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INTERACTIONS OF AN INSECTICIDE WITH COMPETITION AND POND DRYING IN AMPHIBIAN COMMUNITIES

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Abstract. Amphibian populations are often imbedded in agricultural landscapes. Therefore the potential for contamination of their habitat is considerable. Our study examined the effects of an insecticide (carbaryl, a neurotoxin), on larval amphibian communities experiencing natural stresses of competition for resources, predation, and pond drying. In a set of experimental ponds, tadpoles of three anuran species (southern leopard frog [*Rana sphenocephala*], plains leopard frog [*R. blairi*], and the Woodhouse's toad [*Bufo woodhousii*]) were added to 1000-L ponds containing leaf litter, plankton, two newts (*Notophthalmus viridescens*), and four overwintered green frog (*R. clamitans*) tadpoles. We manipulated the overall tadpole density (low or high), pond hydroperiod (constant or drying), and chemical exposure (0, 3.5, 5.0, or 7.0 mg/L carbaryl) of the ponds. We measured mass, time, and survival to metamorphosis to determine treatment effects. Carbaryl positively affected Woodhouse's toad survival, although it had a negligible effect on both leopard frog species. Tadpole density interacted with the chemical treatment: proportionately more Woodhouse's toads survived to metamorphosis in high-density environments than in low-density or control environments. Greater survival may be an indirect effect of increased algal food resources from carbaryl exposure. Most newts lost mass over the course of the experiment, although ponds with drying hydroperiods and high anuran density were the least favorable environments. Overwintered green frogs exposed to carbaryl had longer larval periods on average than did green frogs in control ponds. Our study demonstrated that even sublethal, short-lived contaminants can alter natural communities in ways that cannot be predicted from simple, one-factor studies.

Key words: amphibian decline; anuran; Bufo woodhousii; *contaminant; interactive effects;* Notophthalmus viridescens; Rana blairi; Rana clamitans; Rana sphenocephala; *tadpoles.*

INTRODUCTION

Widespread use of chemicals for insect control likely means many nontarget populations will be exposed to contaminants. The effect contaminants have on nontarget wildlife is generally unclear although they may influence both community dynamics and biodiversity. Each year 540 × 10^6 kg of pesticides are sold, 70% of which are used for agricultural purposes (Ramade 1988). Therefore, the potential for communities to experience exposure is notable. Many amphibian species breed in ephemeral and permanent ponds imbedded within or around agricultural areas and may be exposed to contaminants directly in their habitat (Sanders 1970) in both the aquatic or terrestrial life stages. Because field concentrations are often not great enough to cause direct mortality, the consequences of sublethal levels need to be examined and may be more relevant in explaining population declines (Bridges 1997) where alterations in the food web may have a greater consequence than direct chemical effects.

Under the Federal Insecticide, Fungicide, and Rodenticide Act (1972) all chemicals are tested to determine what levels are safe to humans and nontarget wildlife (Jenkins et al. 1989, Rand 1995). Because of financial and time constraints, acute laboratory studies employing a few model organisms are often used to determine acceptable standards. The majority of these studies use LC50s (i.e., the lethal concentration causing 50% mortality) to determine safety limits (Rand 1995) because this type of mortality-based data are relatively pellucid. Laboratory studies looking at subtle, sublethal effects (e.g., behavioral changes) are becoming more prevalent (Little et al. 1985, Bridges 1997), although extrapolating the consequences of these effects in the field can be difficult. In general, there has been a movement away from large-scale outdoor toxicological experiments, due to high cost and time commitments, as well as increased variability that makes final results difficult to decipher. Though large-scale studies can show high variability among treatments and replicates, they have a high degree of realism lacking in laboratory studies (Diamond 1986). Use of outdoor cattle-tank ponds for larval amphibians, particularly in ecological studies, has been an effective method to bridge the gap between large-scale natural experiments and laboratory studies by combining elements of both: realistic exposure to physical factors with maximum control of

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treatments and adequate replication (Rowe and Dunson 1994).

Most studies, whether in the laboratory or field, look at a chemical's effect on individuals and populations in isolation of natural stresses known to influence communities when the presence of a contaminant should be viewed as an additional stress in an environment. Our premise is that natural biotic and abiotic factors are essential components of ecotoxicological studies if the goal of research is to determine the impact of a chemical at the community level in natural environments. Factors like tadpole density and predator presence (Wilbur 1980, 1987, Morin 1983, Scott 1990), changes in pond hydroperiod (Semlitsch 1987, Wilbur 1987, Semlitsch and Wilbur 1988, Pechmann et al. 1989, Semlitsch et al. 1996, Wellborn et al. 1996), and alterations in pH (Warner et al. 1991, Sadinski and Dunson 1992, Kiesecker 1996) are known to influence population and community dynamics of amphibians and therefore may influence the potency of a contaminant. Under some environmental conditions, the presence of a chemical may not alter community processes, while in other conditions its effect may be profound. Subtle interactions caused by a chemical that do not directly induce mortality could indirectly lead to mortality. For instance, in a study on the effect of an organophosphate (a neurotoxin) on fiddler crabs, the contaminant had an indirect effect on survival by altering predator avoidance behaviors (Ward and Busch 1976, Ward et al. 1976). In this case, although the chemical did not directly cause mortality, it made individuals more vulnerable to mortality via predation. If a chemical caused subtle changes, the outcome on the community may be substantial over time. If a chemical increased the length of an amphibian's larval period by slowing development as some studies have shown (e.g., Fioramonti et al. 1997), then tadpoles could suffer indirect mortality due to pond desiccation or predation. Such outcomes may be superficially ascribed to natural consequences of high density, short hydroperiod, or high density; however, the cause of mortality would be an indirect, anthropogenic factor. The objectives of our study were to determine how realistic levels of the insecticide carbaryl affect natural communities of amphibian species in terms of body size, length of larval period, and survival to metamorphosis when exposed to carbaryl early in the larval period and when experiencing natural stresses of predation, competition, and pond drying.

