

PESTICIDES AND AMPHIBIANS: THE IMPORTANCE OF COMMUNITY CONTEXT

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Abstract. The widespread application of pesticides has attracted the attention of ecologists as we struggle to understand the impacts of these chemicals on natural communities. While we have a large number of laboratory-based, single-species studies of pesticides, such studies can only examine direct effects. However, in natural communities, species can experience both direct and indirect effects. We conducted an outdoor mesocosm experiment on aquatic communities containing three tadpole species (*Hyla versicolor*, *Bufo americanus*, and *Rana pipiens*), zooplankton, and algae. We then manipulated a factorial combination of predators (no predators; newts, *Notophthalmus viridescens*; and larval beetles, *Dytiscus* sp.) and pesticides (no pesticides, the insecticide malathion, and the herbicide Roundup). We found that Roundup (1.3 mg of active ingredient/L) had substantial direct negative effects on the tadpoles, reducing total tadpole survival and biomass by 40%. However, Roundup had no indirect effects on the amphibian community via predator survival or algal abundance. Malathion (0.3 mg/L) had few direct effects on the tadpoles. Malathion caused no indirect effects with one of the predators (red-spotted newts) but caused substantial positive effects on amphibians (a five-fold increase in total tadpole survival and biomass) due to the sensitivity of the predatory beetles to the insecticide. Thus, while high concentrations of malathion can directly kill larval anurans, more ecologically relevant concentrations can have large positive effects in mesocosms by removing predatory insects. These results make it clear that pesticides can have both direct and indirect effects in natural communities and that these effects critically depend upon the composition of the community.

Key words: *amphibian decline; Bufo americanus; Dytiscus; food web; Hyla versicolor; malathion; non-target species; Notophthalmus viridescens; Rana pipiens; Roundup; synergy; trophic cascades.*

INTRODUCTION

Anthropogenic chemicals are pervasive in nature and biologists are faced with the challenge of understanding how these chemicals impact ecological communities. Pesticides in aquatic communities are an excellent case in point. A diversity of pesticides and their residues are present in a wide variety of aquatic habitats (McConnell et al. 1998, LeNoir et al. 1999, Kolpin et al. 2002). While pesticides have the potential to affect many aquatic taxa, the impacts on amphibians are of particular concern in the past decade because of the apparent global decline of many species (Blaustein and Wake 1990, Alford and Richards 1999, Houlihan et al. 2001, Kiesecker et al. 2001). The list of possible causes of amphibian declines are numerous, and pesticides have been implicated in at least some of these declines. Pesticides occur in amphibian habitats (Harris et al. 1998, McConnell et al. 1998, LeNoir et al. 1999), amphibians living with insecticides in these habitats exhibit physiological signatures of these pesticides (i.e., reduced acetylcholine esterase activity; Sparling et al. 2001), and declining populations are correlated with

greater amounts of upwind agriculture where pesticide use is common (Davidson et al. 2001, 2002). While these correlative studies suggest that pesticides may affect amphibian communities, there are few rigorous experiments to confirm that pesticides are altering amphibian communities.

To understand the effects of pesticides on amphibians, the natural first step is to understand the direct toxicity of pesticides. There is a growing number of excellent laboratory-based, single-species toxicology experiments that can inform us about the LC₅₀ values of different pesticide concentrations (the concentration expected to kill 50% of a test population; e.g., Mann and Bidwell 1999, Perkins et al. 2000, Fordham et al. 2001). However, amphibians in nature live in much more complex environments that include assemblages of amphibian species as well as predators, competitors, zooplankton, attached algae (periphyton), and suspended algae (phytoplankton). What we ultimately wish to know is how pesticides influence amphibians not only under short-term, single-species laboratory conditions, but under longer-term, more realistic community conditions.

