

## COMPETITION AND PREDATION MEDIATE THE INDIRECT EFFECTS OF AN INSECTICIDE ON SOUTHERN LEOPARD FROGS

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**Abstract.** Pesticides are widely used by humans to eliminate or reduce populations of unwanted species. These pesticides often cause collateral damage by killing nontarget species and altering biological communities. Our study examined the relative importance of direct and indirect effects of the insecticide carbaryl on southern leopard frog tadpoles, *Rana sphenocephala*, in a simple aquatic community. We did not detect any significant direct effects of carbaryl on the anuran life-history traits examined in this study. Rather, environmentally relevant concentrations of carbaryl indirectly affected *R. sphenocephala* life-history traits by causing changes in the intensity of competition and predation within the community. Carbaryl generally increased the survival of *R. sphenocephala* tadpoles. However, the increase in survival was greatest in mesocosms containing predators, largely due to pesticide-induced mortality of the predators. Carbaryl also exacerbated the effects of competition by decreasing periphyton abundance, which resulted in smaller metamorphs. Thus, we conclude that investigations of the effects of pesticides on a species of interest should go beyond standard toxicological protocols and examine the effects of the pesticide within the context of the community, placing special emphasis on understanding how competition and predation mediate indirect effects.

**Key words:** carbaryl; community structure; competition; declining amphibians; direct effect; indirect effect; insecticide; 1-naphthyl-N-methylcarbamate; pesticide; population regulation; predation; *Rana sphenocephala*.

### INTRODUCTION

Pesticides can affect the life-history traits of a species through two general pathways. Pesticides can affect a species' physiology directly (direct effect), or they can alter the community of which a species is a part, which in turn affects that species (indirect effect). Even sublethal concentrations that do not directly harm a given species can affect that species' competitors, predators, or prey, thus changing species interactions and community structure and potentially either benefiting or harming that species (Abrams 1992).

To understand how pesticides affect nontarget species, it is necessary to know both the direct effects of the chemical and how the chemically induced changes in community structure affect that species. Although toxicologists have recognized the importance of species interactions and community structure, they have generally focused on single species in isolation of the species interactions and community structure of which they are normally a part (Cairns 1995, Halfman 1995). Thus, toxicologists have generally been unable to make explicit conclusions about the indirect effects of chemicals on individual species. The failure to adequately examine indirect effects is unfortunate because factors such as competition and predation are major determi-

nants of community structure, which can only be understood within the context of stressors (such as pesticides) present in the environment (Menge and Sutherland 1987).

We examined how a pesticide interacted with competition and predation to alter amphibian life-history traits. Our study is particularly timely given the current concerns about worldwide amphibian declines (Houlihan et al. 2000) and given that one of the proposed reasons for the decline is environmental contamination by anthropogenic chemicals such as pesticides (Relyea and Mills 2001, Sparling et al. 2001, Davidson et al. 2002).

We used the insecticide carbaryl as our model chemical. Carbaryl (1-naphthyl-N-methylcarbamate) is a relatively short-lived, broad-spectrum carbamate that acts through acetylcholinesterase inhibition (Hill 1995). It is widely used throughout the spring and summer to control insect populations on rangelands and forests and on a variety of fruit and vegetable crops. The effects of carbaryl on amphibians have been comparatively well studied. Ninety-six-hour LC50s for anurans in the midwestern United States range from 9 to 15 mg/L (Bridges 1997, 1999, 2000), which exceeds concentrations expected to occur in the environment (as high as 3.5 mg/L; Peterson et al. 1994). However, carbaryl has a number of sublethal direct effects on tadpoles in the laboratory. Carbaryl reduces tadpole activity levels (Bridges 1997, 1999) and reduces the ability of tadpoles to assimilate ingested food (Marian et

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al. 1983), both of which may reduce larval growth rates (e.g., Skelly 1992). Carbaryl may make tadpoles more susceptible to predation by reducing swimming speed and endurance (Bridges 1997) and by reducing tadpole use of refugia when a predator is present (Bridges 1999). Finally, low-level exposures to carbaryl can cause an increase in malformations among resulting metamorphs (Bridges 2000). However, there is evidence that tadpoles may recover within a few days after short-term exposure to carbaryl (Bridges 1997, 2000). Thus, with the exception of increased malformations, most of the direct effects may be temporary. In contrast to the above laboratory studies, mesocosm studies indicate that exposure to carbaryl early in the larval period alters later life-history traits of anurans, although in ways not necessarily predicted based on single-species laboratory studies. For example, tadpoles in mesocosms dosed with carbaryl frequently survive better (Boone and Semlitsch 2001), have shorter larval periods (Boone et al. 2001, Boone and Semlitsch 2002), and are larger at metamorphosis than tadpoles from control mesocosms (Boone and Semlitsch 2001).

The carbaryl-induced changes in anuran life-history traits seen in previous mesocosm studies appear to be positive for the anurans, in contrast to findings in laboratory studies, and suggest that indirect effects of pesticides are very important. Therefore, the objectives of our study were to determine (1) the relative magnitude of carbaryl's direct and indirect effects on anuran survival, size at metamorphosis, and length of the larval period; (2) how carbaryl influences both intra- and interspecific competitive interactions; and (3) how carbaryl influences predator-prey interactions.

#### MATERIALS AND METHODS

This study is composed of two experiments with similar designs but emphasizing different questions. During the summer of 2000 (experiment 1), we compared the relative magnitude of carbaryl's direct and indirect effects on *Rana sphenocéphala* and determined whether the intensity of these effects varied in response to competition. Results from experiment 1 indicated that indirect effects were quite strong, but we failed to detect significant direct effects of carbaryl. Thus, during the summer of 2001 (experiment 2), we concentrated on understanding the indirect effects of carbaryl with an emphasis on elucidating how predators might interact with competitors to alter the indirect effects of carbaryl on *R. sphenocéphala*. Because the methods are similar between the two experiments, the methods are described once with differences between the experiments noted as appropriate.

##### *Experimental system*

Polyethylene stock tanks (1500 L; 48 and 54 tanks in experiments 1 and 2, respectively) were arranged in a rectangular array at the University of Missouri at Columbia, Research Park and divided into three spatial

blocks. The tanks were cleaned, and no residues from previous toxicological studies were present. The tanks were then filled with ~1000 L of tap water (day zero). Lids made from vinyl window screen (1.6-mm mesh) were kept over each tank once water was added to prevent uncontrolled colonization by anuran competitors and predators. One kilogram of air-dried deciduous leaf litter was added to each tank the following day (day 1). Over the next 30 days, the tanks were inoculated repeatedly with pond water containing concentrated periphyton, phytoplankton, and zooplankton from several natural ponds in Boone County, Missouri and with cladocerans (*Daphnia* and *Ceriodaphnia*) from cultures maintained at the Columbia Environmental Research Center (CERC).

Once the periphyton, phytoplankton, and zooplankton were well established (approximately day 30), the tanks in experiment 1 were assigned to an interspecific competitor treatment (presence or absence of *Pseudacris crucifer* [spring peeper] tadpoles). *P. crucifer* (270 tadpoles per tank) were added to all tanks assigned to the interspecific competitor treatment to compete with *R. sphenocéphala* tadpoles. In experiment 2, the tanks were assigned to both an interspecific competitor treatment (presence or absence of *P. crucifer* tadpoles) and a predator treatment (no predator, *Anax* spp. dragonfly larvae, or *Notophthalmus viridescens* louisianensis [central newt, hereafter *N. viridescens*]) in a randomized block design (Table 1). *P. crucifer* (270 tadpoles per tank) were added to all tanks assigned to the interspecific competitor treatment. Tanks assigned to predator treatments received either seven *Anax* larvae or three adult *N. viridescens* to act as predators on the *P. crucifer* and the *R. sphenocéphala* that would be added later.

All *P. crucifer*, *Anax* larvae, and *N. viridescens* used in these experiments originated from natural ponds in Boone County, Missouri. *P. crucifer* tadpoles were obtained by collecting the egg of amplexing adults. Once the eggs hatched, the *P. crucifer* tadpoles were immediately added to the tanks. Central newts (*N. viridescens*) and dragonfly larvae (*Anax* spp.) were immediately added to the tanks when collected.

