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Chemical cues from multiple predator-prey interactions induce changes in behavior and growth of anuran larvae

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Abstract Chemical signals are used as information by prey to assess predation risk in their environment. To evaluate the effects of multiple predators on prey growth, mediated by a change in prey activity, I exposed small and large bullfrog (*Rana catesbeiana*) larvae (tadpoles) to chemical cues from different combinations of bluegill sunfish (*Lepomis macrochirus*) and larval dragonfly (*Anax junius*) predators. Water was regularly transferred from predation trials (outdoor experiment) to aquaria (indoor experiment) in which activity and growth of tadpoles was measured. The highest predation mortality of small bullfrog larvae in the outdoor experiment was due to *Anax*, and it was slightly lower in the presence of both predators, probably resulting from interactions between predators. There was almost no mortality of prey with bluegill. The activity and growth of small bullfrog larvae was highest in the absence of predators and lowest in the presence of *Anax*. In the presence of bluegill only, or with both predators, the activity and growth of small bullfrog tadpoles was intermediate. Predators did not affect large tadpole activity and growth. Regressing mortality of small bullfrog tadpoles against activity and growth of bullfrog tadpoles revealed a significant effect for small bullfrog larvae but a non-significant effect for large bullfrog larvae. This shows that the response of bullfrog tadpoles to predators is related to their own body size. The experiment demonstrates that chemical cues are released both as predator odor and as alarm substances and both have the potential to strongly alter the activity and growth of prey. Different mechanisms by which chemical cues may be transmitted to species interactions in the food web are discussed.

Key words Predation · Chemical signals · *Rana* · *Lepomis* · *Anax*

Introduction

Predators may affect many different prey traits such as behavior (Lima and Dill 1990; Lima 1998), morphology (Dodson 1989; Adler and Harvell 1990), and life history (Crowl and Covich 1990; Skelly and Werner 1990; Scrimgeour and Culp 1994). Changes in these traits may affect prey fitness because predators may decrease prey foraging rates or habitat use. This in turn, can depress growth and alter species interactions (Diehl and Eklöv 1995; Persson and Eklöv 1995; Werner and Anholt 1996).

The most dramatic effect of predators on prey fitness is direct consumption. To avoid predation, reliable signals are required that alert the prey to the presence of predators. These include mechanical, visual, and chemical signals, which have been reported as major types of signals used to detect predators (Smith 1977; Kats et al. 1988; Keisecker et al. 1996). Accumulating evidence has shown that for aquatic animals, water-borne chemicals are particularly important cues for assessing predation risk, as prey often hide in structurally complex refuges, in the bottom sediment, or in other habitats that restrict the visual detection of approaching predators (Peckarsky and Dodson 1980; Stoddart 1980; Stauffer and Semlitsch 1993). Amphibians may react both to the odor of predators and to cues released by injured conspecifics, and the extent to which prey respond to these cues is highly correlated with predation risk (Kats et al. 1988; Petranka 1989; McCollum and Leimberger 1997).

Chemical signals may produce uncertainty about predation risk in the environment because it affects prey behavior even when predators are locally no longer present (Wiesenden et al. 1994). Such behavioral time lags may be important for prey since it may restrain prey from other activities such as foraging (Petranka 1989). The behavioral options for prey are further complicated in

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natural environments by the presence of multiple predators that may impose conflicting demands on prey behavior (Kotler et al. 1992; Matsuda et al. 1993; Sih et al. 1998). Predators differ in risk and to date we know little about the ability of prey to adaptively respond to chemical cues released by multiple predators (see review in Lima 1998).

To evaluate the effects of multiple predators on growth, mediated by a change in the behavior, of bullfrog (*Rana catesbeiana*) larvae, I exposed bullfrog larvae (tadpoles) to water from different combinations of bluegill sunfish (*Lepomis macrochirus*) and larval dragonfly predators (*Anax junius*). These species co-occur in permanent waters. Bullfrog larvae are unpalatable to bluegills, whereas *Anax* is an important predator on bullfrog larvae (Werner and McPeck 1994). Bullfrog larvae may react to tactile, visual, and/or chemical cues. However, chemical cues associated with predation by conspecifics are of major importance for bullfrogs and other ranids (Kats et al. 1988; Werner 1991; Stauffer and Semlitsch 1993; Werner and Anholt 1996; Relyea and Werner 1999). Although bluegills do not eat bullfrog larvae, while *Anax* does, both predators induce a strong behavioral response by reducing activity or spatial avoidance by bullfrog larvae (Werner 1991; Eklöv and Werner 2000). Hence, there seem to be different mechanisms that could explain the behavioral responses of bullfrog larvae to predators.