Researchers have begun to move toward more realistic testing environments by examining the impact of pesticides on aquatic organisms in outdoor mesocosms that contain many components of real ponds and

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wetlands (Hanazato and Yasuno 1990, Landis et al. 1997, Pratt et al. 1997, Taub 1997, Barry and Logan 1998, Diana et al. 2000, Boone and Semlitsch 2001). For example, Boone and colleagues (Boone and Semlitsch 2001, 2002, Boone et al. 2001, Boone and Bridges 2003, Boone and James 2003) have been investigating the effects of the insecticide carbaryl on larval amphibians in mesocosm communities. Although differing in detail, the experiments have used 0, 3.5, or 7.0 mg/L of carbaryl crossed with low and high competition and found that carbaryl can have inconsistent effects on tadpole survival and mass. We lack a mechanistic understanding of why we can arrive at qualitatively different outcomes across different species within the same experiment and within the same species across different experiments. Further, because our understanding of pesticide effects on amphibians in mesocosms is largely limited to the effects of carbaryl (but see Diana et al. 2000), we need to examine additional globally common pesticides (both insecticides and herbicides) to develop mechanistic generalities about their impacts on amphibian communities.

By embedding amphibians into a community consisting of predators, competitors, zooplankton, and algae, we can begin to understand the effects of pesticides under more natural conditions. Under such conditions, it is imperative that we include predators because they can have an overwhelming impact on community biomass and diversity (Wilbur 1972, Morin 1981, Wilbur and Fauth 1990). Mesocosm experiments conducted thus far have included only salamander and crayfish predators (Boone and Semlitsch 2001, 2002, 2003). However, because insects are also important predators of tadpoles and many pesticides target insects, we need to examine the potential trophic cascades that may occur when insecticides are applied.

In this study, we addressed these challenges by testing the impacts of globally common pesticides (the insecticide malathion and the herbicide Roundup) on outdoor mesocosm communities (Fig. 1) containing either no predators, predatory newts (*Notophthalmus viridescens*), or predatory beetle larvae (*Dytiscus* sp.). We tested the following hypotheses: (1) predators will directly reduce the survival of tadpoles; (2) Roundup will have direct lethal effects on the tadpoles and indirect negative effects by reducing algal biomass (Peterson et al. 1994), which should reduce tadpole growth; and (3) malathion will have no direct lethal effect on tadpoles but will be lethal to the insect predators that, in turn, will have a positive indirect effect on the tadpole assemblage.

Pesticide background

Malathion and Roundup were selected as the focal pesticides because they represent two of the most common pesticides used in the world. Malathion acts by inhibiting acetylcholine esterase and it is the number one insecticide (and the number seven pesticide) used

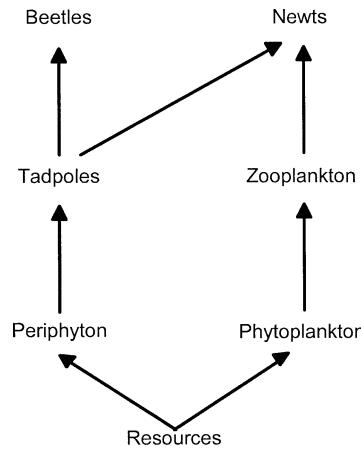


FIG. 1. Trophic connections in the simple aquatic community used in the mesocosm experiment. Arrows indicate the direction of energy flow.

in the United States. It is commonly applied to both terrestrial and aquatic habitats to control insect pests, including the mosquitoes that act as vectors for malaria and the West Nile virus (Gratz and Jany 1994). Total annual application is $13\text{--}15 \times 10^6$ kg with annual applications of $>800\,000$ ha of cropland (Donaldson et al. 2002; National Pesticide Use Database, *available online*).² Moreover, malathion has been detected in aquatic habitats at concentrations up to 0.6 mg/L (California Department of Fish and Game 1982, USDA 1997). The half-life of malathion is dependent on pH; at a pH of 8 the half-life is 2 d whereas at a pH of 6 the half-life is 26 d (Guerrant et al. 1970, Wang 1991).

Roundup is a commercial formulation of glyphosate (the active ingredient) that is combined with a surfactant (POEA; polyethoxylated tallowamine) which improves the penetration of leaf cuticles. Glyphosate works by inhibiting the synthesis of aromatic amino acids in plants. Roundup is currently the second most commonly applied herbicide in the United States with annual usage increasing rapidly. Total annual application is $30\text{--}33 \times 10^6$ kg of active ingredient (AI) applied to both homes and gardens and 8.2×10^6 ha of cropland (Donaldson et al. 2002, National Pesticide Use Database [see footnote 2]). Glyphosate has been observed in aquatic habitats at concentrations up to 2.3 mg AI/L and is predicted to be as high as 3.7 mg AI/L (Newton et al. 1984, Goldsborough and Brown 1989, Feng et al. 1990, Giesy et al. 2000; L. M. Horner, *unpublished report* MSL-9940 [1990; to Monsanto Company, Saint Louis, Missouri, USA]). The half-life of glyphosate in pond water depends on site conditions and ranges from 7 to 70 d (USEPA 1992, Giesy et al. 2000).