METHODS

Chemical contaminant

Carbaryl (1-naphthyl N-methyl carbamate) is a widespread, short-lived (hours to weeks) carbamate insecticide that inhibits acetylcholinesterase (Cox 1993), thereby disrupting nervous system function. Carbaryl affects organisms similarly to other carbamate and organophosphate insecticides and is representative of these classes of pesticides (Rand 1995). Environmental levels of this chemical are known to affect amphibian behavior, although vulnerability to pesticides is known to vary widely among species and within populations (Power et al. 1989, Bridges 1999, Bridges and Semlitsch 2000). Therefore, exposure may have consequences that outlive the direct effects of the contaminant (Bridges 1997, 2000).

Carbaryl can enter aquatic systems by direct application or run-off from agricultural lands. Expected environmental field concentrations have been estimated up to 4.8 mg/L (Norris et al. 1983). However, because application rates can vary, environmental concentrations could be greater for short periods of time (Peterson et al. 1994). The persistence and potency of carbaryl varies with soil type, amount of rainfall, pH, temperature, ultraviolet radiation, and environmental complexity (Wauchope and Haque 1973, Gibbs et al. 1984, Zaga et al. 1998, Boone and Bridges 1999). Carbaryl affects anuran feeding and swimming behavior at sublethal levels (2.0 mg/L in Marian et al. [1983], 3.5 mg/L in Bridges [1997]) that are consistent with field levels. Bridges (1997) acknowledged that there was a slight recovery in activity after exposure to carbaryl, although tadpole swimming performance (i.e., tadpole sprint speed and distance) did not recover even 48 h after exposure indicating that the effects of direct exposure may persist after chemical levels are negligible.

Species collection

We collected four anuran species (Woodhouse's toad [*Bufo woodhousii*], green frog [*Rana clamitans*], plains leopard frog [*R. blairi*], and southern leopard frog [*R. sphenocephala*]) and one caudate species (red-spotted newt [*Notophthalmus viridescens*]) in central Missouri, USA in April 1998. We used three to five egg masses of the plains leopard frog (Boonville, Howard County), the southern leopard frog (Jefferson City, Callaway County), and the Woodhouse's toad (Jefferson City) to stock ponds. Eggs hatched in the laboratory at 23–25°C. We dip-netted for adult newts and overwintered green frog tadpoles at the Baskett Wildlife Area (Ashland, Boone County) to use as predators and competitors, respectively.

Experimental design

We assembled amphibian communities in 50 polyethylene ponds (1.85 m in diameter; 1480 L volume) to which we added 1000 L of well water, 1 kg of leaf litter, and plankton from natural ponds (500 mL of plankton per pond at six different times) in early April 1998. Screen-mesh lids covered each pond to exclude unwanted predators and anuran colonists. The ponds were located in a fenced area at the University of Missouri-Columbia Research Park (Columbia, Boone County).

We experimentally manipulated three factors in a fully crossed design with three replicates: total initial larval density (low [80] or high [240]), pond hydroperiod (constant or drying), and chemical concentration (absent, 3.5 mg/L, 5.0 mg/L, or 7.0 mg/L carbaryl). We mixed multiple egg masses within each anuran species before adding them to the ponds to homogenize genetic differences among families. We assigned tadpoles to the ponds randomly when they were free-swimming (Gosner stage 25; Gosner 1960) at realistic field densities (14–4238 per 1000 L; e.g., Morin 1983, Petranka 1989). Tadpoles of each species were added to the ponds at a constant low or high density based upon availability in the field: 23 or 69 southern leopard frogs on 15 April; 45 or 135 plains leopard frogs on 17 April; and 12 or 36 Woodhouse's toads on 24 April. All ponds contained the same ''background community'' of two adult newts (sex ratio 1:1; male, 2.18 ± 0.09 g; female, 2.77 ± 0.21 g [mean ± 1 SE]) and four overwintered green frog tadpoles (two small, 1.18 ± 0.03 g; two large, 3.38 ± 0.11 g) that were added on 22 April and 24 April, respectively.