The interspecific competitors and predators were given 5–10 d to acclimate, and then the tanks were assigned to a tank carbaryl-treatment or a control group in a randomized block design. At the time the tanks were dosed with carbaryl, the aquatic community consisted of a variety of periphyton, phytoplankton, and zooplankton, along with *P. crucifer* (in one-half of the tanks) and predaceous *Anax* larvae or *N. viridescens* (each in one-third of the tanks in experiment 2). *R. sphenocéphala* had not been added to the tanks yet, and thus any effects of tank carbaryl-treatment on *R. sphenocéphala* would be due to changes in this community.

Tanks assigned to carbaryl treatments were dosed with Sevin (Ortho, Columbus, Ohio, USA; Sevin Liq-

TABLE 1. Comparison of the treatments in experiments 1 and 2.

Variables and statistics	Treatment levels	
	Experiment 1	Experiment 2
Variables		
Tank carbaryl treatment (mg/L)	0, 2	0, 2, 5
Tadpole carbaryl treatment (mg/L)†	0, 2	0‡
Interspecific competition (no. <i>P. crucifer</i> per tank)§	0, 270	0, 270
Intraspecific competition (no. <i>R. sphenoccephala</i> per tank)	20, 60	50‡
Predation (0, none; 1, <i>Anax</i> ; 2, <i>Notophthalmus</i> )§	0‡	0, 1, 2
Statistics		
Treatment combinations	16	18
Blocks (replicates)	3	3
Total tanks	48	54

† Exposure of *R. sphenoccephala* tadpoles to carbaryl for 48 hours prior to introducing them to the tanks.

‡ Not varied during the experiment.

§ Interspecific competitors and predators were introduced to the tanks prior to the tanks being dosed with carbaryl; they were a part of the aquatic community altered by carbaryl.

|| Number of *R. sphenoccephala* introduced to the tanks after all carbaryl had dissipated.

uid Formula; 21.3% carbaryl). In experiment 1, tanks were assigned to a no-carbaryl control or 2 mg/L carbaryl treatment using a randomized block design and were dosed with 0.0 or 9.4 g of Sevin to yield nominal carbaryl concentrations of 0 and 2 mg/L. In experiment 2, tanks were assigned to a no-carbaryl control, 2 mg/L or 5 mg/L carbaryl treatment using a randomized block design and were dosed with 0.0, 9.4, or 23.5 g of Sevin to yield nominal carbaryl concentrations of 0, 2, and 5 mg/L (Table 1).

Two hours after the tanks were dosed with carbaryl, composite water samples, containing an equal volume of water from each tank in a treatment, were collected for analyses of carbaryl concentrations. Water samples were taken every 5–7 d thereafter until analyses indicated there was no carbaryl or its toxic breakdown product,  $\alpha$ -naphthol, present in the water. All water samples were analyzed within 48 hours by the Mississippi State Chemical Laboratory (Mississippi State, Mississippi, USA) using high pressure liquid chromatography (HPLC).

In experiment 1, *R. sphenoccephala* tadpoles (the focal species in these experiments) were prepared for introduction to the tanks after all carbaryl and  $\alpha$ -naphthol were gone from the tanks (21 d after dosing). Forty-eight 15-L glass jars were arranged in three blocks at the CERC corresponding with the three blocks of tanks at the Research Park. All jars were filled with 15 L of well water and dosed with 0.0 or 0.14 mL of Sevin to yield nominal carbaryl concentrations of 0 and 2 mg/L, respectively. These nominal concentrations were intended to be identical to the initial tank nominal concentrations. Composite water samples were collected for analysis of carbaryl concentrations 30 min and 48 h after dosing.

Once all jars were dosed, either 20 or 60 *R. sphenoccephala* tadpoles were introduced into the jars. The tadpoles were exposed to their respective carbaryl

treatments for 48 h. Jars were checked every 12 h for dead tadpoles, which were removed to prevent fouling of the water. The *R. sphenoccephala* tadpoles were then transported to the Research Park where they were introduced into the tanks to produce all possible treatment combinations (neither tanks nor tadpoles exposed to carbaryl, only tanks exposed to carbaryl, only tadpoles exposed to carbaryl, or both tanks and tadpoles exposed to carbaryl) in a full factorial design.

In experiment 2, 50 *Rana sphenoccephala* tadpoles were introduced to the tanks as soon as all carbaryl and  $\alpha$ -naphthol were gone from the tanks (18 d after dosing). None of the *R. sphenoccephala* tadpoles were exposed to carbaryl prior to introduction to the tanks because there were no significant direct effects detected in experiment 1 and our emphasis was on understanding the indirect effects (Table 1).

All *R. sphenoccephala* tadpoles used in these experiments originated from Boone County, Missouri. They were obtained by collecting egg clutches from natural ponds and maintaining them in polyethylene containers until needed for the experiments. Tadpoles were used within seven days of hatching and were at Gosner stage 25 (Gosner 1960) when added to the experiments.

#### Anuran data collection

Tanks were monitored daily for both *P. crucifer* and *R. sphenoccephala* metamorphs. The onset of metamorphosis was defined as the emergence of a front leg (stage 42; Gosner 1960). Once a tadpole had begun to metamorphose, it was captured and maintained in a 1-L plastic box for 1–3 d until the completion of metamorphosis (stage 46; Gosner 1960), at which time it was weighed. Metamorphs never exposed to carbaryl were returned to their natal ponds, while metamorphs exposed to carbaryl were euthanized using chlorotone. After most tadpoles had metamorphosed, the tanks

were drained, and all remaining tadpoles were collected and counted.

*Sampling of zooplankton, phytoplankton,  
and periphyton*

During experiment 1, zooplankton and phytoplankton were sampled four times at approximately one-month intervals (days 27, 64, 93, and 127) starting just prior to the introduction of carbaryl into the tanks (day 35). During experiment 2, zooplankton and phytoplankton were sampled on day 110, which corresponded with the timing of the first *R. sphenoccephala* metamorphs and was after 99% of surviving *P. crucifer* had metamorphosed. Samples were collected by taking five 1-L samples of water from five preselected locations within each tank. After homogenizing the 5 L of water, 0.5 L of water was removed for phytoplankton chlorophyll *a* analysis and the remaining 4.5 L were filtered through an 80- $\mu\text{m}$  Wisconsin net (Wildco, Buffalo, New York, USA) to collect zooplankton. The zooplankton were stored in 80% ethanol for identification. Fluorometric analysis of phytoplankton for chlorophyll *a* was performed following the procedures in *Standard Methods* (American Public Health Association et al. 1998).

Periphyton abundance was sampled by suspending five  $2 \times 7$  cm rectangles of scrimweave (Sto-Cote Products, Richmond, Illinois, USA)  $\sim 1$  cm below the water's surface in the center of each tank immediately after the leaf litter was added. At the same time plankton samples were taken, a rectangle of scrimweave was removed from the tank and placed in 15 mL of buffered acetone that was immediately refrigerated. Fluorometric analysis of periphyton for chlorophyll *a* was performed following the procedures in *Standard Methods* (American Public Health Association et al. 1998). The scrimweave was frequently disturbed while collecting *R. sphenoccephala* metamorphs. Thus, data measuring periphyton abundance after *R. sphenoccephala* metamorphosis began were not analyzed.

*Analyses of Rana sphenoccephala data*

*General.*—Data on the focal species, *R. sphenoccephala*, were analyzed using analysis of variance (GLM; SAS Institute 1999–2001). The dependent variables were percentage survival, metamorph size, days to metamorphosis, and percentage metamorphosis. All tadpoles that metamorphosed or that were still alive in the tanks at the termination of the experiment were considered survivors. Percentage survival was derived by dividing the total survival by the number of tadpoles initially added to each tank. Metamorphs were weighed at the conclusion of tail resorption, and the mean from each tank was used as an estimated size at metamorphosis. Length of the larval period was monitored in two ways. The number of days from introducing the tadpoles into the experiment to the onset of metamorphosis (i.e., emergence of a front leg) was recorded for

each tadpole that metamorphosed. The mean from each tank was used as an estimated time to metamorphosis. However, not all surviving tadpoles metamorphosed. Any differences in the proportion of surviving tadpoles that metamorphosed would also indicate an effect on the length of the larval period. Therefore, percentage metamorphosis was calculated by dividing the number of tadpoles that metamorphosed by the number surviving. Differences in either time to metamorphosis or percentage metamorphosis would indicate that treatments changed the length of the larval period. Percentage survival was arcsine square-root transformed, mass was log-transformed, and percentage metamorphosis was either squared (experiment 1) or arcsine square-root transformed (experiment 2) to meet assumptions of normality and homogeneity of variances.

