EFFECT OF PREDATOR DIET ON LIFE HISTORY SHIFTS OF RED-LEGGED FROGS, *Rana aurora*

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Abstract—Larval red-legged frogs (*Rana aurora*) are known to exhibit antipredator behavior in response to both chemical alarm cues released from injured conspecifics and chemical cues of predators. In some cases, the response to predators is dependent on the predator's diet. In this experiment, we tested whether long-term exposure to predator chemical cues and alarm cues resulted in alteration of life history characteristics of red-legged frogs. We raised groups of tadpoles in the presence of chemical cues of predators that were either fed conspecifics or heterospecific caddis-fly larvae, chemical cues of injured conspecifics, or a no-cue control. Tadpoles raised in the presence of either a predator fed conspecifics or cues of injured conspecifics metamorphosed earlier and at a smaller size than those exposed to predators fed heterospecifics or the no-cue control. The result suggests that red-legged frogs exhibit a life history shift in response to predatory cues and that this response is dependent on the diet of predators.

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INTRODUCTION

Interactions among predators, prey, and factors that modify the strength of predator–prey interactions are important for determining the structure of many biological communities (e.g., Menge and Sutherland, 1976; Wellborn et al., 1996). Although many predators can reduce prey abundance, predator effects on prey survival can be modified through a variety of strategies employed by prey. Such strategies include alterations of behavioral responses (e.g., Sih, 1986; Kiesecker et al., 1996; Chivers et al., 1996b), changes in morphology (Appleton and Palmer, 1988; Bronmark and Miner, 1992; McCollum and VanBuskirk, 1996), and shifts in the timing of life-history events (e.g., Dodson and Havel, 1988; Skelly and Werner, 1990; Chivers et al., 1999). Prey that initiate antipredator strategies often have an increased probability of surviving predation events (e.g., Hews, 1988). However, antipredator strategies often take time and energy that would otherwise be available for other activities such as foraging and reproduction (Lima and Dill, 1990). As a result, there should be strong selection on prey to determine vulnerability to predation and to adjust antipredator response accordingly.

The vulnerability of prey to predators may also be influenced indirectly by the presence of heterospecifics that share the same predator. For example, apparent competition describes situations where the increase of one prey species negatively impacts another through increased predation intensity (Vandermeer, 1969; Levine, 1976; Abrams, 1987). Alternatively, increased heterospecific density may have positive effects on prey species when it decreases predation intensity as a result of predator switching (e.g., Murdoch, 1969; Abrams, 1987). In turn, selection should act to either sensitize or desensitize prey to cues of heterospecific predation events, depending on whether the presence of heterospecifics increases or decreases predation intensity.

Many aquatic prey animals, including amphibians, use chemical signals to assess predation risk (e.g., Petranka et al., 1987; Dodson et al., 1994; Kiesecker et al., 1996, 1999; Chivers and Smith, 1998). Cues may arise from the predators or they may be released by prey animals when they are captured by a predator (Chivers and Smith, 1998; Kats and Dill, 1998). However, the response of prey to predators may be context dependent. For example, many prey species only respond to chemical cues of a predator when the predator is fed a diet that contains conspecifics (e.g., Mathis and Smith, 1993; Wilson and Lefcort, 1993; Chivers et al., 1996a). Dietary cues of predators may be an effective way for prey to assess their vulnerability to the predators they encounter (Mathis and Smith, 1993; Wilson and Lefcort, 1993; Chivers et al., 1996; Stabell and Lwin, 1997). For example, Wilson and Lefcort (1993) found that tadpoles of the red-legged frog, *Rana aurora*, exhibit antipredator behaviors in response to predatory rough-skinned newts, *Taricha granulosa*, when newts are fed a diet of conspecifics, but not when newts are fed heterospecifics.

In this study, we raised tadpoles of the red-legged frog in the presence of chemical cues from predatory rough-skinned newts fed conspecifics, newts fed heterospecifics, injured conspecifics only, or a no-cue control. We tested whether tadpoles shifted aspects of the life history in response to potential predation risk. Specifically, we measured whether larval red-legged frog altered the timing of metamorphosis or size at metamorphosis in response to predation risk. While short-term behavioral decisions of prey to predatory chemical cues have been studied extensively, relatively little is known regarding life-history responses of prey to chemical cues that indicate increased predation risk (Chivers and Smith, 1998).

