration over time, while water from different ponds will certainly have different microbe concentrations, we can expect that the cue duration could be either longer or shorter than that observed in this experiment depending on the origin and handling of the water.

The results suggest that the phenotypic response of animals to chemical cues from the same predator–prey interaction can vary spatially or temporally, even if the concentration of the chemical cue is initially equivalent. In addition to the widespread nature of phenotypic responses of prey to predators via chemical cues in aquatic systems, ecologists are becoming increasingly aware of the complexity and richness of the phenotypic responses. For example, many prey respond differentially to different predators (Chivers & Smith, 1998; Relyea, 2003), and even between predators fed different prey (reviewed in Chivers & Smith, 1998; Kats & Dill, 1998; Schoeppner & Relyea, 2005;). Such differences suggest that organisms can detect signals arising from predator scent that is both dependent and independent of the predator's diet. These results are consistent with an organism detecting and interpreting multiple chemicals simultaneously (Weber, 2003). Because different chemicals will be broken down at different rates, the relative abundance of the chemicals could change, and thus the nature of the chemical composition would change. This study suggests that the change could be a function of factors that will vary between systems, and thus the composition of a chemical signal, in addition to its concentration, could vary spatially and temporally even if initiated from equivalent predator–prey interactions.

The results also indicate a limitation in the tadpole's ability to assess factors that affect chemical cues, and hence a limitation in the ability to assess predation risk. Given the ability of organisms to recognize subtle differences in chemical cues and the threat they represent (reviewed in Chivers & Smith, 1998; Kats & Dill, 1998; Schoeppner & Relyea, 2005), it should not be a forgone conclusion that the tadpoles would respond differently in the pond and well water even if there are different concentrations of chemical cues due to different degradation rates; it is possible that in order to accurately assess the predation cue, that organisms could take into account factors that affect the cue concentration. For example, if higher concentrations of microbes (or organic matter) cause faster cue degradation, then equivalent cue concentrations in an environment with higher microbe density (or organic matter) would indicate higher predation risk. In this study, the chemical signal in the two water types arose from the same density of predators foraging, but the tadpoles responded similarly to new chemical cue in pond water and well water (Fig. 1). This suggests that tadpoles did not use factors other than chemical cue concentration when assessing predation risk, including factors that lead to cue degradation. I am unaware of studies that have shown that animals use information that affects the concentration of chemical cues when assessing predation risk. Another factor that would likely affect the duration of the chemical is temperature, and it would be interesting to examine whether organisms incorporate temperature into the assessment of predation risk through chemical cues.

Finally, this study has implications for empirical studies. Many laboratory and mesocosm studies last on the order of days or weeks. This study indicates that if predator chemical cue was introduced every other day (e.g., by feeding the predators which is known to increase the magnitude of the chemical cue), then the cue level may fluctuate moderately through time, and for longer time intervals greater than or equal to 4 days, such fluctuations may be large. Further, if comparisons are made between experiments (e.g., to compare the chemical signals from different predators, or the same predator fed different prey), then the origin and handling of the experimental water could influence the results and their inferences.

Conclusion

In conclusion, this study quantified the degradation rate of chemical cues (kairomones) that bullfrog tadpoles perceive from an invertebrate predator. Cue degradation rate depended on the type of water containing the responding tadpoles and the cue. The similar responses to new cue but the different responses in water having properties that differentially affected cue degradation, suggests that tadpoles are limited in their ability to assess the interaction of chemical cues and factors that affect their magnitude. Water condition or origin is not typically considered in studies of phenotypic responses of aquatic prey to predators. Given the growing recognition that such responses can strongly affect the fitness of prey, and indirectly affect other species that the prey interact with, it is important to better understand the processes that affect the magnitude of the chemical cue. Such studies, like this one, have implications to the evolution of phenotypic plasticity, the theory of species interactions, and experimental designs that explore the ecological significance of phenotypic plasticity in aquatic systems.

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