² (www.ncfap.org/database/default.htm)

METHODS

We conducted the experiment at the University of Pittsburgh's Pymatuning Laboratory of Ecology in northwestern Pennsylvania (USA). We began by filling 45, 1200-L cattle watering tanks with 1000 L of well water on 21–23 April 2003. During the next week, we added the following components to each tank: 300 g of dry leaves (primarily *Quercus* spp.), 25 g of commercial rabbit chow, and a 0.5 L aliquot of water collected from a mixture of five nearby ponds to serve as a source of algae and zooplankton (for prior usage of this experimental venue, see Werner and Anholt 1996, Peacor and Werner 1997, Relyea 2002b, Relyea and Yurewicz 2002). Thus the tanks contained many of the components found in natural ponds and wetlands. Each tank was also equipped with a single clay tile (225 cm^2) facing south in each tank to assess periphyton growth. To prevent colonization by amphibians and insects, we covered each tank with a 60% shade cloth lid.

After allowing the tank algal and zooplankton communities to establish for 3 wk, we added to each tank a community of larval amphibians consisting of American toads (*Bufo americanus*), leopard frogs (*Rana pipiens*), and gray tree frogs (*Hyla versicolor*). For all three amphibian species we collected the animals as newly deposited eggs from nearby ponds and hatched the eggs in outdoor wading pools. We collected 10 egg masses of leopard frogs (on 29 March), 8 egg masses of toads (on 21 April), and 10 egg masses of tree frogs (on 7 May). The hatchlings were fed rabbit chow ad libitum until used in the experiment. On 20 May we added 20 individuals of each species to each tank (8 tadpoles per species per square meter), which is well within natural densities for these species (R. A. Relyea, *personal observations*). All tadpoles were still early in their development (initial mass was 55 ± 6 mg for leopard frogs, 19 ± 2 mg for toads, and 9 ± 1 mg for gray tree frogs [means \pm SE]). A sample of 20 tadpoles from each species was set aside to quantify survivorship due to handling; 24-h survivorship was 100%.

The experiment employed a completely randomized design with a factorial combination of three predator treatments (no predators, adult newts, and larval beetles) crossed with three pesticide treatments (no pesticide, the insecticide malathion, and the herbicide Roundup). Thus, there were nine treatment combinations, each replicated 5 times, for a total of 45 experimental units. For tanks assigned the predator treatments, we added either two newts or two beetle larvae on 22 May. These predators were collected from nearby ponds and weighed prior to being added to the experiments. The density of each predator species (0.8 individuals/ m^2) was similar to the densities of the predators observed in natural wetlands (0.2 to 2.5 ind./ m^2 ; E. E. Werner, R. A. Relyea, D. K. Skelly, and K. L. Yurewicz, *unpublished data*). All adult newts were fairly similar in mass (3.3 ± 0.1 g [mean \pm SE]). However,

there was variation in the mass of beetle larvae. Thus, for the two beetles assigned to each tank, we added one large and one small individual (0.23 ± 0.01 g and 0.10 ± 0.01 g, respectively). To allow the tadpoles to acclimate to their new mesocosm environment, we caged all predators for the first 6 h and then released them into the tanks on 22 May. While mesocosms containing leaf litter do mimic many types of ponds in terms of the type of refuges that are available, additional natural refuges including vegetation could produce different predation rates.

After releasing the predators from their cages and removing the cages from the tanks, we applied the pesticide treatments (22 May). For both the insecticide (malathion) and herbicide (Roundup), our goal was to simulate the direct overspray of a wetland or flooded agricultural field in which many amphibian species lay their eggs. Thus, for malathion, we followed the manufacturer's recommended application rate (0.262 mL/ m^2) for malathion (50% active ingredient). Given that our tanks had a surface area of 2.4 m^2 , we added 0.63 mL of malathion to each appropriate tank, producing a concentration of 0.315 mg/L of malathion in the tanks. We used a commercial form of malathion whose commercial concentration (50.6% active ingredient) was independently confirmed by the Mississippi State Laboratory (Mississippi State, Mississippi, USA) using high-pressure liquid chromatography. For Roundup, the recommended application rate was 6.4 mL AI/ m^2 . However, from previous experiments (Relyea 2005a), we knew that this application rate was capable of killing nearly every tadpole in a mesocosm. Thus, we reduced the Roundup application rate to approximately one third this amount. Using Roundup with 13% active ingredient (confirmed by the Mississippi State Laboratory), we added 10 mL (10 g) of Roundup to each appropriate tank, producing a concentration of 1.3 mg AI/L.