We added carbaryl as liquid Sevin (Ortho, San Ramon, California, USA), which contains 21.3% carbaryl, on 1 May (experimental day 0) after all individuals had time to acclimate to the pond environment by diluting it in ~5 L of pond water and pouring it evenly across the pond surface with a water-sprinkling can. We did not stir ponds to minimize the disturbance to the community and because direct application in a natural environment could occur similarly. We chose chemical levels based on expected environmental concentrations and followed a 70% dilution series: 3.5 mg/L (16.43 g/1000 L), 5.0 mg/L (23.47 g/1000 L), and 7.0 mg/L (32.86 g/1000 L). The low and medium levels were near measured or expected environmental levels (Norris et al. 1983, Peterson et al. 1994), and the high level was a ''worst-case'' scenerio that is near the LC50 for some anuran species (Boone and Bridges 1999, Bridges 1999). At the time of chemical addition, water temperature was 22°C and pond pH was 8.5. Results from high performance liquid chromatography (HPLC) analysis (conducted at Mississippi State Chemical Laboratory) indicated that carbaryl had a half-life of ~4 d in our ponds. In drying treatments, water level was lowered with a movable standpipe every third day for a total of 77 d, following the drying regime of a natural pond (Semlitsch 1987). We searched ponds daily for dead newts or tadpoles that might have been killed by carbaryl application.

Response variables and statistical analyses

We searched ponds daily for metamorphs (defined by the emergence of at least one forelimb [stage 42, Gosner 1960]). We removed metamorphs from ponds and held them separately in the laboratory until tail resorption at which time they were weighed to the nearest milligram. Over days 76–77 (9–10 July 1998), we ended the experiment because the water level in drying treatments was at a minimal depth of 3 cm. We drained ponds and thoroughly searched for any remaining tadpoles or metamorphs. Many ephemeral ponds in the area had dried by this date, suggesting that our experiment mimicked natural conditions. Many of our ponds still contained leopard frog tadpoles (but no toad tadpoles) at the close of the experiment, although southern and plains leopard frog tadpoles could not be distinguished. Only 28 of the 50 ponds still held newts at the end of the experiment. We observed newts actively attempting to escape during the last month of the experiment. We suspected that many newts crawled out of the ponds during this time because ponds were searched daily and, likely, any mortalities would have been noticed. However, tadpole mortality occurred earlier in the larval period before tadpoles outgrew the gape of newts, so any escapes of newts toward the end of the study should not have affected tadpole mortality.

Length of the larval period (days to metamorphosis), body size (mass) at metamorphosis, proportion surviving to metamorphosis, and total pond survival (including both metamorphs and tadpoles) were used to measure the response of each anuran species to treatments. We tested the effects of density, hydroperiod, chemical, and their interaction on these responses by species using analyses of variance (ANOVA; SAS Institute 1988). The total number of green frogs that metamorphosed or survived to the end of the experiment often had a significant effect, therefore we used it as a covariate in all analyses. We did not use the number of newts in each pond (which differed after a few individuals died, apparently from chemical exposure early in the study) as a covariate because it was never significant. For mass and days to metamorphosis, we used survival to metamorphosis as a covariate because it was often significant and strongly affected these responses.

For the Woodhouse's toads, very few or no individuals survived in chemical controls, therefore we could not test the density by chemical, chemical by hydroperiod, or density by chemical by hydroperiod interactions for mass or days to metamorphosis (because of missing cells). In these cases, we tested only the effects of density, hydroperiod, chemical, and the density by hydroperiod interactions. In all other cases, we used the full model in analyses for anurans. Proportion surviving to metamorphosis and total proportion surviving were angularly transformed; mass at metamorphosis and days to metamorphosis were log transformed before analysis to normalize the data (Snedecor and Cochran 1980). Pairwise comparisons were performed using Scheffé's multiple comparison tests.

We determined the response of newts to chemical, initial tadpole density, and hydroperiod treatments with ANOVA. We weighed newts at the beginning and end of the experiment to determine their change in mass. As well, we found newt larvae in some ponds and determined the percent of ponds within a treatment that contained larvae to test for main treatment effects. Percent reproduction in treatments was angularly transformed. Although we found four dead newts 10–11 d after chemical exposure, the newts had spent 18 d in the ponds prior to chemical exposure and may have reproduced during that time. For this reason, we did not exclude any ponds from the analysis on newt reproduction even though in one case, both newts died, presumably from carbaryl exposure. Interactions were not significant in preliminary analyses, therefore they were removed to conserve degrees of freedom. We also tested for correlations between the change in newt mass and survival to metamorphosis for each species.