Survival was highly variable among tanks. Because survival influences intraspecific competition for resources, survival has a strong effect on metamorph size and length of the larval period (e.g., Travis 1983, Wilbur 1997). Therefore, percentage survival was included as a covariate in the analysis of the other dependent variables following the example of Parris and Semlitsch (1998). Including percentage survival as a covariate allowed us to partition variability in the data due to differences in survival and thus determine if there was an effect of the experimental treatments on metamorph size and length of larval period independent of survival.

Alpha was set at 0.05. The dependent variables of metamorph size, days to metamorphosis, and percentage metamorphosis are all based on measurements of the same surviving individuals within each tank. Consequently, the analyses of these three dependent variables are not independent. Thus, alpha for these three variables was set at 0.017 using a Bonferroni adjustment (Sokal and Rohlf 1995) to prevent the experiment-wide probability of a Type I error ( $\alpha$ ) from increasing above 0.05. All results are presented as the mean  $\pm$  one standard error.

The independent variables in experiment 1 (Table 1) were block, interspecific competition (0 or 270 *P. crucifer*), intraspecific competition (20 or 60 *R. sphenoccephala*), tank treatment (0 or 2 mg/L carbaryl), and tadpole treatment (0 or 2 mg/L carbaryl). One of the central goals of experiment 1 was to quantify the relative strength of direct and indirect effects. Thus, partial regression coefficients of determination ( $r^2$ ) were calculated for the effects of tank and tadpole carbaryl-treatments on the dependent variables as an estimate of the variability explained by each.

The independent variables in experiment 2 (Table 1) were block, interspecific competition (0 or 270 *P. crucifer*), predation (none, *Anax* larvae, or *N. viridescens*), and tank treatment (0, 2, or 5 mg/L carbaryl). Very few *R. sphenoccephala* tadpoles survived in tanks containing predatory *Anax* larvae when the tanks were not dosed with carbaryl, and we were unable to gather

sufficient data on the effects of *Anax* larvae on metamorph size and larval period. Thus, tanks containing *Anax* larvae were excluded from analyses of metamorph size and length of larval period.

In both experiments, we thought the response of *R. sphenoccephala* to the independent variables might be interdependent. Therefore, a decision was made a priori to include all two- and three-way interactions in the initial models but to only include interactions with *P*  $\leq 0.1$  in the final models.

#### *Analyses of Pseudacris crucifer data*

*P. crucifer* survival was analyzed using analysis of variance. The independent variables in experiment 1 were block, tank carbaryl-treatment, interspecific competition from *R. sphenoccephala* (20 or 60 tadpoles), and the interaction between tank carbaryl-treatment and interspecific competition. The independent variables in experiment 2 were block, tank treatment, predation, and the interaction between tank carbaryl-treatment and predation.

#### *Analyses of zooplankton, phytoplankton, and periphyton data*

*Experiment 1.*—The effects of tank treatment (0 or 2 mg/L carbaryl), *R. sphenoccephala* (20 or 60 tadpoles), and *P. crucifer* (0 or 270 tadpoles) on cladoceran and copepod abundance over time were analyzed using a repeated-measures analysis of variance (GLM; SAS Institute 1999–2001). Cladoceran and copepod data were log-transformed but still did not satisfy the assumption of sphericity, which states that the variances of the differences between treatments must be equal (Field 1998, Maxwell and Delaney 1999). Thus, reported *P* values are adjusted using the Huynh-Feldt estimate of epsilon (SAS Institute 1990). The effects of tank carbaryl-treatment, number of *R. sphenoccephala* tadpoles, and number of *P. crucifer* tadpoles on phytoplankton and periphyton chlorophyll *a* abundance over time were also analyzed using repeated-measures analysis of variance. Periphyton data were square-root transformed and phytoplankton data were log-transformed to meet the assumption of sphericity.

*Experiment 2.*—The effects of tank treatment (0, 2, or 5 mg/L carbaryl), *P. crucifer* (0 or 270 tadpoles), and predation (none, *Anax* larvae, or *N. viridescens*) on cladoceran and copepod abundance and on phytoplankton and periphyton chlorophyll *a* abundance were analyzed using an analysis of variance. All data were log-transformed to meet assumptions of normality and homogeneity of variances.

#### *Analyses of predator data*

Data were collected on the survival of adult *N. viridescens* and *Anax* larvae in experiment 2. No predators survived in the 5 mg/L tank carbaryl-treatment. Therefore, a nonparametric analysis of survival was conducted by first ranking the data and then conducting

an analysis of variance. The independent variables were block, tank carbaryl treatment, *P. crucifer* (0 or 270 tadpoles), and the interaction between tank treatment and *P. crucifer*. The survival endpoints used for the two predators differed. *N. viridescens* survival was quantified as the percentage introduced to a tank that survived until the study was terminated. All *Anax* larvae either died or metamorphosed prior to the end of the experiment. Thus, their survival was quantified as the percentage introduced to a tank that metamorphosed.

## RESULTS

### *Carbaryl concentration*

In experiment 1, carbaryl concentrations in the tanks two hours after dosing were 0.0 and 1.7 mg/L in the 0 and 2 mg/L treatments, respectively. Carbaryl concentrations to which *Rana sphenoccephala* tadpoles were exposed in the laboratory prior to introduction to the tanks were 0.0 and 1.9 mg/L. In experiment 2, tank concentrations were 0.0, 2.4, and 6.0 mg/L in the 0, 2, and 5 mg/L treatments, respectively. When *R. sphenoccephala* tadpoles were introduced to the tanks 18–24 d after dosing the tanks, there was no carbaryl detected (detection limit = 1  $\mu$ g/L).

### *Response of R. sphenoccephala to the carbaryl-altered environment*

*Survival.*—In experiment 1, tank carbaryl-treatment did not affect survival of *R. sphenoccephala* tadpoles (Table 2; tank, *P* = 0.5879), which averaged 55% (*n* = 48 tanks) but was highly variable among tanks (3–95%). In experiment 2, *R. sphenoccephala* generally survived better in tanks dosed with carbaryl (Table 3; tank, *P* < 0.0001), but the increase in survival was dependent on predation (Fig. 1; Table 3; tank  $\times$  predation, *P* < 0.0001). This interactive effect was most noticeable in tanks containing predaceous *Anax* larvae. When the tanks were not exposed to carbaryl, <1% of *R. sphenoccephala* tadpoles survived to metamorphosis in tanks with *Anax* larvae, whereas 35% of *R. sphenoccephala* tadpoles survived to metamorphosis in tanks without *Anax* larvae. However, when the tanks were exposed to carbaryl, *R. sphenoccephala* survival in tanks containing *Anax* larvae (50% and 61% in the 2 and 5 mg/L treatments, respectively) was similar to survival in tanks not containing *Anax* larvae (52% and 54% in the 2 and 5 mg/L treatments, respectively). This interaction was due to carbaryl-induced death of *Anax* larvae, which are more sensitive to carbaryl than amphibians. *Rana sphenoccephala* survival was generally lower in tanks containing *N. viridescens* than in tanks not containing predators, and the response to *N. viridescens* was unaffected by carbaryl.

*Size at metamorphosis.*—In experiment 1, exposing tanks to carbaryl decreased metamorph size from  $1.12 \pm 0.05$  g to  $0.92 \pm 0.04$  g (Table 2; tank, *P* = 0.0057).

TABLE 2. ANOVA results for effects of the independent variables on *R. sphenoccephala* life-history traits in experiment 1.