On 12 June (after 23 d), the experiment was terminated because few tadpoles were observed in the predator treatments. We removed the periphyton tiles and brushed off all attached algae (from the top surface) onto oven-dried, pre-weighed filters (70-mm diameter Whatman GF/C filters). These filters were oven-dried again at 80°C for 15 h and reweighed to assess the dry mass of periphyton in each tank. We also quantified phytoplankton by collecting 1.12-L of water from each tank and filtering the water through Whatman 25-mm GF/C glass fiber filters using vacuum filtration techniques. The filters were wrapped in foil and frozen to break the algal cell walls. The thawed filters were placed in film canisters containing 10 mL of 95% ethanol solution to solubilize the chlorophyll, and these solutions were then analyzed for chlorophyll *a* concentration (in parts per billion [10⁻⁹]) using a fluorometer. Finally, we removed all tadpoles and predators from the tanks; the recovered animals were counted and weighed. Growth rate of the tadpoles and their

TABLE 1. Results of a MANOVA that examined the impact of pesticides and predators on aquatic communities.

A) Multivariate tests		df		Wilks' <i>F</i>		<i>P</i>	
Predators		14, 58		15.8		<0.001	
Pesticides		14, 58		4.7		<0.001	
Predators × Pesticides		28, 106		3.0		<0.001	
B) Univariate tests							
Variable	Predators		Pesticides		Predators × Pesticides		<i>P</i>
	<i>P</i>	<i>F</i> _{2,35}	<i>P</i>	<i>F</i> _{2,35}	<i>P</i>	<i>F</i> _{4,35}	
Periphyton abundance	0.246	1.5	0.138	2.1	0.588	0.7	
Phytoplankton abundance	0.693	0.4	0.731	0.3	0.554	0.8	
Total tadpole survival	<0.001	120.2	<0.001	20.8	<0.001	12.6	
Total tadpole biomass	<0.001	80.6	<0.001	10.8	0.001	5.6	
Tree frog survival	<0.001	92.4	0.372	1.0	0.015	3.6	
Toad survival	<0.001	139.5	<0.001	1.0	<0.001	18.9	
Leopard frog survival	<0.001	34.7	<0.001	15.6	0.002	5.5	

predators was calculated as (final mass – initial mass)/23 d. Because we had already demonstrated the impact of these two pesticides on the zooplankton assemblage (malathion reduces zooplankton abundance but Roundup does not; Relyea 2005a), we did not quantify the impact on zooplankton in the current experiment.

Statistical analyses

We conducted three analyses on the data. In the first analysis, we conducted a multivariate analysis of variance (MANOVA) of the effect of pesticides and predators on the following response variables: total tadpole survival, total tadpole biomass, tree frog survival, toad survival, leopard frog survival, periphyton mass, and phytoplankton (*chlorophyll a*) abundance. Because of heterogeneous errors that could not be transformed (many treatments had 0% survival), we analyzed survival data by first ranking the data.

We conducted a separate analysis on the impacts of the treatments on tadpole growth. Because predators frequently eliminated gray tree frogs and toads (i.e., 0% survival), we could not assess growth in the predator treatments. However, we could assess the impacts of the pesticides treatments across all three tadpole species in the absence of predators. Thus, we conducted a MANOVA on the growth of the three tadpole species under the three treatments lacking predators. The impact of both the pesticides and predators on tadpole growth could be assessed for leopard frogs, so we conducted a separate ANOVA on leopard frog growth across all nine treatments.

In the third analysis, we tested the impacts of the pesticide treatments on the predatory newts and beetles. We conducted an ANOVA to examine the impacts of the pesticides on the survival and growth of the two predator species (survival data were ranked due to heterogeneous errors). For all analyses, mean comparisons were conducted using Fisher's LSD tests.