RESULTS

Woodhouse's toads.—Toads in high-density ponds had a greater probability of survival to metamorphosis ($F_{1,33} = 8.09$, $P = 0.0053$), but a smaller mass at metamorphosis ($F_{1,20} = 43.61$, $P < 0.0001$) than those in low-density environments. Carbaryl exposure had a positive effect on survival to metamorphosis ($F_{1,33} = 19.99$, $P < 0.0001$) with survival increasing from essentially zero in control ponds to up to 30% in ponds with the highest carbaryl exposure (Fig. 1A). A significant chemical by density interaction showed that the biotic and chemical environment altered survival at metamorphosis ($F_{3,33} = 5.17$, $P = 0.0049$) with individuals in high-density ponds showing greater survival than those in low-density ponds, particularly at the highest carbaryl level (Fig. 1B). Mass at metamorphosis showed a positive trend from carbaryl exposure ($F_{3,20} = 2.82$, $P = 0.0650$) with all chemical ponds producing larger metamorphs (Fig. 1C).

Southern and plains leopard frogs.—Tadpole density did not change the probability of metamorphosis for the southern leopard frog ($F_{1,32} = 1.43$, $P = 0.2407$), although there was a trend of greater survival for the plains leopard frog in low-density ponds ($F_{1,32} = 3.70$, $P = 0.0634$). High density did increase total proportion of tadpoles and metamorphs surviving to metamorphosis (with the southern and plains leopard frogs considered together; $F_{1,32} = 60.46$, $P < 0.0001$) compared to low-density ponds. For both leopard frog species, high density led to longer larval periods (southern, $F_{1,28} = 39.44$, $P < 0.0001$; plains, $F_{1,28} = 47.36$, $P < 0.0001$) and smaller mass at metamorphosis (southern, $F_{1,28} = 191.90$, $P < 0.0001$; plains, $F_{1,28} = 314.60$, $P < 0.0001$). Constant water depth also resulted in slightly larger size at metamorphosis ($F_{1,28} = 4.54$, $P = 0.0420$) for plains leopard frogs. The length of the larval period of the plains leopard frog was generally shorter in carbaryl treatments ($F_{3,28} = 3.09$, $P = 0.0430$) than controls (Fig. 2). In general, the carbaryl treatment did not have a significant or profound influence on either leopard frog species (Fig. 3), nor did it interact with natural stresses of density or hydroperiod.

Green frogs.—One overwintered green frog tadpole died in the 7.0 mg/L carbaryl treatment 10 d after exposure. High tadpole density resulted in lower mass at metamorphosis ($F_{1,32} = 58.32$, $P < 0.0001$), shorter larval periods ($F_{1,32} = 5.04$, $P = 0.0317$), and reduced survival to metamorphosis ($F_{1,32} = 5.08$, $P = 0.0310$). Carbaryl exposure had a significant effect on days to metamorphosis ($F_{3,32} = 5.44$, $P = 0.0039$), with tadpoles in chemical treatments generally having longer larval periods (Fig. 4); however, even with longer lar-

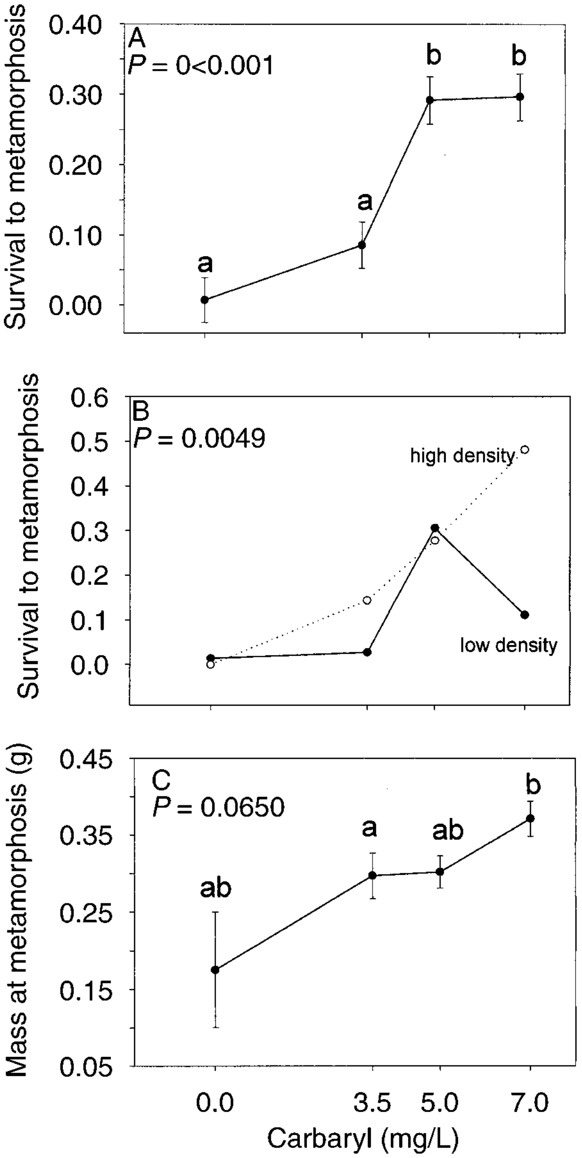

FIG. 1. Proportion of Woodhouse's toads (*Bufo woodhousii*) (A) surviving to metamorphosis across chemical treatments and (B) surviving to metamorphosis in low- and high-density ponds across chemical treatments, and (C) mass at metamorphosis across chemical treatments with proportion survival used as a covariate in experimental ponds at the University of Missouri–Columbia Research Park, Columbia, Missouri, USA. Plotted values are least-square means ± 1 SE. Different letters indicate significant differences among treatments according to Scheffé's multiple comparison tests.