In order to test the effects of single and multiple predators on the activity and growth of bullfrog larvae I exposed them in indoor aquaria to water from outdoor pools containing bullfrog larvae together with bluegills, dragonflies, or both. I quantified the activity and growth of the tadpoles in the indoor aquaria and related this to tadpole mortality in the outdoor pools. By using two sets of experiments I was able to estimate the separate and combined effects of predators on prey growth mediated by a behavioral response to chemical cues. The questions asked were:

1. How do waterborne cues from predators affect behavior and growth of bullfrog larvae?
2. Do prey responses to predators change with prey size?
3. Do effects of multiple predators alter behavior and growth of bullfrog larvae?

Methods

Bullfrog eggs and large bullfrog tadpoles were collected from the Michigan Department of Natural Resources' Saline Fish Hatchery (eggs) and from the University of Michigan's E.S. George Reserve (large tadpoles). Eggs were hatched and tadpoles reared in outdoor wading pools for approximately 2 weeks before the experiment was initiated. During this time tadpoles were fed Purina Rabbit Chow ad libitum.

Bluegill sunfish (total length: 76.2 ± 1.8 mm) were seined from a pond at the University of Michigan's E.S. George Reserve 2 weeks before the experiment and were held in 95-l aquaria. During the acclimation period, the fish were fed a mixture of green frog tadpoles, *Anax*, and earthworms. Final-instar *Anax* were ob-

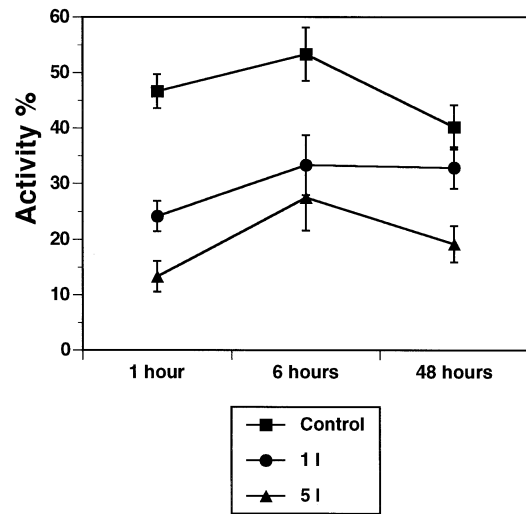


Fig. 1 Activity (%) (means \pm ISE) of bullfrog larvae at 1, 6 and 48 h after receiving different volumes of water from a pool with free *Anax* and small bullfrog tadpoles

tained from ponds on the E.S. George Reserve 2 weeks prior to the experiment, placed in cups, and fed bullfrog tadpoles. All experimental predators were starved for 24 h before they were transferred to the experimental containers to avoid a release of pre-experimental chemical cues.

The study consisted of two related experiments that were conducted simultaneously. In one experiment (outdoor pools), I assessed the mortality rate of bullfrog tadpoles in different predator treatments. In the other experiment (indoor aquaria), I assessed the behavioral and growth responses of bullfrog tadpoles to chemical cues released in the first experiment, by moving water from the first to the second experiment.

In order to determine an appropriate water volume to transfer from pools to aquaria to produce a long-term effect (persisting for at least 24 h), I conducted a preliminary experiment. I added different volumes of water from predation trials in outdoor pools to 37-l indoor aquaria stocked with 20 bullfrog tadpoles (mean individual mass \pm 1 SD, 27.3 ± 1.6 mg). The pools were filled with 50 l of tap water and each received 50 tadpoles from the same stock population as those in the indoor aquaria. All tadpoles were fed at a ratio of 7.5% of individual tadpole body mass per day with a 3:1 mixture (by mass) of finely ground Purina Rabbit Chow and Tetra-min Fish Flakes. A control treatment (no predators) and an *Anax* treatment (one final-instar *Anax*) were assigned to the pools and each treatment was replicated four times. The experiment started when all animals had been in their containers for 24 h. Either 1 or 5 l of water were taken out from each aquarium and the same volume was transferred from each pool to a corresponding aquarium. The pools were then filled to their original volume. After 1 h, the activity of tadpoles was measured by slowly approaching an aquarium and counting all swimming tadpoles for 5–10 s. Activity was measured again 6 and 48 h after the water transfer. A repeated-measures ANOVA revealed a significant difference in activity between different volumes of water transferred (Fig. 1, $F_{2,9}=15.08$, $P=0.0013$) and times ($F_{2,24}=8.82$, $P=0.0027$). By the end of the experiment, the activity of tadpoles in the 1-l treatment was approaching the activity in the control whereas tadpoles still showed a strong response to the cue in the 5-l treatment (Fig. 1, ANOVA on the 48 h data, treatment effect: $F_{2,9}=7.0$, $P=0.016$). This suggests that 5 l of pool water added to an aquarium every 24 h was sufficient to sustain a chemical cue concentration high enough to study long-term behavior and growth responses of tadpoles in the experiment.