RESULTS

Effects on tadpoles and algae

We found a significant multivariate effect of the pesticides, predators, and their interaction on the mesocosm community (Table 1). In examining each response variable, we found that pesticides and predators had no impact on periphyton or phytoplankton. In contrast, pesticides and predators had interactive effects on the total number of tadpoles surviving in the communities and the total biomass of tadpoles in the communities (Fig. 2). In the absence of predators, total tadpole survival and biomass were high with no pesticides and malathion ($P > 0.12$), but reduced by 39–41% with Roundup ($P \leq 0.008$). In the presence of newts, survival and biomass were much lower than the no-predator/no-pesticide control treatment ($P < 0.001$). Compared to tanks containing newts and no pesticides, adding malathion had no further effect ($P > 0.28$) while Roundup caused significant reductions in total survival ($P = 0.030$) and marginal reductions in total biomass ($P = 0.098$). In the presence of beetles without pesticides, total survival and biomass were also lower than the control treatment ($P < 0.001$). Compared to tanks containing beetles and no pesticides, adding malathion caused a five-fold increase in total survival and biomass ($P = 0.001$) while adding Roundup had no effect on either response variable ($P > 0.78$).

The MANOVA also included univariate tests of tadpole survival by species. The pesticide and predator treatments had significant interactive effects on all three species (Table 1, Fig. 2). In the absence of predators and pesticides, survival was relatively high for tree frogs (71%), toads (94%), and leopard frogs (98%). Adding malathion had no impact on the survival of tree frogs or leopard frogs ($P > 0.3$) but caused a small reduction in toads (11%; $P = 0.011$). Adding Roundup had no impact on the survival of tree frogs ($P > 0.2$)

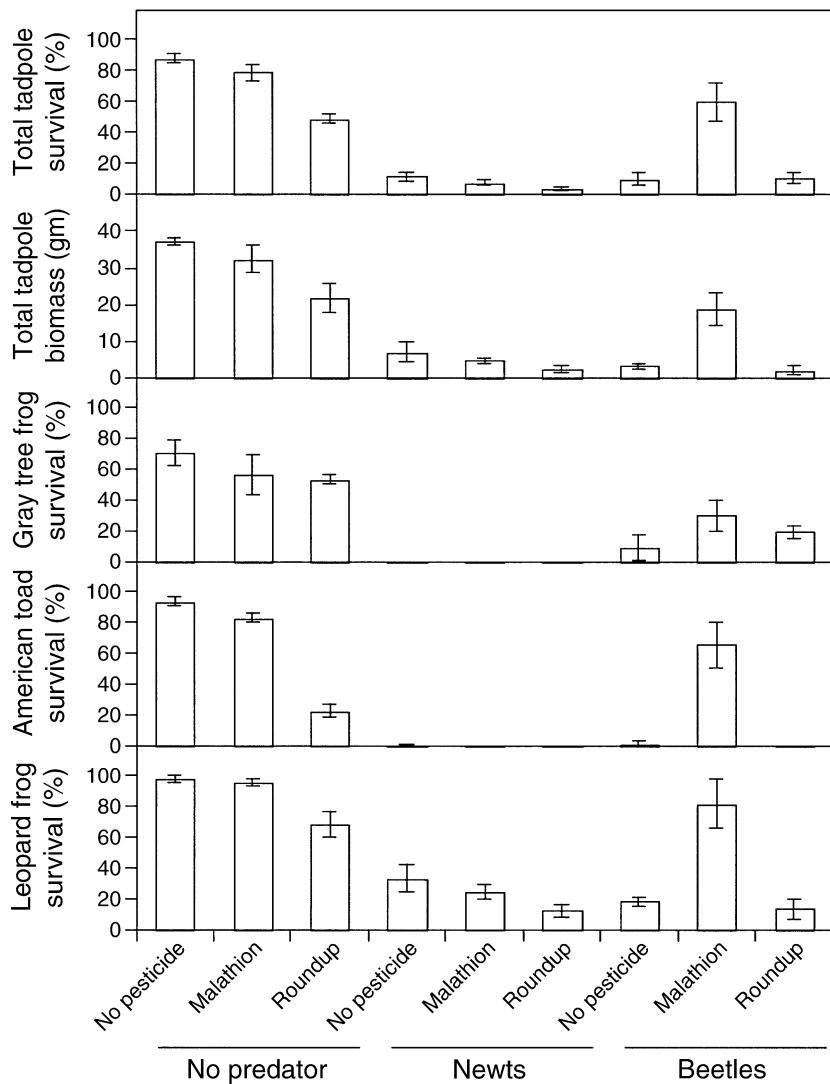


FIG. 2. Total survival and total biomass of all tadpoles pooled, and the survival of three individual species of tadpoles living in aquatic mesocosms with different pesticides and predators present. Data are means \pm SE.

but caused a 71% reduction in toad survival ($P < 0.001$) and a 29% reduction in leopard frog survival ($P = 0.002$).