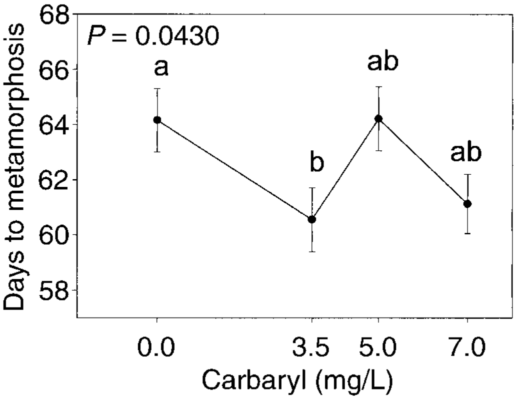

FIG. 2. Days to metamorphosis of the plains leopard frog (*Rana blairi*) across chemical treatments. Plotted values are least-square means ± 1 SE. Differing letters indicate significant differences among treatments according to Scheffé's multiple comparison tests.

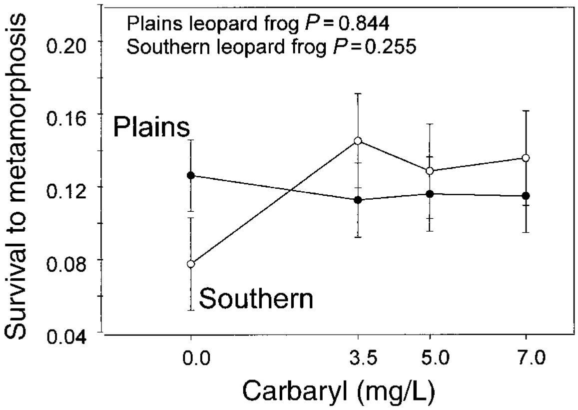

FIG. 3. Proportion surviving to metamorphosis for plains leopard frog (*Rana blairi*) and southern leopard frog (*R. sphenocephala*) across chemical treatments. Plotted values are least-square means ± 1 SE.

val periods, individuals were still slightly smaller at metamorphosis ($F_{3,32}$ = 0.54, P = 0.6601) than individuals leaving control ponds.

Red-spotted newts.—A total of four newts died 10–11 d after the addition of carbaryl in three ponds (4% of all newts): three at the highest concentration of 7.0 mg/L (in two ponds; two in a low-density pond and one in a high-density pond) and one at 5.0 mg/L carbaryl (at high density). At the end of the experiment, 28 ponds still contained newts; however, we found no newts in ponds with high chemical exposure (7.0 mg/L carbaryl). We believe these newts escaped the ponds because ponds were checked daily and no newts, besides those mentioned above, were found dead. Remaining newts weighed an average of 1.86 ± 0.06 g (mean ± 1 SE); overall, newts lost an average of 0.77 ± 0.11 g or 29% of their initial body mass over the 77 d of the experiment.

Hydroperiod had a negative effect on mass for newts ($F_{1,16}$ = 9.00, P = 0.0085); however, the effect of hydroperiod was mediated by the tadpole density ($F_{1,16}$ = 5.00, P = 0.0399) and to a lesser extent by the chemical treatment ($F_{2,16}$ = 3.11, P = 0.0721). Newts in drying ponds (−1.10 ± 0.14 g) lost 2.8 times as much mass on average as newts in ponds with a constant hydroperiod (−0.39 ± 0.17 g). Tadpole density mediated this effect, with newts in a constant environment nearly maintaining their mass at high density and newts in drying ponds losing more mass in high-density ponds than low-density ponds (Fig. 5A). Across chemical treatments, newts lost more mass in low-density ponds than in high-density ponds (Fig. 5B). In general, there was no effect of carbaryl ($F_{2,16}$ = 1.19, P = 0.3294) on newt mass, although generally newts weighed less in chemical treatment ponds (control, −0.59 ± 0.13; 3.5 mg/L, −0.90 ± 0.16; 5.0 mg/L, −0.74 ± 0.22).

We found newt larvae in 32% of the ponds. Presence or absence of reproduction was influenced only by tadpole density ($F_{8,10}$ = 8.82, P = 0.014) with newt larvae 2.2 times more likely in high-density than low-density ponds. Although the chemical treatment was not a significant factor influencing presence or absence of newt larvae ($F_{8,10}$ = 2.17, P = 0.1542), chemically treated ponds were less likely on average to contain newt larvae (47% of control, 25% of 3.5 mg/L, 18% of 5.0 mg/L, and 36% of 7.0 mg/L ponds contained newt larvae). Change in mass of newts was not significantly correlated with survival to metamorphosis for any anuran species (Woodhouse's toad, r^2 = −0.277, P = 0.1706; southern leopard frog, r^2 = 0.171, P = 0.4026; plains leopard frog, r^2 = −0.136, P = 0.5072).