Response	Source of variation	df	MS	F	P
Percentage survival ( $\alpha = 0.05$ )	Block	2	0.2852	4.72	0.0142
	Tank	1	0.0180	0.30	0.5879
	Tadpole	1	0.0573	0.95	0.3357
	Intraspecific competition	1	0.1133	1.88	0.1782
	Interspecific competition	1	0.1385	2.29	0.1375
	Error	41	0.0604		
Size ( $\alpha = 0.017$ ) <sup>†</sup>	Block	2	0.1498	2.78	0.0744
	Tank	1	0.4606	8.54	0.0057
	Tadpole	1	0.0006	0.01	0.9172
	Intraspecific competition	1	2.4874	46.14	<0.0001
	Interspecific competition	1	0.7137	13.24	0.0008
	Survival (covariate)	1	0.1112	2.06	0.1589
	Error	39	0.0539		
Percentage metamorphosis ( $\alpha = 0.017$ ) <sup>†</sup>	Block	2	0.0593	1.24	0.2997
	Tank	1	0.2355	4.94	0.0322
	Tadpole	1	0.0013	0.03	0.8691
	Intraspecific competition	1	1.1211	25.53	<0.0001
	Interspecific competition	1	0.4799	10.07	0.0030
	Tank $\times$ intraspecific competition	1	0.2271	4.77	0.0352
	Intra- $\times$ interspecific competition	1	0.7523	15.79	0.0003
	Survival (covariate)	1	0.0326	0.68	0.4135
	Error	38	0.0476		
	Days ( $\alpha = 0.017$ ) <sup>†‡</sup>	Block	2	118.39	1.01
Tank		1	323.41	2.76	0.1048
Tadpole		1	99.70	0.85	0.3621
Intraspecific competition		1	2.21	0.02	0.8914
Interspecific competition		1	312.52	2.67	0.1107
Tank $\times$ interspecific competition		1	409.43	3.50	0.0693
Intra- $\times$ interspecific competition		1	946.95	8.08	0.0071
Survival (covariate)		1	1751.23	14.95	0.0004
Error		38	117.14		

<sup>†</sup> A Bonferroni adjustment of alpha was used to prevent the experiment-wide probability of a Type I error ( $\alpha$ ) from increasing above 0.05. See *Methods*.

<sup>‡</sup> Days refers to the length of the larval period.

In addition, both intraspecific and interspecific competition caused a decrease in metamorph size independent of tank carbaryl-treatment (Table 2; intraspecific competition,  $P < 0.0001$ ,  $1.29 \pm 0.06$  g and  $0.80 \pm 0.04$  g in the 20 and 60 tadpole treatments, respectively; interspecific competition,  $P = 0.0008$ ,  $1.16 \pm 0.06$  g and  $0.90 \pm 0.04$  g in the absence and presence of *P. crucifer*, respectively). In experiment 2, exposing tanks to carbaryl caused *R. sphenoccephala* metamorphs to be smaller, but only if there were *P. crucifer* competitors present (Fig. 2; Table 3; tank  $\times$  interspecific competition,  $P = 0.0140$ ).

*Larval period.*—Tank carbaryl-treatment did not have a statistically significant effect on the length of the larval period, although there is some evidence in experiment 1 that under highly competitive situations exposing the tanks to carbaryl might lengthen the larval period (Fig. 3; Table 2; percentage metamorphosis, tank  $\times$  intraspecific competition,  $P = 0.0352$ ; days to metamorphosis, tank  $\times$  interspecific competition,  $P = 0.0693$ ).

#### *Response of R. sphenoccephala to direct carbaryl exposure*

Exposing tadpoles to carbaryl prior to introduction to the tanks (i.e., tadpole carbaryl-treatment) did not cause any changes in the life-history traits examined in this study (Table 2). In addition, there was no evidence that the effects of tank carbaryl-treatment, intraspecific competition, or interspecific competition were altered by tadpole carbaryl-treatment, and interaction terms containing tadpole treatment were not included in the final models.

#### *Response of R. sphenoccephala to competition and predation*

Competition and predation both influenced *R. sphenoccephala* life-history traits independent of any carbaryl treatments.

*Survival.*—The effect of competition on *R. sphenoccephala* survival was mixed. Neither intra- nor interspecific competition altered *R. sphenoccephala* survival in experiment 1 (Table 2; intraspecific competition,  $P$

TABLE 3. ANOVA results for effects of the independent variables on *R. sphenoccephala* life-history traits in experiment 2.

Response	Source of variation	df	MS	F	P
Percentage survival ( $\alpha = 0.05$ )	Block	2	0.0404	2.08	0.1372
	Tank	2	1.0502	54.13	<0.0001
	Interspecific competition	1	0.3837	19.78	<0.0001
	Predation	2	0.1835	9.46	0.0004
	Tank $\times$ predation	4	0.2679	13.81	<0.0001
	Error	42	0.0194		
Size ( $\alpha = 0.017$ ) <sup>†</sup>	Block	2	0.0252	0.88	0.4265
	Tank	2	0.0915	3.21	0.0581
	Interspecific competition	1	0.1187	4.16	0.0525
	Predation	1	0.2700	9.47	0.0052
	Tank $\times$ interspecific competition	2	0.1460	5.12	0.0140
	Tank $\times$ predation	2	0.0750	2.63	0.0925
	Survival (covariate)	1	0.9910	34.76	<0.0001
	Error	24	0.0285		
Percentage metamorphosis ( $\alpha = 0.017$ ) <sup>†</sup>	Block	2	0.0678	1.06	0.3610
	Tank	2	0.0541	0.84	0.4411
	Interspecific competition	1	0.5263	8.21	0.0078
	Predation	1	0.0511	0.80	0.3797
	Survival (covariate)	1	0.1527	2.38	0.1340
	Error	28	0.0641		
Days ( $\alpha = 0.017$ ) <sup>†‡</sup>	Block	2	4.3208	0.66	0.5246
	Tank	2	6.0081	0.92	0.4111
	Interspecific competition	1	120.1272	18.37	0.0002
	Predation	1	4.3083	0.66	0.4241
	Interspecific competition $\times$ predation	1	47.1013	7.20	0.0123
	Survival (covariate)	1	15.0125	2.30	0.1413
	Error	27	6.5392		

<sup>†</sup> A Bonferroni adjustment of  $\alpha$  was used to prevent the experiment-wide probability of a Type I error ( $\alpha$ ) from increasing above 0.05. See *Methods*.

<sup>‡</sup> Days refers to the length of the larval period.

= 0.1782; interspecific competition,  $P = 0.1375$ ). However, interspecific competition from *P. crucifer* did decrease *R. sphenoccephala* survival from  $44.0 \pm 2.6\%$  to  $27.9 \pm 2.4\%$  in experiment 2 (Table 3; interspecific competition,  $P < 0.0001$ ).

*Size at metamorphosis.*—In experiment 1, both intra- and interspecific competition caused a decrease in metamorph size (Table 2; intraspecific competition,  $P < 0.0001$ ,  $1.29 \pm 0.06$  g and  $0.80 \pm 0.04$  g in the 20 and 60 tadpole treatments, respectively; interspecific competition,  $P = 0.0008$ ,  $1.16 \pm 0.06$  g and  $0.90 \pm 0.04$

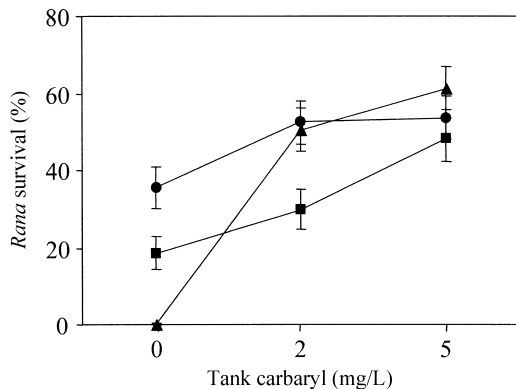


FIG. 1. Exposing tanks to carbaryl generally increased the survival of *Rana sphenoccephala*, although the increase was dependent on predation in the tanks. Key to symbols: solid circle, predators absent; solid square, *Notophthalmus viridescens louisianensis* (central newt) present; solid triangle, *Anax* dragonfly larvae present. Error bars represent  $\pm 1$  SE.

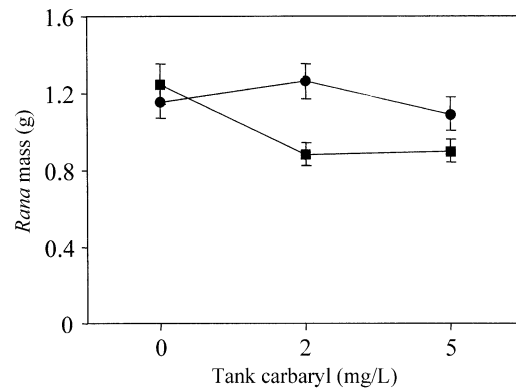


FIG. 2. Exposing tanks to carbaryl resulted in smaller *R. sphenoccephala* metamorphs, but only in the presence of interspecific competition. Key to symbols: solid circle, *Pseudacris crucifer* (spring peeper) absent; solid square, *P. crucifer* present. Error bars represent  $\pm 1$  SE.