Red-legged frogs and rough-skinned newts can be found together in many of the same breeding habitats where their ranges overlap west of the Cascade–Sierra Nevada mountains from British Columbia, Canada, to northern California, USA (Stebbins, 1985). Rough-skinned newts are effective predators of red-legged frog larvae, although the presence of alternative prey may in some instances lower predation risk for red-legged frogs (Nussbaum et al., 1983; Wilson and Lefcort, 1993). Thus, the presence of newts alone may not accurately predict the level of predation that red-legged frog larvae may expect to experience. We predicted that red-legged frog larvae should only respond to cues of rough-skinned newts when they are associated with chemical cues that give a reliable indication of predation risk experienced by conspecifics.

METHODS AND MATERIALS

We collected *R. aurora* embryos on January 15, 1996, from a pond located 18 km south of Waldport, Oregon, USA. Embryos were collected and transported to a laboratory at Oregon State University where they were reared in 38-liter aquaria. After hatching, tadpoles were raised at approximately 16°C on a 12L:12D photoperiod and fed alfalfa pellets and fish food *ad libitum*. We began tests when all tadpoles reached Gosner (1960) stage 25.

Experiments took place in 24, 38-liter aquaria that were divided widthwise with a fiberglass mesh screen to create two compartments, each measuring 25 × 30 × 25 cm. One compartment of each tank contained a 24-cm-diam. opaque plastic chamber. The side of the chamber facing away from the other compartment was perforated with small (500 μm) holes to allow movement of chemical cues out of the chamber.

We placed 12 randomly selected tadpoles into each of the 24 test tanks, on the side opposite the large plastic chambers. The experiment consisted of four treatments in a randomized block design with six replicates. Treatments 1 and 2 were predator treatments. In treatment 1, tadpoles were exposed to chemical cues of one predatory rough-skinned newt (*T. granulosa*) that was fed caddisfly larvae;

treatment 2 tadpoles were exposed to chemical cues of a predatory *T. granulosa* that was fed conspecifics. Treatment 3 consisted of exposing tadpoles to alarm cues from injured conspecifics. Treatment 4 was a control where tadpoles were exposed to a blank control containing no cues. Within a given treatment, we rotated newts among each of the tanks so that tadpoles were exposed to all newts fed a particular diet.

Throughout the experiment, tanks were cleaned weekly. Newts were fed either three tadpoles or three caddisfly larvae each week. There was always at least one prey item available for each newt. We prepared the alarm cue stimulus by grinding a single tadpole with a mortar and pestle in 60 ml of distilled water. Ten milliliters of the solution was added to each of the six alarm cue treatment containers. Alarm cues were added to the tanks three days per week. On all occasions, the cues were introduced into the plastic chambers. Test tadpoles were fed ground rabbit chow *ad libitum.* All test animals that reached metamorphosis (Gosner stage 41) (Gosner, 1960) were removed from the aquaria, and their mass was determined to the nearest milligram.

Statistical Analysis. For each aquarium, we calculated the mean time tadpoles took to reach metamorphosis and their mean mass at metamorphosis. Because individual responses are not independent of one another, we used tank means for all statistical analyses. We used multivariate analysis of variance (MANOVA) to evaluate the treatment effects on the dependent variables: mean mass at metamorphosis and mean time to metamorphosis (Tabachnick and Fidell, 1989). After MANOVA, we used Bonferroni-adjusted univariate analysis of variance (ANOVA) on each response variable to interpret patterns uncovered by the MANOVA. Tukey (HSD) tests were used to compare treatment means where significant ($P < 0.05$) differences were found with the ANOVA.

RESULTS

The results of MANOVA revealed strong treatment effects on *R. aurora* life-history parameters (Table 1). We used univariate tests (ANOVA) and *post hoc* comparisons (Tukey tests) to interpret patterns of response of time and mass at metamorphosis due to our manipulations.