DISCUSSION

The effect of a chemical contaminant on an amphibian population already experiencing natural stresses such as drying environments, competition, and predation pressure has not been previously documented

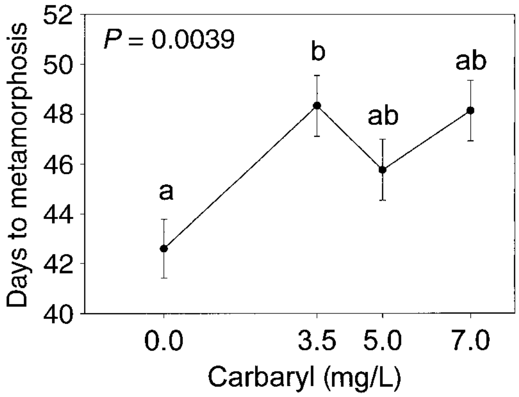

FIG. 4. Days to metamorphosis of the overwintered green frogs (*Rana clamitans*) across chemical treatments. Plotted values are least-square means ± 1 SE. Differing letters indicate significant differences among treatments according to Scheffé's multiple comparison tests.

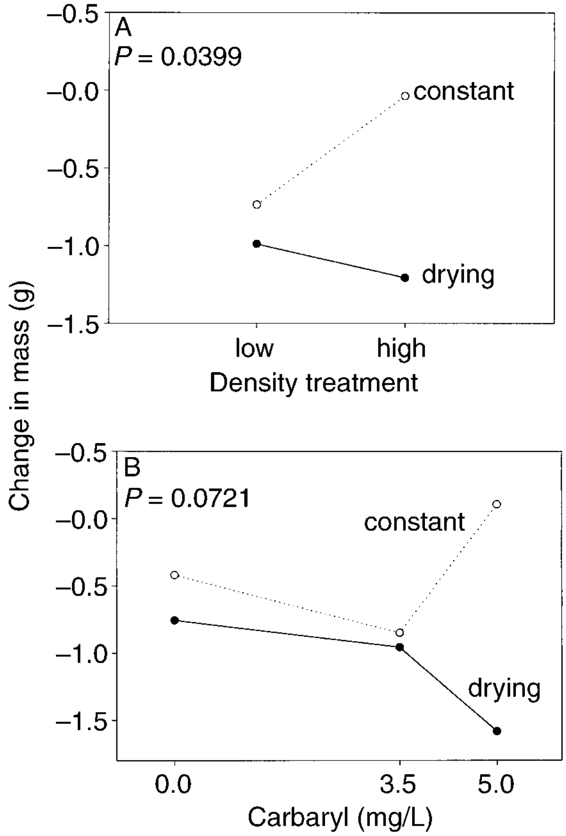

FIG. 5. Change in mass of red-spotted newts (*Notophthalmus viridescens*) from the beginning to the end of the study in ponds with drying and constant hydroperiods (A) across density levels and (B) across chemical treatments. No newts were recovered at the end of the experiment in ponds exposed to 7.0 mg/L carbaryl. Plotted values are least-square means.

and is generally unknown. Such an effect is presumed trivial based on traditional studies (e.g., LC50s) conducted in the laboratory. Traditional mortality studies would suggest that realistic levels of carbaryl that are currently applied should not influence amphibian abundance because expected levels are lower than LC50s for anurans (Bridges 1997, 1999). Sublethal tests with carbaryl, however, suggest that effects of exposure may have important consequences for amphibian populations. For instance, Bridges (1997) showed that swimming and feeding behavior were reduced by carbaryl exposure and that there was no recovery after 48 h in fresh water; this study suggested that carbaryl exposure could lead to lengthened larval periods, increased mass at metamorphosis, and potentially greater mortality by increasing the amount of time tadpoles were vulnerable to aquatic predators (Bridges 1997). Our study, however, indicated the short-term effects of carbaryl exposure on tadpoles that influence behavior are either not significant or are less significant than natural stresses like larval density. Similar cattle tank studies designed to test for direct and indirect effects of carbaryl exposure have shown that carbaryl's indirect effect on the food web has the greatest impact on the community (N. Mills, *personal communication*). Neither mortality nor behavior studies would necessarily predict the outcome of our experiment where in some cases species were either not influenced or were positively affected by exposure to carbaryl. Even though carbaryl is short-lived in the environment, it does appear to indirectly affect the population dynamics of the Woodhouse's toad. Our experiment demonstrated that chemical exposure in the larval environment can alter species abundances in ways that are unexpected based on laboratory studies.