The design of the main study was based on the results from the preliminary experiment and consisted of two related experiments.

An outdoor experiment was constructed consisting of circular pools (0.73 m²) filled with 50 l of well water and arranged in a single block. The bottom of the pools was covered with small pieces of fine-mesh aluminum screen to provide structural complexity. The pools were covered with fine-mesh aluminum screen to prevent overheating and insect oviposition. The outdoor pools were assigned to the following treatments and replicated four times: (1) control (no predators present), (2) *Anax*, (3) bluegill, (4) *Anax*+bluegill.

The indoor experiment was conducted in 37-l aquaria filled to the top with well water and randomly assigned the same treatments and same number of replicates as the outdoor pools. The aquaria were illuminated by overhead fluorescent lamps on a 12 h light/12 h dark cycle. The purpose was to test the effects of chemical cues released in the outdoor pools on bullfrog larvae performance (activity and growth) in the indoor aquaria. Therefore, an equal volume of water (5 l) was transferred daily from the pools in the outdoor experiment to the corresponding aquaria in the indoor experiment.

Fifty bullfrog tadpoles (29.4±2.5 mg) and a single predator were added to each outdoor pool except to the *Anax*+bluegill treatment to which one individual of each predator species was added. Controls received no predators. The day after the animals were added to the outdoor pools, 20 small (individual mass±1 SD 30.0±0.9 mg) and two large (3.73±0.18 g) bullfrog tadpoles were added to each indoor aquarium. Every day after the start of the experiment, 5 l of water were siphoned from each aquarium and replaced with an equal amount from the corresponding outdoor treatments. The water was sieved through a fine-mesh nylon screen to prevent food and large particles entering the indoor aquaria. All equipment used for transferring water from pools to aquaria was rinsed thoroughly with tap water between treatments. When the transfer of water from pools to aquaria was completed, 5 l of well water were added to each pool, tadpoles were counted, and killed tadpoles were replaced by new individuals. These individuals were taken from the same stock population as the 50 individuals at the start of the experiment. At the same time, food was added to both aquaria and pools at a ratio of 7.5% of individual tadpole body mass per day, consisting of a 3:1 mixture (by mass) of finely ground Purina Rabbit Chow and Tetramin Fish Flakes. Every 7th day, I measured mean individual mass for these calculations, across treatments for the small tadpoles and individual mass for the large tadpoles. At the same time tadpole feces were removed from the aquaria and pools.

Statistical analyses were conducted using ANOVA on bullfrog survival, activity, and growth. Survival and activity were based on pool and aquarium means respectively, over the whole experimental period. Growth of tadpoles in the aquaria was tested using weight of tadpoles on four occasions as repeated measures. To test whether the mortality of tadpoles in the outdoor pool experiment predicted activity and growth of tadpoles in the indoor experiment, I regressed mortality in the pool experiment against activity and growth of tadpoles in the indoor experiment. The causal relationship between prey mortality and activity and growth of small bullfrog larvae was analyzed using ANCOVAs with mortality as a covariate. All values were ln- or (ln+1)-transformed except proportions, which were angular-transformed.

Results

Mortality in outdoor and indoor experiments

Anax caused a high mortality whereas bluegill had no effect on small bullfrog larvae in the outdoor experiment (Table 1a, Fig. 2). The significant *Anax*×bluegill interaction illustrates that their combined effects were not additive but rather lower than the effect of *Anax* alone (Table 1a, Fig. 2). All small bullfrog larvae survived in

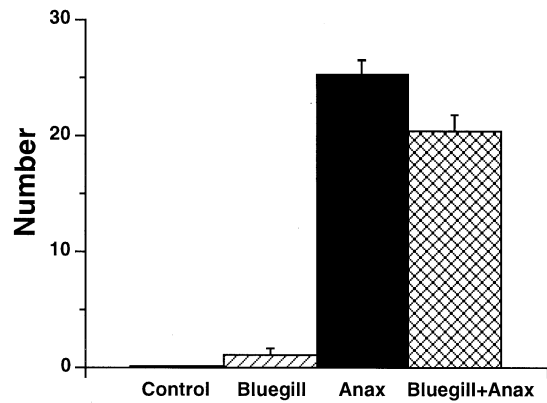


Fig. 2 Number (means±1SE) of small bullfrog larvae killed in the outdoor pools after 24 h in the control, bluegill, *Anax*, and bluegill+*Anax* treatments