There were strong treatment effects on the timing of metamorphosis by red-legged frogs (Table 1, Figure 1). They responded to exposure to chemical cues of predatory newts fed conspecifics by initiating metamorphosis more than six days earlier than those exposed to chemical cues of newts fed caddisflies (Table 2, Figure 1). Moreover, frogs exposed to chemical cues of injured conspecifics initiated metamorphosis more than 7 days earlier than those exposed to control conditions (Table 2, Figure 1). Similarly, there were strong treatment effects on frog mass at metamorphosis (Table 1, Figure 1). Frogs exposed to predatory newts

TABLE 1. RESULTS OF MANOVA FOR TREATMENT EFFECTS ON LIFE-HISTORY ATTRIBUTES OF *Rana aurora* (MASS AT AND TIME TO METAMORPHOSIS) AND ANOVAS FOR EACH RESPONSE VARIABLE[a]

MANOVA	$F_{2,19}$	P
Constant	7339.543	<0.001
Treatment	7.423	<0.001
ANOVAs	$F_{3,20}$	P
Mass		
Treatment	13.167	<0.001
Time		
Treatment	9.563	<0.001

[a] Response variables are mass at metamorphosis (mass) and time to metamorphosis (time). Significance level for univariate tests is 0.025 (Bonferroni-adjusted for two response variables).

fed conspecifics were 20% smaller than their counterparts exposed to newts fed caddisflies (Table 2, Figure 1). Additionally, mass at metamorphosis of frogs exposed to chemical cues of injured conspecifics was 14% lower than control animals (Table 2, Figure 1).

DISCUSSION

The results suggest that red-legged frogs alter aspects of their life history in response to chemical cues that indicate predation. Larval frogs initiate metamorphosis earlier and at a smaller size when exposed to cues that denote predation of conspecifics. By decreasing time to metamorphosis, larvae limit their exposure to predatory newts. However, this type of life-history shift likely incurs costs. For example, red-legged frogs that initiate metamorphosis earlier are also smaller. Smaller size at metamorphosis can decrease both reproductive success and survival in the terrestrial environment (e.g., Woodward, 1983). By metamorphosing earlier, frogs also may be exposed to higher levels of terrestrial predation and/or may experience slower growth rates. The cues responsible for initiation of metamorphosis are complex and need further investigation regarding the costs of early metamorphosis.

The experimental design provided insight regarding the specific mechanism responsible for the alterations of life history. Wilson and Lefcort (1993) showed that red-legged frog tadpoles do not initiate antipredator behaviors in response to cues of predatory newts unless newts are fed a diet of conspecifics. In this experiment, we
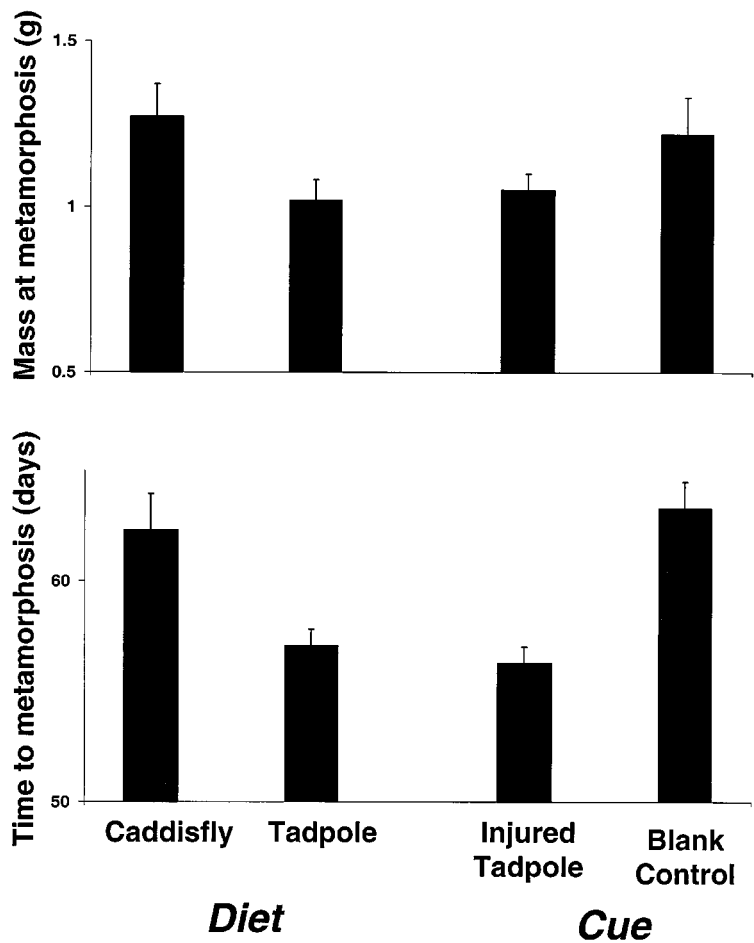
FIG. 1. Mean (+SE) time to metamorphosis (days) and mass at metamorphosis (grams) for *Rana aurora* exposed to chemical cues of predatory newts fed caddisflies, predatory newts fed conspecifics, injured conspecifics, or a blank control.