Effects of carbaryl alone

Our study suggests that both leopard frog species may be less affected by environmental levels of carbaryl than Woodhouse's toads. Amphibian species are expected to be differentially affected by any stress, including a chemical contaminant, based on differences in competitive ability, vulnerability to predation, or length of the larval period (Wilbur 1980, Warner et al. 1993, Fioramonti et al. 1997, Bridges 2000). Toads showed a dramatic increase in survival with carbaryl exposure, whereas leopard frog survival was essentially the same across chemical levels even though 7.0 mg/L of carbaryl would be expected to induce some mortality based on LC50 data (Boone and Bridges 1999, Bridges 1999). The increase in toad survival could have resulted from decreased predation by newts exposed to carbaryl, or more likely, a competitive release from zooplankton in the presence of carbaryl (discussed below, *Interaction of carbaryl with natural stresses*). Different responses to carbaryl may stem from differences in tolerance (Mayer and Ellersieck 1986, Bridges 1999) or length of the larval period (Werner 1986, Boone and Semlitsch 2001). As well, the three anuran species in our study came from the floodplains of the Missouri River in agricultural areas. These areas have likely been exposed to contaminants in the past, and species may have been selected for some chemical tolerance (Bridges 1999). Our study may have essentially tested the effect of a contaminant on a relatively resistant species with a history of exposure, as Bridges and Semlitsch (2000) suggested in their study with the southern leopard frog (*R. sphenocephala*) from the same flooded agricultural field as our experiment.

Three of the species in our study (*R. sphenocephala*, *R. blairi*, and *B. woodhousii*) showed no direct negative effect when exposed to carbaryl, although other experiments have shown that carbaryl reduces survival of some species at 3.5 and 7.0 mg/L (e.g., gray treefrogs [*Hyla versicolor*], Woodhouse's toads [*B. woodhousii*]; Boone and Semlitsch 2001). Differences in a chemical's effect can vary among families within a population (Bridges and Semlitsch 2000) and among species

(Bridges 1999); furthermore, carbaryl was added later in larval development by Boone and Semlitsch (2001) than in the present study. Both of these factors may help explain the different outcomes between these similar studies.

We must note that chemical control ponds had few to no toad metamorphs. Although unexpected, this result may be explained by priority effects with differences in hatching as small as 6 d having strong effects on responses at metamorphosis (Alford and Wilbur 1985). Alford and Wilbur (1985) found that when toads were added to ponds after ranid species, they tended to do worse than when added at the same time or before ranids likely due to reduced competitive ability. We added Woodhouse's toads ~8 d after leopard frogs, so under control conditions they may have had a severe competitive disadvantage.

Because natural interactions in control ponds were not conducive to toad survival, low or no survival in control ponds makes it difficult to interpret the chemical effect negatively, especially when the control ponds produced essentially no juveniles for recruitment into the adult population. It is critical, however, to consider the potential ramifications of an apparent positive effect. An increase in the toad population could have relatively little effect at the community level because a hard winter or high predation rates may obliterate the increased toad population to levels equivalent of a natural, undisturbed pond. Likewise, if contamination was relatively infrequent or adventitious, an increase in the population may have little effect on a community that typically shows a high degree of variation in abundance among years and within ponds in the same year (Pechmann et al. 1991, Semlitsch et al. 1996). The increase in survival could be interpreted as a preferable outcome to the ''natural'' outcome that permits no juvenile recruitment. It might even be suggested that carbaryl exposure may be a useful way to increase some amphibian population sizes when populations are declining.

We agree that such conclusions are possible. However, in a field where scientists have often concluded initially that expected environmental levels were safe (i.e., DDT) when they were later shown to be perilous to nontarget populations, it is essential to give consideration to both sides equally, although both negative and positive ramifications may be equally speculative at this point. It is clear from this study that carbaryl increased toad survival significantly. However, the increase in survival may not actually benefit the toad population. Individuals that benefit from carbaryl exposure may be resistant to the pesticide, which would be beneficial in a contaminated environment; however, Semlitsch et al. (2000) found that individuals that had greater tolerance to carbaryl came from families that were inferior under natural field conditions without contamination. For this reason, individuals that survive the aquatic stage may have low adult survival or may produce offspring that are less likely to survive the aquatic phase, particularly when contaminant exposure is erratic or dissipates. As well, chemical exposure may suppress the immune system function of organisms and make individuals more vulnerable to disease (Carey et al. 1999). Further, if carbaryl can consistently favor one species over another, it may disrupt species interactions. If the toad populations increased dramatically over time, they may outcompete other species for food resource and reduce biodiversity in that community. Potentially, even an advantage during one year for one species may significantly affect the degree of competition for food resources in the terrestrial environment (Pechmann 1995) and in the larval environment. As well, while our focus here is on larval anurans, the zooplankton and invertebrate communities are severally affected and altered by even the lowest level of contaminants, which could have important ramifications in the food web. Exactly how an increase in one species may affect the community is unclear, but there are a number of possibilities and for this reason, we advocate the interpretation that any unnatural change in the community is undesirable.