Table 1 Two-way ANOVAs for the effect of predator on **a** mortality of small bullfrog larvae in the outdoor experiment, **b** activity of small bullfrog larvae and **c** activity of large bullfrog larvae in the indoor experiment

Source of variation	SS	df	F	P
a				
<i>Anax</i>	1771.88	1	181.05	<0.0001
Bluegill	2.30	1	0.24	N.S.
<i>Anax</i> ×Bluegill	66.21	1	6.76	0.023
Error	117.44	12		
b				
<i>Anax</i>	0.38	1	25.25	<0.0001
Bluegill	0.12	1	8.03	0.015
<i>Anax</i> ×Bluegill	0.39	1	26.21	<0.0001
Error	0.18	12		
c				
<i>Anax</i>	0.061	1	2.765	N.S.
Bluegill	<0.001	1	<0.001	N.S.
<i>Anax</i> ×Bluegill	0.012	1	0.528	N.S.
Error	0.266	12		

the absence of predators. All predators survived during the experiment.

In the indoor experiment, large bullfrog tadpoles had a 100% survival. Survivorship of small tadpoles was also high (88%) and did not differ between treatments ($F_{3,12}=2.013$, $P=0.166$).

Activity and growth of small bullfrog larvae in indoor experiment

The activity level of small bullfrog larvae decreased both in the single and combined predator treatments (Table 1b, Fig. 3a). The activity level of small bullfrog larvae was highest in the control (about 35%) and lowest in the *Anax* treatment (about 15%, Fig. 3a). The significant *Anax*×bluegill interaction resulted from a much

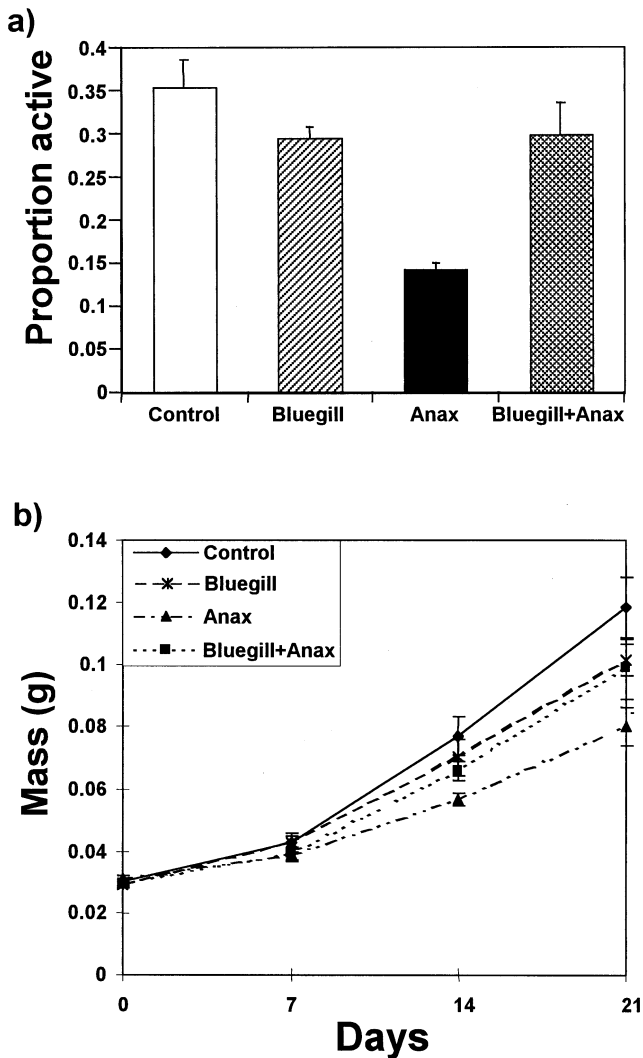


Fig. 3 a Proportion (means \pm 1SE) of small bullfrog larvae active and b average mass (g) of small bullfrog larvae over 3 weeks in response to control, bluegill, *Anax*, bluegill+*Anax* treatments

lower activity level of small bullfrog larvae in the *Anax* treatment compared to the other treatments (Fig. 3a).