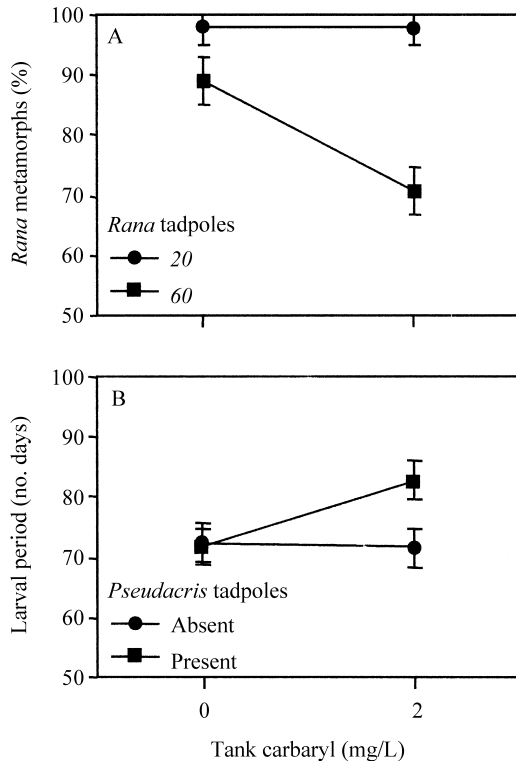


FIG. 3. Although not statistically significant, it appeared that exposing tanks to carbaryl might lengthen the larval period of *R. sphenoccephala* if there was intense (A) intraspecific competition (20 vs. 60 *Rana* tadpoles) or (B) interspecific competition with *Pseudacris* tadpoles. Error bars represent  $\pm 1$  SE.

g in the absence and presence of *P. crucifer*, respectively). In experiment 2, competition again caused a decrease in metamorph size, but only if the tanks had been exposed to carbaryl (Fig. 2; Table 3; tank  $\times$  interspecific competition,  $P = 0.0140$ ). Predation by *N. viridescens* caused a general increase in *R. sphenoccephala* size from  $0.98 \pm 0.04$  g to  $1.18 \pm 0.05$  g (Table 3; predation,  $P = 0.0052$ ).

**Larval period.**—Competition caused an increase in the length of the larval period, which was indicated by a decrease in the percentage of surviving tadpoles that metamorphosed prior to termination of the experiment. In experiment 1,  $98 \pm 2\%$  and  $96 \pm 2\%$  of surviving tadpoles metamorphosed in tanks containing low intraspecific competition or no interspecific competition, respectively. However,  $81 \pm 3\%$  and  $84 \pm 3\%$  of surviving tadpoles metamorphosed in tanks containing high intraspecific competition or containing interspecific competition, respectively (Table 2; intraspecific competition,  $P < 0.0001$ ; interspecific competition,  $P = 0.0030$ ). In addition, the effects of intra- and interspecific competition on the percentage of tadpoles reaching metamorphosis were interdependent (Fig. 4A; Table 2; intra-  $\times$  interspecific competition,  $P = 0.0003$ ). Thus, the effect of intraspecific competition

was greater in the presence of interspecific competitors than in the absence of interspecific competition. In experiment 2, interspecific competition again caused a decrease in the percentage of surviving tadpoles that metamorphosed from  $98 \pm 2\%$  to  $89 \pm 5\%$  (Table 3; interspecific competition,  $P = 0.0078$ ).

Among *R. sphenoccephala* tadpoles that reached metamorphosis prior to termination of the experiments, time to metamorphosis was dependent on the amount of competition present. In experiment 1, time to metamorphosis was dependent on an interaction between intra- and interspecific competition (Fig. 4B; Table 2; intra-  $\times$  interspecific competition,  $P = 0.0071$ ). In the absence of *P. crucifer* competitors, the days required to reach metamorphosis decreased in response to increased intraspecific competition. However, in the presence of *P. crucifer* competitors, the days required to reach metamorphosis increased in response to increased intraspecific competition. In experiment 2, the timing of metamorphosis was dependent on an interaction between interspecific competition and predation (Fig. 5; interspecific competition  $\times$  predation,  $P = 0.0123$ ). Interspecific competition caused a greater increase in the days to metamorphosis in the absence of *N. viridescens* than in the presence of *N. viridescens*.

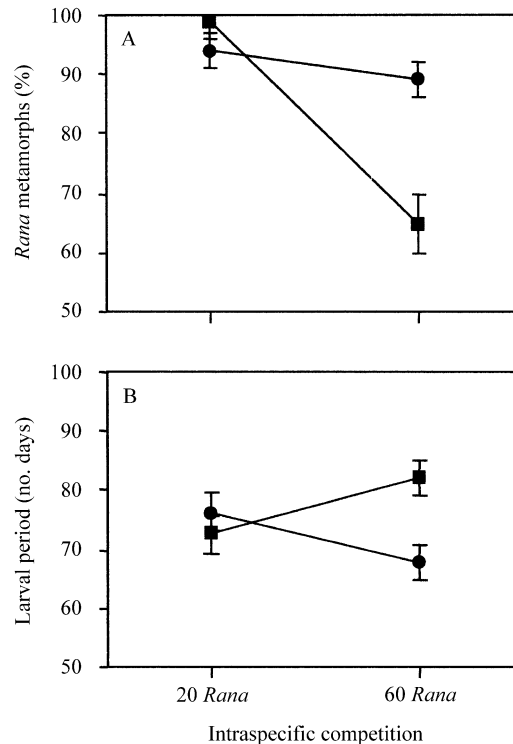


FIG. 4. Intraspecific competition (20 vs. 60 *Rana* tadpoles) and interspecific competition with *P. crucifer* interact to modify (A) the percentage of *Rana* metamorphs and (B) the duration of the larval period. Key to symbols: solid circle, *P. crucifer* absent; solid square, *P. crucifer* present. Error bars represent  $\pm 1$  SE.

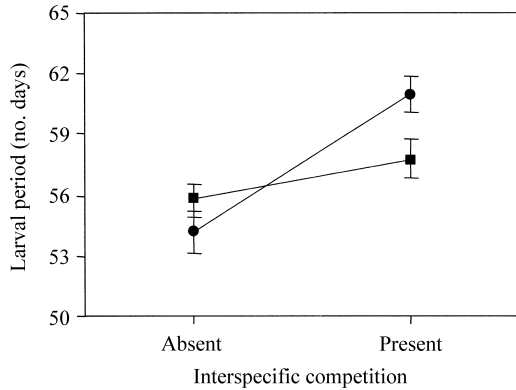


FIG. 5. Interspecific competition from *P. crucifer* caused an increase in the length of the larval period, but the increase was greater in the absence of predaceous *N. viridescens* (solid circles) than in the presence of *N. viridescens* (solid squares). Error bars represent  $\pm 1$  SE.

#### Response of the aquatic community to carbaryl

In an effort to understand which species were involved in mediating observed indirect effects, we examined the effects of carbaryl on components of the aquatic community.

**Predators.**—Carbaryl caused a decrease in the survival of both predators. *Anax* survival decreased from  $31 \pm 4\%$  in control tanks to  $7 \pm 4\%$  and  $0 \pm 0\%$  in the 2 and 5 mg/L tank carbaryl-treatments, respectively (carbaryl,  $F_{2,10} = 15.81$ ,  $P = 0.0008$ ). The difference in *Anax* survival between the control and carbaryl treatments could be accounted for by observed *Anax* mortality within 48 hours of dosing the tanks with carbaryl. *N. viridescens* survival decreased from  $89 \pm 7\%$  in control tanks to  $61 \pm 7\%$  and  $0 \pm 0\%$  in the 2 and 5 mg/L tank carbaryl-treatments, respectively (carbaryl,  $F_{2,10} = 31.54$ ,  $P < 0.0001$ ). No *N. viridescens* mortality was observed at the time the tanks were dosed. Rather, *N. viridescens* in carbaryl-treated tanks slowly became emaciated over time and died several months after the tanks were dosed, apparently due to starvation.