In the background community, green frogs also responded to carbaryl exposure despite their greater development and larger size than other species in our study. Green frog metamorphs exposed to carbaryl had longer larval periods and left the ponds at smaller sizes than those in control ponds, effects that may have negative fitness consequences. In contrast, other experiments with green frogs have suggested that carbaryl exposure later in development can stimulate precocious metamorphosis (Boone 2000, Boone et al. 2001) rather than delayed metamorphosis. This difference may be attributable to the time of chemical application in the tadpoles' development (Boone 2000, Bridges 2000, Boone et al. 2001) with more-developed tadpoles more likely to be stimulated to metamorphosis with chemical stress (Boone et al. 2001). The timing of exposure in terms of development or time of year may be important in determining the outcome of this effect. In our present experiment, however, differences in green frog larval period were relatively small.

Interaction of carbaryl with natural stresses

To our knowledge, there is no community study that documents a chemical contaminant interacting with stresses known to be important in the natural environment of amphibians and few with other species (Ward and Busch 1976, Ward et al. 1976). Our study demonstrated that the biotic and abiotic conditions of the environment modified the effect of the contaminant in unexpected ways, suggesting that acute and chronic laboratory studies may not be useful litmus tests for determining environmental levels that would have no effect.

Although survival of the Woodhouse's toad increased with carbaryl exposure, this effect varied with overall tadpole density. Survival may have increased due to reduction in predation, reduced competition, or both, but the chemical by density interaction suggested it was likely due to a competitive release. If a reduction in predation alone explained increased toad survival, we would predict that toads in high- and low-density treatments would have similar per capita survival rates. Survival of toads differed between density treatments, suggesting that the ultimate cause was most likely a competitive release. Survival of toads increased in both high- and low-density ponds up to 5.0 mg/L, but at 7.0 mg/L low-density ponds had a three-fold reduction in toad survival, whereas high-density ponds had nearly a two-fold increase in survival compared to survival at 5.0 mg/L. Even at 3.5 mg/L, survival of toads in high-density ponds was nearly 7.5 times greater than survival in low-density ponds. This suggests that survival is not enhanced from decreased predation, but rather through increased food resources.

Low levels of insecticides often have a direct negative impact on zooplankton populations, with indirect, positive effects on periphyton and phytoplankton (Hanazato and Yasuno 1987, Fairchild et al. 1992; N. Mills, *personal communication*). Therefore, the addition of carbaryl increases the food supply of anurans because it reduces competing zooplankton populations (Hanazato and Yasuno 1987, 1990) that can feed on similar food resources as anurans. In high-density ponds, an increase in the food base could enhance survival by reducing mortality due to starvation, an effect seen in other studies (Boone and Semlitsch 2001; N. Mills, *personal communication*). Low-density ponds may not have increased survival, because food was already plentiful in this environment. Of course, both reduced predator and competitor pressure may interact to influence survival of the species in the community, although our design precludes determination of the precise mechanism of this effect.

Conclusions and management implications

Our experiment demonstrated that even a short-lived chemical with a half-life of only 4 d that was present early in the larval development of anurans can impact responses at metamorphosis, particularly for the species with a short larval period (Woodhouse's toad). Carbaryl also interacted with density, but not pond hydroperiod, to produce unexpected interactive effects. Had this experiment been conducted only at a low density, we might not have detected a significant chemical effect. A simpler design might have suggested that the chemical had no impact on natural populations.

The effects of carbaryl appeared advantageous for the Woodhouse's toads and generally indifferent for the leopard frogs. However, some newt mortality resulted, apparently due to carbaryl exposure. Because newts are voracious predators on tadpoles, this effect may allow tadpole abundance to increase, resulting in longer larval periods, smaller mass, and greater mortality, all of which can have negative fitness consequences for anurans (Berven and Gill 1983, Smith 1987). As well, effects on larval salamanders may be more dramatic because contaminants like carbaryl typically reduce or eliminate zooplankton and invertebrates that they feed upon for short periods of time.

Carbaryl is short-lived, does not bioaccumulate, and has relatively low toxicity to vertebrates (Cox 1993), making this a relatively favorable contaminant. Even though carbaryl may be more benign than many other contaminants in the environment, it was still able to alter responses at metamorphosis that could affect population dynamics through time. The goal of those setting safety standards should be to minimize the impact on the community. Whether or not this study indicates that the impact of carbaryl at realistic levels is minimal or that these low-level effects are cause for concern is probably moot. Ideally, the natural population dynamics of nontarget species should not be altered by contaminants. Any seemingly positive effects, such as increased toad survival, should be interpreted cautiously for a number of reasons. First, animals used in our experiment were from an agricultural area and may be less sensitive to pesticides than individuals in more pristine habitats. Additionally, because individuals that are tolerant to contaminants are not the most fit under natural field conditions (Semlitsch et al. 2000), individuals that flourish in a contaminated area may be more susceptible to viruses or diseases via immunosuppression (Carey et al. 1999), and therefore these populations may not have long-term viability. Most importantly, chemical contamination could reduce biodiversity over time by selecting for more tolerant species (Burnett 1997, Kupferberg 1997), which could then outcompete less tolerant or unaffected species over time. In any case, our study demonstrates that anthropogenic factors, such as chemical contaminants, can alter the structure of amphibian communities through direct or indirect effects on individual species, which may disrupt natural community regulation over time.

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