Anax had a strong negative effect on growth of small bullfrog tadpoles, and the significant *Anax* \times bluegill interaction was due to a higher growth of small bullfrog tadpoles when predators were combined compared to *Anax* alone (Table 2a, Fig. 3b). Bluegill did not affect growth of small bullfrog larvae (Table 2a, Fig. 3b). Overall, mass of small bullfrog larvae increased over time but there was no treatment difference related to time (Table 2a). A separate ANOVA on the second sampling date revealed a significant effect of *Anax* and an almost significant *Anax* \times bluegill interaction, suggesting that treatment differences were already established after 1 week (ANOVA on growth of small bullfrog tadpoles after 1 week, *Anax*: $F_{1,12}=8.66$, $P=0.012$, bluegill: $F_{1,12}=0.166$, $P=0.69$, *Anax* \times bluegill: $F_{1,12}=0.079$, $P=0.079$).

Table 2 Repeated-measures ANOVA for the effect of predator on growth of a small bullfrog larvae and b large bullfrog larvae

Source of variation	SS	df	F	P
a				
<i>Anax</i>	0.002	1	7.898	0.016
Bluegill	<0.001	1	0.035	N.S.
<i>Anax</i> \times Bluegill	0.001	1	4.711	0.051
Error	0.004	12		
Time	0.008	2	63.979	<0.0001
Time \times <i>Anax</i>	<0.001	2	1.644	N.S.
Time \times Bluegill	<0.001	2	1.431	N.S.
Time \times <i>Anax</i> \times Bluegill	<0.001	2	1.802	N.S.
Error	0.001	24		
b				
<i>Anax</i>	0.002	1	0.558	N.S.
Bluegill	<0.001	1	0.202	N.S.
<i>Anax</i> \times Bluegill	0.01	1	2.687	N.S.
Error	0.046	12		
Time	0.133	2	138.0	<0.0001
Time \times <i>Anax</i>	<0.001	2	0.528	N.S.
Time \times Bluegill	<0.001	2	0.019	N.S.
Time \times <i>Anax</i> \times Bluegill	<0.001	2	0.059	N.S.
Error	0.01	24		

Activity and growth of large bullfrog larvae

The activity of large tadpoles ranged between 34% (*Anax* treatment) and 54% (bluegill treatment), but there was no significant predator effect (Table 1c). Large tadpoles increased in mass during the experiment but predators did not affect tadpole growth (Table 2b).

Mortality dependent activity and growth

There was a strong positive correlation between activity and growth of small bullfrog tadpoles (activity vs. growth, $r^2=0.60$, $F_{1,14}=8.10$, $P=0.013$). Furthermore, activity and growth of small bullfrog larvae in the indoor experiment showed significant negative correlations with mortality of small bullfrog larvae in the outdoor experiment (Fig. 4a, activity vs. mortality, $r^2=0.40$, $F_{1,14}=9.16$, $P=0.009$, growth vs. mortality, $r^2=0.48$, $F_{1,14}=13.06$, $P=0.003$). *Anax*, which caused the highest mortality in the outdoor experiment, also caused the largest reduction in growth of small bullfrog larvae in the indoor experiment (Fig. 4a). A separate regression of mortality caused by *Anax* against small bullfrog tadpole activity showed that the number of tadpoles killed by *Anax* almost perfectly explained the variation in small tadpole activity ($r^2=0.92$, $F_{1,6}=70.77$, $P=0.0002$). Also, the variation in the growth response was significantly explained by number of tadpoles killed by *Anax* ($r^2=0.49$, $F_{1,6}=5.79$, $P=0.05$). Separate ANCOVAs using small bullfrog mortality as a covariate on their activity and growth in the indoor experiment revealed no effect of *Anax* whereas bluegill affected activity but not growth (Table 3). This demonstrates that small bullfrog larvae indeed reacted to cues that were related to numbers of prey killed by *Anax*,

Fig. 4 Relation between mortality of small bullfrog tadpoles in the outdoor experiment and proportion active and growth (mg day^{-1}) of **a** small bullfrog tadpoles and **b** large bullfrog tadpoles in the control, bluegill, *Anax* and bluegill+*Anax* treatments in the indoor experiment