***Pseudacris crucifer*.**—In experiment 1, the effect of carbaryl on *P. crucifer* survival was dependent on the intensity of competition from *R. sphenoccephala* (carbaryl  $\times$  competition,  $F_{1,18} = 5.55$ ,  $P = 0.0301$ ). Carbaryl did not reduce *P. crucifer* survival in tanks with low densities of *R. sphenoccephala* ( $58 \pm 3\%$  and  $59 \pm 3\%$  in the 0 and 2 mg/L carbaryl treatments, respectively), but carbaryl did reduce *P. crucifer* survival in tanks containing high densities of *R. sphenoccephala* ( $62 \pm 3\%$  and  $47 \pm 3\%$  in the 0 and 2 mg/L carbaryl treatments, respectively). In experiment 2, the effect of carbaryl on *P. crucifer* survival was dependent on the presence of predators (Fig. 6; carbaryl  $\times$  predation,  $F_{4,16} = 8.20$ ,  $P = 0.0008$ ). Carbaryl decreased survival in tanks without predators, but increased survival in tanks containing predators. The increase in *P. crucifer* survival in carbaryl-treated tanks containing predators

was presumably due to carbaryl's adverse effect on predator survival.

**Cladocerans.**—Cladoceran abundance changed significantly over the duration of experiment 1 and was dependent on tank carbaryl-treatment (time  $\times$  carbaryl,  $F_{3,126} = 18.51$ ,  $P < 0.0001$ ). In control tanks, cladoceran abundance generally exceeded 30 cladocerans/L. However, in carbaryl-treated tanks, cladoceran abundance dropped to  $0.9 \pm 0.04$  cladocerans/L  $\sim 30$  days after the tanks were dosed (day 64 of the experiment) and was still only  $3.7 \pm 2.6$  cladocerans/L  $\sim 60$  days after the tanks were dosed (day 93 of the experiment). Cladoceran populations were just recovering when the last samples were taken  $\sim 90$  days after the tanks were dosed with carbaryl (day 127;  $20.7 \pm 7.3$  cladocerans/L). Neither the presence of *P. crucifer* nor the number of *R. sphenoccephala* (20 or 60 tadpoles) in the tanks significantly affected cladoceran abundance over time (time  $\times$  intraspecific competition,  $F_{3,126} = 1.49$ ,  $P = 0.2246$ ; time  $\times$  interspecific competition,  $F_{3,126} = 2.62$ ,  $P = 0.0617$ ).

In experiment 2, carbaryl again caused a significant reduction in cladoceran abundance ( $F_{2,46} = 20.87$ ,  $P < 0.0001$ ). Approximately 60 days after the tanks were dosed with carbaryl (day 110), control tanks averaged  $125.7 \pm 22.3$  cladocerans/L while the populations in the 2 and 5 mg/L treatments were  $11.9 \pm 7.1$  and  $26.1 \pm 11.4$  cladocerans/L, respectively. Neither the presence of *P. crucifer* nor the predaceous *Anax* and *N. viridescens* significantly altered the overall abundance of cladocerans (*P. crucifer*,  $F_{1,46} = 0.70$ ,  $P = 0.4066$ ; predation,  $F_{2,46} = 0.25$ ,  $P = 0.7834$ ). However, the presence of predators did change cladoceran species composition. In tanks without predators,  $>90\%$  of the cladocerans were large *Daphnia* (2–3 mm long), while the remaining cladocerans were primarily a mix of *Ceriodaphnia* and *Scapholeberis* (both  $<1$  mm long). However, in tanks containing either *Anax* larvae or *N.*

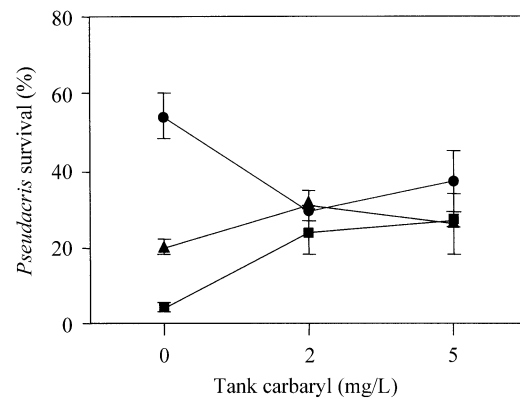


FIG. 6. Effect of carbaryl on survival of *P. crucifer* in the presence and absence of predators. Key to symbols: solid circle, predators absent; solid square, *N. viridescens* present; solid triangle, *Anax* larvae present. Error bars represent  $\pm 1$  SE.

*viridescens*, *Daphnia* were completely absent, and cladoceran populations were dominated by *Ceriodaphnia* and *Scapholeberis*.

**Copepods.**—Copepod abundance changed significantly over the duration of experiment 1 and was dependent on tank carbaryl-treatment (time  $\times$  carbaryl,  $F_{3,126} = 3.40$ ,  $P = 0.0213$ ). Copepod populations in carbaryl-treated tanks were generally larger than in control tanks. However, copepod abundance in carbaryl-treated tanks ( $7.8 \pm 1.9$  copepods/L) was significantly elevated relative to control tanks ( $2.4 \pm 0.4$  copepods/L) at only a single point in time  $\sim 60$  days after the tanks were dosed with carbaryl (day 93;  $F_{1,42} = 10.87$ ,  $P = 0.0020$ ). Neither the presence of *P. crucifer* nor the number of *R. sphenoccephala* (20 or 60 tadpoles) in the tanks significantly changed copepod abundance over time (time  $\times$  intraspecific competition,  $F_{3,126} = 2.07$ ,  $P = 0.1109$ ; time  $\times$  interspecific competition,  $F_{3,126} = 0.30$ ,  $P = 0.8159$ ).

In experiment 2, carbaryl again altered copepod abundance ( $F_{2,46} = 6.62$ ,  $P = 0.0030$ ). Approximately 60 days after the tanks were dosed with carbaryl (day 110), tanks in the 2 mg/L treatment contained significantly larger copepod populations ( $5.6 \pm 0.7$  copepods/L) than either control tanks ( $0.9 \pm 0.3$  copepods/L) or tanks in the 5 mg/L treatment ( $1.3 \pm 0.3$  copepods/L). Neither the presence of *P. crucifer* nor the predaceous *Anax* larvae and *N. viridescens* significantly altered the overall abundance of copepods (*P. crucifer*,  $F_{1,46} = 0.73$ ,  $P = 0.3980$ ; predation,  $F_{2,46} = 2.92$ ,  $P = 0.0638$ ).

**Periphyton.**—In experiment 1, periphyton abundance changed significantly over the duration of the experiment and was dependent on tank carbaryl-treatment (time  $\times$  carbaryl,  $F_{2,84} = 14.36$ ,  $P < 0.0001$ ). Periphyton abundance was consistently greater in control tanks than in tanks treated with 2 mg/L carbaryl. Approximately 60 days after the tanks were dosed (day 93), and about the time *R. sphenoccephala* began to metamorphose, there was three times as much periphyton in control tanks as there was in tanks dosed with 2 mg/L carbaryl ( $0.057 \pm 0.012$   $\mu\text{g}/\text{cm}^2$  and  $0.017 \pm 0.004$   $\mu\text{g}/\text{cm}^2$ , respectively;  $F_{1,42} = 22.14$ ,  $P < 0.0001$ ). In addition, the presence of *P. crucifer* had a significant effect on periphyton abundance over time (time  $\times$  interspecific competition,  $F_{2,84} = 39.94$ ,  $P < 0.0001$ ). Approximately one month after *P. crucifer* were added to the tanks and about the time *R. sphenoccephala* tadpoles were added to the tanks, *P. crucifer* had reduced periphyton abundance to less than half its abundance in tanks without *P. crucifer* (day 64;  $0.098 \pm 0.006$   $\mu\text{g}/\text{cm}^2$  and  $0.245 \pm 0.019$   $\mu\text{g}/\text{cm}^2$ , respectively;  $F_{1,42} = 97.67$ ,  $P < 0.0001$ ). Approximately two months after *P. crucifer* were added to the tanks and after 80% of them had metamorphosed, there was no longer any difference in periphyton abundance between tanks with and without *P. crucifer* (day 93;  $0.028 \pm 0.006$   $\mu\text{g}/\text{cm}^2$  and  $0.040 \pm 0.013$   $\mu\text{g}/\text{cm}^2$ , respectively;  $F_{1,42} =$

$1.75$ ,  $P = 0.1928$ ). The number of *R. sphenoccephala* (20 or 60 tadpoles) in the tanks did not change periphyton abundance over time ( $F_{2,84} = 0.91$ ,  $P = 0.4073$ ).