In the presence of newts, no tree frog tadpoles and few toad tadpoles survived across all three pesticide treatments (Fig. 2). Leopard frogs were not completely exterminated, although their survival was considerably lower than leopard frogs reared in the control treatments ($P < 0.001$). Compared to tanks containing newts and no pesticides, adding malathion had no further impact on leopard frog survival ($P = 0.491$) but adding Roundup caused an additional 21% reduction in leopard frog survival ($P = 0.026$).

In the presence of beetles, there was substantially lower survival for all three tadpole species when pesticides were absent compared to the control treatments ($P < 0.001$; Fig. 2). However, compared to tanks con-

taining beetles and no pesticides, adding malathion increased survival by 21% for tree frogs, 66% for toads, and 63% for leopard frogs ($P \leq 0.01$). Adding Roundup to tanks containing beetles caused a small (10%) increase in tree frog survival ($P = 0.042$) but had no effect on the survival of toads and leopard frogs ($P > 0.25$). For all three species, survival with the combination of malathion and beetle larvae was not different than survival with malathion and no predators ($P > 0.1$).

We conducted two analyses on tadpole growth. In the first analysis, we examined the impact of the pesticide treatments on the three tadpole species in the no-predator treatments. There was no multivariate effect of pesticides on tadpole growth (Wilks' $F_{6,18} = 1.6$, $P = 0.214$) nor were there any significant univariate effects for any of the three species ($P > 0.14$). In the

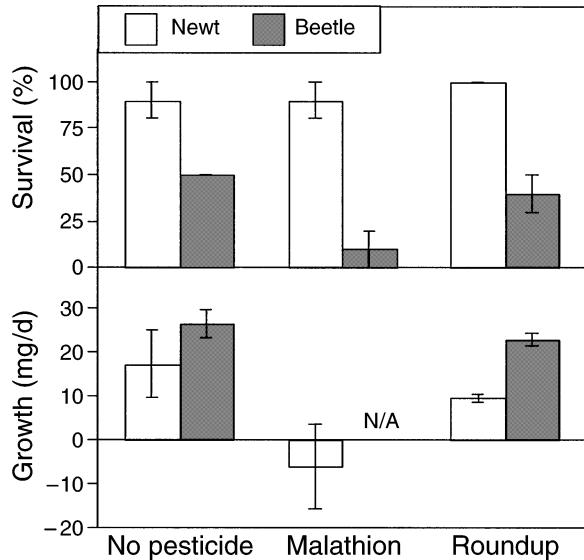


FIG. 3. The impact of three pesticide treatments on the survival and growth of predatory newts and predaceous diving beetles. "N/A" indicates treatments in which there was insufficient survival to reliably estimate growth. Data are means \pm SE.

second analysis, we examined the impact of pesticides and predators on leopard frog growth (because leopard frogs were the only species that had some survivors in all predator treatments). Leopard frog growth was affected by the predator treatments ($F_{2,43} = 16.9$, $P < 0.001$) but not by the pesticide treatments ($F_{2,43} = 0.6$, $P = 0.563$) nor by the predator–pesticide interaction ($F_{3,43} = 0.6$, $P = 0.674$). Leopard frog growth was significantly lower with newts (31% reduction) and beetles (46% reduction) than with no predators ($P < 0.001$); growth was marginally lower with beetles than with newts ($P = 0.071$). In short, predators affected tadpole growth but the pesticides did not.

Effects on the predators

The predators were also affected by the addition of the pesticides (Fig. 3). Pesticides had no impact on newt survival ($F_{2,12} = 0.5$, $P = 0.619$), which remained high across all treatments ($93 \pm 5\%$ [mean \pm SE]). Newt growth was marginally affected by the pesticides ($F_{2,10} = 3.1$, $P = 0.090$). This marginal effect occurred because Roundup had no effect on newt growth ($P = 0.415$) whereas malathion caused a 23 mg/d reduction in newt growth ($P = 0.035$).