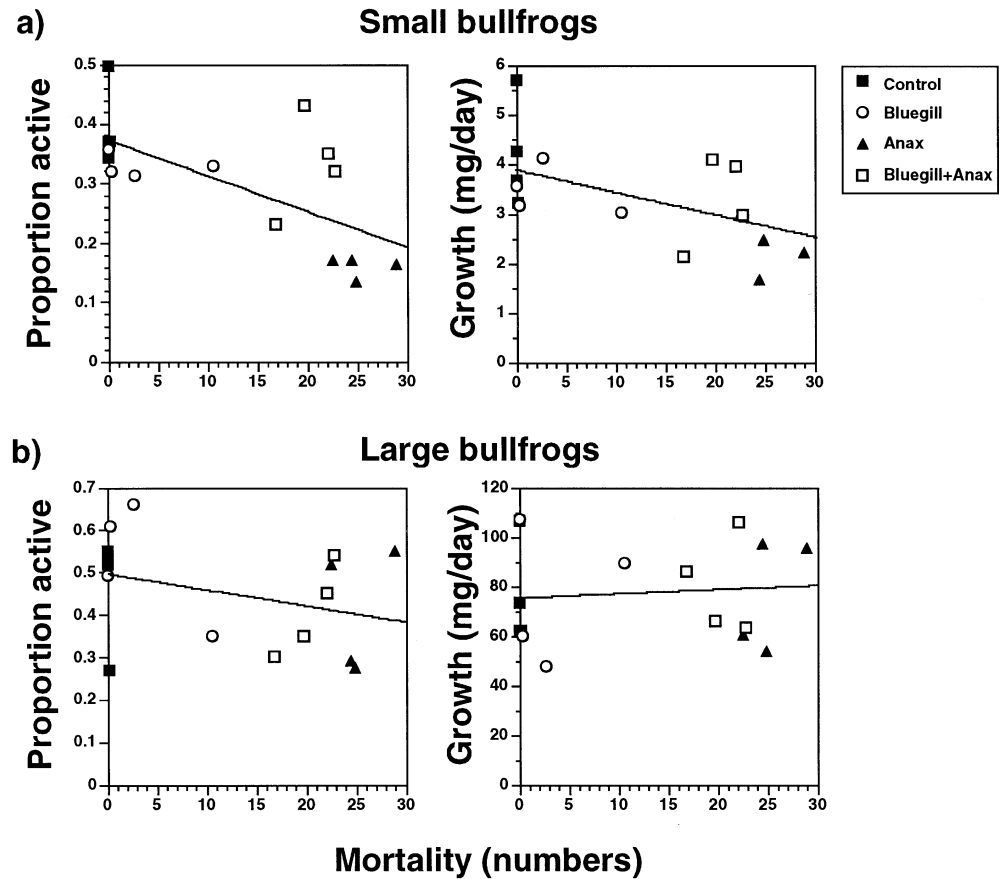


Table 3 Two-way ANCOVAs for the effect of predator and prey mortality (covariate) on **a** activity and **b** growth of small bullfrog larvae in the indoor experiment

Source of variation	SS	df	F	P
a				
Prey mortality	0.095	2,3	22.77	0.043
<i>Anax</i>	0.045	1	2.76	N.S.
Bluegill	0.106	1	6.53	0.026
<i>Anax</i> ×Bluegill	0.279	1	17.05	0.002
Error	0.180	11		
b				
Prey mortality	0.944	2,13	18.54	0.052
<i>Anax</i>	<0.001	1	1.26	N.S.
Bluegill	<0.001	1	1.13	N.S.
<i>Anax</i> ×Bluegill	<0.001	1	0.14	N.S.
Error	<0.001	11		

whereas the reaction of small bullfrog larvae to bluegill was related to other factors (effects on activity), or was absent (effects on growth).

No effects of tadpole mortality on activity and growth of large bullfrog tadpoles were found (Fig. 4b, activity vs. mortality $r^2=0.22$, $F_{1,18}=3.90$, N.S., growth vs. mortality, $r^2=0.003$, $F_{1,18}=0.044$, N.S.).

Discussion

Chemical cues affecting behavior and growth of small bullfrog larvae

Chemical cues released in the presence of *Anax* alone or combined with bluegill indirectly affected the activity and growth of small bullfrog larvae in different ways. *Anax* caused a strong effect both on prey activity and growth, whereas the effect of bluegill was smaller. When both predators were present, the activity and growth of small bullfrog larvae was similar to the treatment with bluegills only. Below, I will first discuss some plausible explanations for the different responses of small bullfrog larvae to the predators and then discuss the consequences of these responses for the direct and indirect interactions between the species in the food web.

Previous studies have shown that bullfrog larvae respond to visual, mechanical, and chemical cues, and all these cues are probably important for their antipredator behavior (Werner 1991; Relyea and Werner 1999; Eklöv and Werner 2000). My experiment demonstrates that the small bullfrog larvae reacted strongly to chemical cues only and this seems also to be the main way by which many species of amphibians recognize predators (Petranka et al. 1987; Kats 1988; Feminella and Hawkins 1994; Kiesecker et al. 1996). The identity of the cue it-

self is unknown, but my preliminary experiment clearly showed a dose response to quantity of the cue over 48 h.