In experiment 2, carbaryl again caused a significant decrease in periphyton abundance ( $F_{2,46} = 10.25$ ,  $P = 0.0002$ ). Periphyton abundance in carbaryl-treated tanks was significantly less ( $0.28 \pm 0.12$   $\mu\text{g}/\text{L}$  and  $0.21 \pm 0.08$   $\mu\text{g}/\text{L}$  in the 2 mg/L and 5 mg/L treatments, respectively) than in control tanks ( $0.84 \pm 0.36$   $\mu\text{g}/\text{L}$ ). Neither the presence of *P. crucifer* or predaceous *Anax* and *N. viridescens* significantly affected periphyton abundance (*P. crucifer*,  $F_{1,46} = 2.29$ ,  $P = 0.1374$ ; predation,  $F_{2,46} = 3.15$ ,  $P = 0.0523$ ).

**Phytoplankton.**—Phytoplankton abundance changed significantly over the duration of experiment 1 and was dependent on tank carbaryl-treatment (time  $\times$  carbaryl,  $F_{3,126} = 3.27$ ,  $P = 0.0236$ ). Phytoplankton abundance was consistently elevated in tanks dosed with 2 mg/L carbaryl relative to control tanks. Approximately 60 days after the tanks were dosed, and about the time *R. sphenoccephala* began to metamorphose, there was three times more phytoplankton in the tanks dosed with 2 mg/L carbaryl than in control tanks (day 93;  $F_{1,42} = 28.12$ ,  $P < 0.0001$ ;  $0.92 \pm 0.28$   $\mu\text{g}/\text{L}$  and  $0.27 \pm 0.07$   $\mu\text{g}/\text{L}$ , respectively). Neither the presence of *P. crucifer* nor the number of *R. sphenoccephala* (20 or 60 tadpoles) in the tanks changed phytoplankton abundance over time ( $F_{3,126} = 0.27$ ,  $P = 0.8471$  and  $F_{3,126} = 2.50$ ,  $P = 0.0625$ , respectively).

In experiment 2, carbaryl again caused a significant increase in phytoplankton abundance ( $F_{2,46} = 8.21$ ,  $P = 0.0009$ ). Phytoplankton abundances in the 2 mg/L and 5 mg/L treatments were  $3.22 \pm 1.00$   $\mu\text{g}/\text{L}$  and  $2.30 \pm 1.47$   $\mu\text{g}/\text{L}$ , respectively; whereas phytoplankton abundance in the control tanks was  $0.78 \pm 0.38$   $\mu\text{g}/\text{L}$ . Neither the presence of *P. crucifer* nor predaceous *Anax* and *N. viridescens* significantly affected phytoplankton abundance (*P. crucifer*,  $F_{1,46} < 0.01$ ,  $P = 0.9553$ ; predation,  $F_{2,46} = 2.00$ ,  $P = 0.1463$ ).

## DISCUSSION

The results of our study demonstrate that indirect effects of carbaryl on amphibians can be more pronounced, and possibly more important, than direct effects (Fig. 7). Carbaryl-induced changes in the aquatic community (i.e., indirect effects) increased tadpole survival and decreased metamorphosis size by modifying the effects of predation and competition. However, there was a complete lack of statistically significant direct effects. Overall, indirect effects explained a much greater percentage of variability in *R. sphenoccephala* life-history traits than was explained by direct effects (Fig. 7). The results convincingly demonstrate that indirect effects can be very pronounced, and thus the overall effect of a pesticide on an organism's life-history traits will likely reflect the relative strength and direction of both direct and indirect effects.

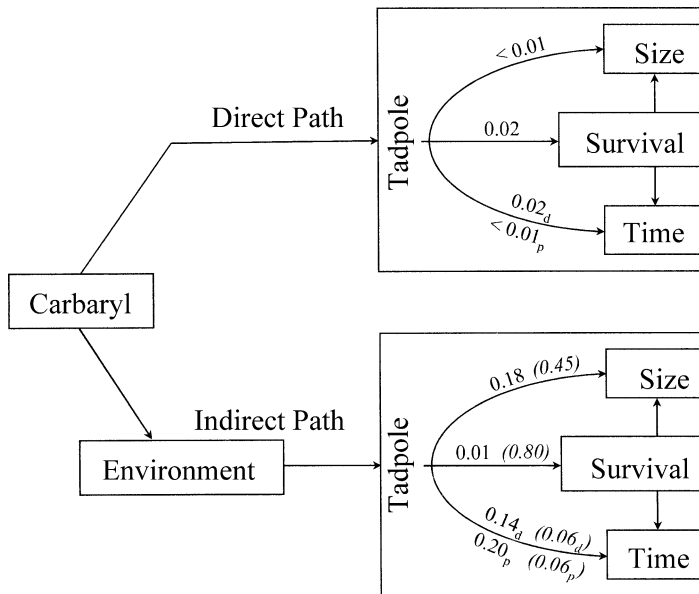


FIG. 7. Diagram of the two routes by which carbaryl can alter the amphibian life-history traits of survival, larval period, and metamorph size. Numbers represent partial regression coefficients of determination ( $r^2$ ) between the direct or indirect effects of carbaryl and each life-history trait. Italicized numbers in parentheses are  $r^2$  values for experiment 2, in which only indirect effects were examined. The arrows pointing to "Time" have  $r^2$  values both above and below the arrows. The  $r^2$  values above the arrows, designated with subscript "d," are for the average days to metamorphosis. The  $r^2$  values below the arrows, designated with a subscript "p," are for the percentage of tadpoles that metamorphosed prior to the conclusion of the experiment.

Metamorph size and larval period, which were used as dependent variables in this study, are important life-history traits for amphibians. Metamorphs that are larger than conspecifics have greater survival and increased reproductive output (Berven and Gill 1983, Howard and Kluge 1985, Smith 1987, Semlitsch et al. 1988). In addition, individuals with shorter larval periods than conspecifics can frequently escape mortality due to pond drying or other unfavorable pond conditions, although metamorphosing quickly to minimize death resulting from unfavorable conditions can come at the cost of smaller size (Berven and Gill 1983). In general, metamorphosis is timed to maximize the survival and growth of an amphibian because they are so closely correlated with future reproductive success and thus fitness (e.g., Ryan and Semlitsch 1998). Any pesticide-induced changes in these life-history traits are potentially very important if we are to understand the role of pesticides in anuran population declines.

#### Indirect mechanisms of action

We attempted to elucidate the mechanisms by which pesticides, such as carbaryl, affect amphibians through alteration of the aquatic community. The aquatic community was limited to potential invertebrate competitors (i.e., zooplankton) and food sources (i.e., periphyton and phytoplankton) of larval anurans, which were monitored in response to carbaryl. Data collected on these components of the community cannot provide cause-and-effect tests of the mechanisms by which changes in the community may affect tadpoles because they were not experimentally manipulated. However, they can provide insights concerning patterns of responses and provide direction for future research that might elucidate the exact mechanisms by which carbaryl indirectly affects tadpoles.

Carbaryl dramatically reduced cladoceran abundance, which is consistent with previous studies that indicate cladocerans are very sensitive to carbaryl (Hanazato and Yasuno 1990, Havens 1994, 1995). Cladocerans are known to alter anuran life histories (Leibold and Wilbur 1992). Periphyton and phytoplankton compete with each other for limited nutrient resources in the mesocosms used in this experiment (Leibold and Wilbur 1992). Cladocerans, by preying on phytoplankton, shift the competitive advantage toward periphyton, which is a primary food source for anurans (Leibold and Wilbur 1992). Thus, we would predict that elimination of cladocerans would be harmful to anurans because it would allow phytoplankton to better compete with periphyton for nutrient resources. The results of our study are consistent with this prediction. In tanks exposed to carbaryl, we observed a decrease in cladoceran abundance, an increase in phytoplankton abundance, and a decrease in periphyton abundance, which resulted in a decrease in the size of *R. sphenoccephala* metamorphs (Fig. 8).