fed newts tadpoles in experimental chambers. Consequently, the predator stimulus likely resulted in a complex chemical array that included both chemical cues of the predator and injured prey cues. We know that predator stimuli and cues of injured conspecifics alone will induce change in life history. Furthermore, it is clear that exposure to predator cues in the absence of alarm cues will not induce life-history shifts. These results imply that the changes we observed are a direct response to the alarm cues of injured prey and not a reaction to the predator.

TABLE 2. MATRIX OF PAIRWISE COMPARISON PROBABILITIES
FOR TUKEY HSD MULTIPLE COMPARISONS[a]

Treatment	1	2	3	4
Mass				
1	1.0	—	—	—
2	<0.001	1.0	—	—
3	0.001	0.961	1.0	—
4	0.728	0.003	0.009	1.0
Time				
1	1.0	—	—	—
2	0.022	1.0	—	—
3	0.007	0.952	1.0	—
4	0.932	0.006	0.002	1.0

[a] Treatment 1 = predatory newts fed caddisflies; treatment 2 = predatory newts fed conspecifics; treatment 3 = injured conspecifics cues; treatment 4 = blank control.

The results parallel those found in other systems. For example, studies of behavioral responses and morphological changes indicate that prey species may only respond to chemical cues of a predator when the predator is fed a diet that contains conspecifics (Mathis and Smith, 1993; Wilson and Lefcort, 1993; Chivers et al., 1996a). The results presented here contribute to a growing body of evidence that suggests prey use chemical alarm cues released from injured conspecifics to alter aspects of their life history. For example, Crowl and Covich (1990) demonstrated that snails, *Physella virgata*, exposed to crayfish feeding on conspecifics were older at first reproduction than *P. virgata* exposed to crayfish not feeding on snails. Moreover, Chivers et al. (1999) showed that western toad tadpoles exposed to chemical cues of injured conspecifics initiated metamorphosis earlier than tadpoles not exposed to injured conspecifics. Future studies should examine the chemical nature of the stimulus that induces the changes (e.g., Wassersug, 1997; Kiesecker et al., 1999), and they should manipulate the timing and frequency of chemical stimuli to assess if alterations of life history can be induced by exposure to stimuli throughout development or whether exposure during certain stages is required to initiate life history shifts.

We expect that initiating antipredator responses by red-legged frog larvae must be costly and only used when predators are focusing their feeding on conspecifics. For example, why would red-legged frogs forgo responding to a potential predator when newts that are feeding on heterospecific caddisflies are still capable of consuming red-legged frog tadpoles? The foraging strategies employed by rough-skinned newts are unknown—whether they focus their feeding on the most abundant prey items or specialize their feeding on particular prey items regardless of relative abundance. However, the lack of response to newts fed heterospecifcs

suggests that the presence of alternative prey such as caddisfly larvae must result in decreased predation intensity on frog larvae. In natural ponds, abundance of heterospecific prey (e.g., caddisflies) varies dramatically between ponds and between years. If heterospecifics offer a release from predation by newts, then red-legged frog tadpoles may not be able to accurately assess predation risk by using cues of newts alone. Thus, using dietary cues of predators, as opposed to using cues directly released from the predators themselves, may provide a more accurate indication of vulnerability.

The ability of prey animals to assess risk and respond flexibly towards different degrees of predation has important ramifications. The failure of a prey animal to recognize and respond to a predator increases the probability that it will be captured during an encounter with a predator. However, a prey animal that gives an antipredator response upon encountering a nonpredator wastes time and energy that would otherwise be available for activities such as foraging and reproduction (Lima and Dill, 1990). Thus, there should be strong selective pressure for prey that can distinguish between predators that pose a risk and those that do not.

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