Chemical cues may have different sources. Prey have been found to react to chemicals that are released both by predators themselves (predator odor) and by prey (alarm substances) (Petranka 1989; Kats et al. 1994; Turner et al. 1999). The response of prey to predator odor may differ with predator search mode, and the magnitude of the response is often related to predation risk. The response of prey to alarm substances occurs only when conspecifics or closely related species are killed (Hews 1988; Kiesecker et al. 1996; Turner et al. 1999). Furthermore, tadpoles may develop an aposematic defense in which chemicals in the skin make the tadpole unpalatable to many predators (Wassersug 1971; Cooke 1974; Brodie et al. 1978; Formanowicz and Brodie 1982). In my experiment, bluegill essentially ignored small bullfrog tadpoles. Bullfrog larvae have been found to be unpalatable to bluegill and that is probably the reason why they have a high overlap in their habitat distribution (Werner and McPeck 1994). Although bullfrog larvae are unpalatable to bluegill, bullfrog larvae responded to bluegills by decreasing their activity, suggesting that there are constraints in the behavioral responses of frog larvae to chemical cues. Such a decrease in activity of bullfrog larvae could in turn affect growth, as feeding activity also decreases with an overall decrease in activity. In fact, the growth of small bullfrog larvae in my experiment was on average 24% lower in the presence of bluegill than in controls (the difference was however not statistically significant). The most plausible explanation for this is that bullfrog larvae respond in general to fish, as bullfrog larvae are vulnerable to other fish species (P. Eklöv, unpublished work).

In contrast to bluegill, *Anax* preyed voraciously on bullfrog larvae. The prey response to *Anax* is likely a result of alarm substances released from the prey during predation, because there was a strong correlation between activity and growth in the indoor experiment and the number of tadpoles killed in the outdoor experiment. In short-term aquarium experiments, bullfrog larvae have been found to decrease activity as a response to alarm substances released at predation of conspecifics, whereas no or small responses were elicited by starved *Anax* or *Aeshna* larvae feeding on heterospecifics (S.A. McCollum, unpublished work). In contrast, Petranka and Hayes (1998) also found strong responses of *Bufo americanus* and *Rana sylvatica* larvae to starved *Anax* nymphs, suggesting that we cannot exclude the possibility that tadpoles react to a background predator odor in addition to alarm substances from conspecifics.

A high sensitivity to chemical cues seems to be adaptive, because the habitats of amphibian larvae are often characterized by turbid water and high structural complexity, in which prey may encounter mainly cryptic or sit-and-wait predators such as odonates or other predatory insects. Amphibians also have low escape capacities and depend highly on crypticity or aposematic defense, rather than on active defenses such as high swimming

speed or schooling (see review in Lima 1998). Therefore, chemical stimuli should be more important than tactile and visual cues, although some studies indicate that a combination of cues gives the strongest response (Stauffer and Semlitsch 1993). Eklöv and Werner (2000) showed in a laboratory experiment that visual cues either did not add or added very little to the overall response of tadpoles to chemical cues.

Activity and growth of large bullfrog larvae

Neither *Anax* or bluegill affected either activity or growth of large bullfrog larvae. This is in congruence with previous studies showing that prey vulnerability and also the antipredator response generally decrease with prey size (Eklöv and Werner 2000). For example, Kats et al. (1994) showed that small California newts (*Taricha torosa*) responded more strongly to predators than large ones. The absence of a significant difference in activity was also reflected in the growth of large bullfrog larvae in my experiment. Because food was supplied in excess, and large tadpoles did not react to predators, there was no difference in the growth rate between treatments. Furthermore, previous studies have shown that when food resources are limited, non-responsiveness of large bullfrog larvae to predators may increase their access to food resources, leading to higher growth, when they are together with small bullfrog larvae (Werner and Anholt 1996). This is because the feeding rate of small bullfrog larvae strongly decreases in the presence of predators.

Chemical cues mediating direct and indirect effects in anuran food webs

I have shown that bullfrog larvae react to predators in different ways by responding to chemical cues and that this response tends to disappear at larger prey sizes. But how can the effects of these cues be transmitted to species interactions in the food web?