*Pseudacris crucifer* were added to the aquatic community to provide competition for *R. sphenoccephala*. In the absence of predators, *P. crucifer* generally experienced decreased survival in response to carbaryl. Because carbaryl reduced *P. crucifer* survival, we might predict that *R. sphenoccephala* would experience competitive release and thus benefit from being placed in a community previously exposed to carbaryl. However, *R. sphenoccephala* did not do as well in tanks populated with *P. crucifer* that had been previously exposed to carbaryl, even though there were fewer *P. crucifer* present. The most likely explanation for this observation is that the reduction in *P. crucifer* numbers was not enough to significantly decrease competition for periphyton. Thus, the combined effects of com-

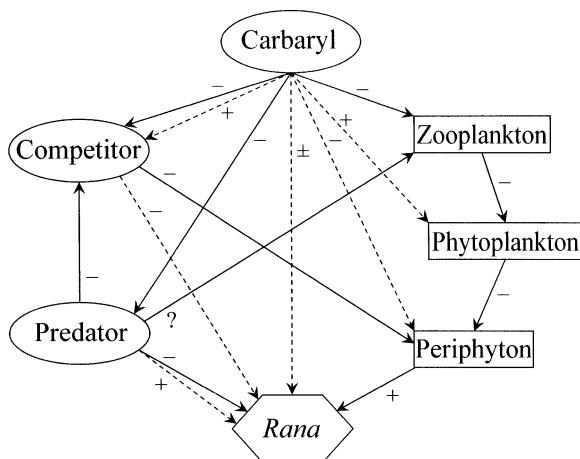


FIG. 8. Interaction diagram of the effects of carbaryl on *R. sphenoccephala* tadpoles in a simple aquatic community. Solid arrows represent direct effects of one component on another. Dashed arrows represent indirect effects of one component on another that are transmitted through other species in the community. Symbols next to each arrow indicate whether a given effect is beneficial (+) or harmful (-).

petition from *P. crucifer* and the carbaryl-induced decrease in periphyton abundance greatly reduced food availability for *R. sphenoccephala* (Fig. 8).

The presence of predators reversed the effect of carbaryl on *P. crucifer* survival. Carbaryl increased *P. crucifer* survival if predators were present in the tanks. This was particularly obvious in tanks containing predaceous *Anax*. The *Anax* were killed almost immediately by carbaryl thus releasing *P. crucifer* from any predatory threat and leading to an increase in survival, which increased competitive pressure on *R. sphenoccephala*. Modification of the effects of carbaryl on *P. crucifer* by predaceous *N. viridescens* was not as obvious, probably for two reasons. First, *N. viridescens* are not as sensitive to carbaryl as *Anax* larvae and thus generally survived exposure to carbaryl. Second, *N. viridescens* are gape-limited predators that only affect *P. crucifer* for a short period when *P. crucifer* tadpoles are relatively small.

Based on the effects of carbaryl on cladocerans and *P. crucifer*, there are two basic pathways by which carbaryl affects periphyton abundance and thus the competitiveness of the environment for *R. sphenoccephala* (Fig. 8). First, carbaryl eliminated cladocerans from the community, which ultimately led to decreased food availability for *R. sphenoccephala* by changing the competitive dynamics between phytoplankton and periphyton. Second, carbaryl altered the number of competitors competing for this food. Carbaryl caused some direct mortality of *P. crucifer*, which would decrease competition for food resources. However, in the presence of predators, the overall effect of carbaryl was to increase competition for food resources by eliminating predators that would have removed some of the com-

petitors. Thus, the effect of carbaryl on *R. sphenoccephala* is dependent on the relative toxicity of carbaryl to a variety of other species in the community and how carbaryl-induced changes in the abundance of these species affect *R. sphenoccephala*'s food resources.

Carbaryl also caused shifts in predatory pressure on *R. sphenoccephala* (Fig. 8). *Rana sphenoccephala* survival increased in response to carbaryl regardless of whether there were predators present or not. However, the increase in survival was much greater in tanks containing predaceous *Anax* than in tanks without predators or in tanks containing predaceous *N. viridescens*. The cause of this shift was due primarily to the fact that carbaryl is very toxic to *Anax* larvae. Carbaryl completely eliminated *Anax* from the tanks, thus creating predator-free communities and resulting in *R. sphenoccephala* survival that was comparable to survival in tanks that had never contained predators.

Although carbaryl also affected the survival of *N. viridescens* (Fig. 8), the survival of *N. viridescens* had little effect on *R. sphenoccephala* survival. Two observations are significant in explaining the lack of a relationship between *N. viridescens* survival and *R. sphenoccephala* life-history traits. First, carbaryl did not immediately kill *N. viridescens*. Rather, over several months the *N. viridescens* in carbaryl-treated tanks became emaciated and eventually died. By the time the *N. viridescens* in carbaryl-treated tanks began to die, the *R. sphenoccephala* were too large to be preyed upon by gape-limited *N. viridescens*. Second, the deaths of *N. viridescens* do not appear to be due to the direct effects of carbaryl. Rather, it appears the *N. viridescens* starved to death due to carbaryl-induced changes in the zooplankton community. In tanks dosed with carbaryl, there were few cladocerans that could be used as an alternative food source to tadpoles (either *P. crucifer* or *R. sphenoccephala*), which were now too large to be eaten. The importance of the larger cladocerans as a food source for the *N. viridescens* is demonstrated by the shift in cladoceran species composition in response to predation. In tanks without predators, the cladoceran community was composed almost entirely of large *Daphnia* species. However, in tanks containing predators, the large *Daphnia* species almost completely disappeared and were replaced by smaller *Ceriodaphnia* and *Scapholeberis* species.

#### Differences among studies

The results of our experiment differed from the results of previous mesocosm experiments, which indicated that anurans could experience seemingly beneficial shifts in life-history traits in response to carbaryl including shorter larval periods (Boone et al. 2001, Boone and Semlitsch 2002) and larger size at metamorphosis (Boone and Semlitsch 2001). The most likely explanation for this difference in outcomes is the lag between dosing the tanks with carbaryl and introducing *R. sphenoccephala* tadpoles into the tanks. In all

previous studies cited above, the anurans were a part of the aquatic community when it was exposed to carbaryl. In our study, the *R. sphenoccephala* were not added to the aquatic community until 18–24 d after the community was dosed with carbaryl. Carbaryl is known to cause a temporary increase in periphyton abundance before periphyton abundance decreases below levels present in control tanks (Mills 2002; M. Boone, *personal communication*). Anurans in previous studies would have been able to take advantage of this temporary increase in food availability. In this study, *R. sphenoccephala* were not added until after periphyton abundance would already have peaked and had dropped below levels found in control tanks.

The differences between our study and previous studies point to a fundamentally important concept related to toxicological research. Many amphibians are terrestrial as adults and only return to aquatic habitats to reproduce. In addition, amphibian species often differ in the phenology of reproduction and in the length of the larval period. These species differences can result in pesticides having differential effects on both the species and on community dynamics. We need to understand not only the effects of pesticides while the tadpoles are present, but also how pesticide contamination prior to amphibian reproduction can affect amphibian larvae due to pesticide induced changes in the community (even if the pesticide is no longer present). This is an avenue of research that to our knowledge has received very little attention.

### Conclusions

Two overarching conclusions can be drawn from the results of this study. First, accurate predictions about the effects of pesticides on amphibian populations must be based on an evaluation of both direct and indirect pathways. This study clearly demonstrates that pesticide induced changes in the community can result in changes in important amphibian life-history traits that would not be detected by more traditional toxicological tests in the laboratory. Second, accurately predicting the indirect effects of pesticides on amphibian populations is dependent on a thorough knowledge of both ecotoxicology and ecology. Most of the indirect effects could be predicted based on known species interactions and the relative toxicity of the pesticide to each individual species. Thus, there is a need for an intensive collaborative effort between ecologists who understand the population and community dynamics of an ecological system and ecotoxicologists who understand how pesticides act in the environment and who have a wealth of knowledge concerning the direct effects of pesticides (Hansen and Johnson 1999). Indeed, integrating the approaches, concepts, and theories of ecology and ecotoxicology will be a productive necessity if we wish to understand the true impact of pesticides on amphibian populations.

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