The impact of the pesticides on beetles was qualitatively different from the impact on newts (Fig. 3). Pesticides had a significant impact on beetle survival ($F_{2,12} = 6.5$, $P = 0.012$). In tanks with no pesticides, only $50 \pm 0\%$ of the beetles survived (i.e., one of the two beetles that were initially added to each tank). We suspected that the high mortality in the no-pesticide treatment was the result of the cannibalistic nature of

these beetle larvae. A small supplemental laboratory experiment confirmed this hypothesis. Putting pairs of large (350 mg) and small (175 mg) beetles together in 10 separate 10-L tubs, the large beetle killed the small beetle in every case. Thus, we could be sure that the single beetle recovered from each tank was the larger of the two beetles that we added initially. Adding malathion to the communities killed nearly every beetle ($P = 0.005$) while Roundup had no effect ($P = 0.403$). By quantifying beetle growth as (final mass of the surviving beetle – initial mass of the larger beetle), we found no difference in beetle growth between the no-pesticide and Roundup treatments (the only treatments in which beetles survived; $F_{1,7} = 0.8$, $P = 0.393$).

DISCUSSION

The results of the experiment demonstrated that pesticides can have diverse direct and indirect effects on aquatic communities. Malathion appeared to have few direct lethal effects on tadpoles (except for the toads). In the absence of predators, there was little impact on tadpole survival (only a small reduction in toad survival) and no effect on tadpole growth. This result is consistent with previous laboratory studies on malathion with amphibians. For example, toad embryos (*Bufo arenarum*) are quite resistant to malathion ($LC50_{5d} = 42$ mg/L; Rosenbaum et al. 1988) and Fordham et al. (2001) found that malathion only causes mortality in bullfrog tadpoles (*Rana catesbeiana*) when it exceeds 2.5 mg/L. For the three species used in the current study, Relyea (2004) found $LC50_{16d}$ values of 2.4 mg/L for leopard frog tadpoles, 5.9 mg/L for American toads, and 2.0–4.1 mg/L for tree frog tadpoles (depending on the presence of predator cues). Because the current experiment used a malathion concentration of 0.3 mg/L, one would expect little impact of malathion on tadpole survival; this was confirmed under the more natural conditions of the pond mesocosms.

In contrast to malathion, Roundup had strong direct effects on the tadpoles. Roundup caused a 40% reduction in total tadpole survival and biomass. The impact of Roundup (with POEA [polyethoxylated tallowamine] surfactant) is consistent with previous laboratory studies in a variety of species. Mann and Bidwell (1999) estimated $LC50_{48h}$ at 3.9 to 15.5 mg active ingredient (AI)/L in four species of Australian tadpoles while Perkins et al. (2000) estimated $LC50_{96h}$ values of 12.4 mg AI/L in the African clawed frog (*Xenopus laevis*). In both studies, it was clear that the high toxicity of Roundup was caused by the POEA surfactant and not from the active ingredient (glyphosate). Lajmanovich et al. (2003) examined the impact of Kleeraway (another formulation of glyphosate that contains the POEA surfactant) on a South American tadpole (*Scinax nasicus*) and found an $LC50_{48h}$ of 1.74 mg AI/L. In North American tadpoles (*Bufo americanus*, *Rana pipiens*, and *R. clamitans*), Edginton et al. (2004) found $LC50_{96h}$ of 1.5–4.7 mg AI/L using Vision (a for-

mulation that also includes the POEA surfactant). For the three species used in our mesocosm experiment, Relyea (2005b) found LC₅₀_{16d} values of 1.4 mg AI/L for gray tree frogs, 2.5 mg AI/L for American toads, and 2.5 mg AI/L for leopard frogs. All of this suggests that Roundup with the POEA surfactant can cause substantial mortality in larval amphibians. However, more recent formulations of glyphosate containing other surfactants (Roundup Biactive) may be less toxic (Tsui and Chu 2003, Howe et al. 2004).