Anax and bluegill, both alone and combined, indirectly affected growth of bullfrog larvae by changing their activity level in different ways. The strong correlation between activity and growth of small bullfrog larvae supports this result. Animals generally have some control over both their access to food and predation risk and there is often a trade-off between these two factors (Werner and Anholt 1993; McNamara and Houston 1994; Leonardsson and Johansson 1997). My experiment demonstrates how this trade-off was mediated by a change in prey activity level which differed in magnitude of response to the two different predators. Tadpoles exposed to *Anax* showed the strongest negative response whereas they responded less to bluegill. Furthermore, the correlation between prey behavioral responses (activity and growth) and prey mortality suggested that prey responded to predators according to a frequency-dependent

mortality rate. For example, there was a strong correspondence between the number of tadpoles killed by *Anax* and tadpole activity ($r^2=0.92$). The causal relationship was confirmed by removing the effect of mortality in an ANCOVA analysis of predator effects on small bullfrog tadpole activity, showing no significant effect of *Anax*. However, in the same analysis bluegill had a significant effect on small bullfrog tadpole activity demonstrating that the response of small bullfrog larvae to bluegill is probably related to other sources. First, activity and growth of bullfrog larvae in response to bluegill was lower than would be expected considering that essentially no prey were killed in this treatment. This is probably because bullfrog larvae reduce activity in response to bluegill even though bluegill pose no risk to them. This has also been shown in a previous experiment in which bullfrog larvae of different sizes decreased activity in the presence of bluegill (Eklöv and Werner 2000). Second, bullfrog larvae in the presence of multiple predators showed similar activity to bullfrog larvae with bluegill alone even though the predators (probably *Anax*) caused a significant mortality. This result is more difficult to explain given that tadpoles respond according to a frequency-dependent mortality rate of prey. A possible explanation however, is that bluegill reduced the activity of *Anax* which potentially would reduce amount of cue released. Eklöv and Werner (2000) showed that bluegill reduced the activity of *Anax* and thereby reduced the predation rate of *Anax* on tadpoles. Although *Anax* caused substantial mortality of the bullfrog tadpoles in my experiment, it was still lower than when *Anax* was alone. However, an explicit evaluation of the relative contribution of signals released at predation and the cues released by *Anax* odor and *Anax* activity to the overall change in tadpole activity and growth would require further experimentation.

Higher growth of bullfrog larvae in the presence of multiple predators compared to the single predator *Anax* indicated that predator interference decreased the predation rate resulting in increased activity and growth of small bullfrog larvae. Effects of multiple predation on prey performance may arise from different mechanisms (review in Sih et al. 1998). Studies have reported that changes in responses of prey may result from both predator facilitation and inhibition (Soluk and Collins 1988; Kotler et al. 1992; Soluk 1993). Predator facilitation may arise if a change in prey behavior to one predator increase capture rate of the other predator (Vandermeer et al. 1985; Soluk 1993). Predator inhibition is often associated with predator-predator interactions that reduce the predation rates on prey by one or both predators (Huang and Sih 1991; Wissinger and McGrady 1993; Diehl 1995). In my study, the predation mortality was not additive and the most likely mechanism was predator inhibition in which bluegill indirectly affected the prey by changing the behavior of *Anax*, which has also been found in a previous experiment (Eklöv and Werner 2000). Eklöv and Werner (2000) also showed that multiple predators could have a positive effect on prey mortality rate via a de-

crease in prey activity in the presence of one predator, reducing the predation rate of the other predator. This mechanism probably also operated in my experiment. Thus, there are behaviorally mediated indirect effects of predators on prey growth, and the magnitude of these effects differs with the presence of single and multiple predators. Recently, it has been acknowledged that these indirect effects can be of large magnitude in food webs, and can in fact be larger than effects mediated by changes in density (e.g., Wilbur and Fauth 1990; Werner and Anholt 1996; Peacor and Werner 1997).

In conclusion, my experiment demonstrates the significance of predator-induced behavioral effects on prey activity and growth, mediated by chemical cues. The strong effects on prey activity and growth indicate that the chemical cues provide very important information for prey assessing predation risk. Not only direct effects of predators, but also indirect effects resulting from predator interactions, were reflected in cues released and prey in turn responding to these cues. In particular, there is a complexity of sources of chemical cues that may have large implications for the behavioral decisions of prey in regard to the risk of predation. For example, a cue magnitude which depends on the numbers of prey killed would reflect a "true" mortality risk, whereas a cue released by a predator only signals the presence of a predator regardless of the risk it may impose on the prey. The residual variation in activity and growth in my study demonstrates that there is a potential for cues unrelated to actual predation to constrain other activities of the prey (e.g., feeding, reproduction). Evaluating the implications of such residual variation would prove especially valuable in understanding how adaptive behavior is constrained in predator-prey interactions.

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