The direct lethal effect of Roundup on amphibian larvae has previously been examined in mesocosm experiments. At higher concentrations than used in the current experiment (3.8 mg AI/L), Roundup caused a 70% reduction in larval amphibian diversity including the complete elimination of leopard frog and gray tree frog tadpoles and only 2% survival of wood frog tadpoles (*R. sylvatica*; Relyea 2005a). A subsequent mesocosm experiment with Roundup found that with the application of 3.8 mg AI/L, tree frog survival was reduced from 75% to 2%, toad tadpole survival was reduced from 97% to 0%, and leopard frog tadpole survival was reduced from 98% to 4% (R. A. Relyea, *unpublished manuscript*). Thus, under the relatively natural conditions of aquatic mesocosms, maximum expected concentrations of Roundup (3.8 mg AI/L) can eliminate several species of North American tadpoles whereas lower concentrations (1.3 mg AI/L), as used in our experiment, can still have a major impact on tadpole survival (40% reduction). The impact of Roundup is likely a direct effect of toxicity (possibly due to damaged respiratory surfaces; Edginton et al. 2004) and not an indirect impact on the tadpole's algal resources. Despite the fact that glyphosate can affect algal growth (Peterson et al. 1994), we found that the algae were not affected by Roundup. Further, laboratory experiments (Relyea 2005b) have found high rates of tadpole mortality with Roundup when tadpoles were fed commercial fish food as a resource, suggesting that the tadpole death was not caused by a lack of algae, but from direct toxicity. Recently, other investigators have applied 1.9 mg AI/L of Vision (glyphosate plus POEA) to field enclosures placed into real wetlands in Canada and found no significant mortality in larval leopard frogs and green frogs (Thompson et al. 2004, Wojtaszek et al. 2004). Clearly, much more work needs to be done to understand the effects of glyphosate and POEA on tadpole survival across different experimental venues and different populations of amphibians.

The other major direct effects were due to the addition of predators to the community. In the absence of pesticides, adult newts and larval *Dytiscus* beetles caused widespread mortality on all three species of tadpoles. Both of these predators are known to cause high rates of tadpole mortality (Morin 1981, Wilbur and Fauth 1990, Boone and Semlitsch 2001, 2002, Relyea 2002b). The higher survival of the leopard frogs

was likely a reflection of the leopard frog's larger initial size and the gape-limitation of predators.

The direct impacts on tadpole survival were both predator and pesticide specific. In the presence of newts, tree frogs and toads were completely eliminated, preventing any combined effects from being exhibited. However, leopard frogs survived well enough to determine how the two pesticides combined with newt predation. Newts alone caused moderate leopard frog mortality while malathion alone had no further effect; when newts and malathion were combined, leopard frog mortality was similar to newts alone. In contrast, newts alone and Roundup alone each caused moderate effects on leopard frog mortality. When newts and Roundup were combined, leopard frog mortality was higher than with newts alone. Thus, in the presence of both newts and Roundup, tadpoles suffer combined effects on mortality.

There also were a number of important indirect effects. Perhaps the most striking indirect effect was the positive impact of malathion on the amphibian assemblage. The addition of malathion to an aquatic community containing insect predators caused the elimination of most insect predators and a five-fold increase in total tadpole survival and total tadpole biomass. However, when malathion was applied to communities containing salamander predators, there was no effect. This result underscores the importance of examining relevant concentrations of pesticides in more complex (i.e., natural) communities. When we examine pesticide effects in the absence of natural community complexity or under high concentrations of pesticides (above those documented in nature), one can often find detrimental effects on amphibians (e.g., Relyea and Mills 2001, Relyea 2003, 2004). However, when we apply more ecologically relevant concentrations to more complex and realistic communities, we arrive at the intriguing result that a globally common insecticide (malathion) can have a large positive effect on amphibian survival (at least in mesocosm communities; for similar results with the insecticide carbaryl, see Boone and Semlitsch 2001, 2003). The contrasting results in this study between communities containing insect predators and salamander predators highlights the importance of predator identity; malathion (and other insecticides that share its mode of action) can have positive indirect effects on amphibians when insects are the major predators in the community but no effect when salamanders (and possibly other vertebrates) are the major predators. Thus, community context is extremely important in assessing pesticide effects on amphibians (and probably many other taxa as well).

There were additional indirect effects that occurred in the experiment. Leopard frog growth was substantially reduced in the presence of both predators. Numerous experiments have demonstrated that larval amphibians (and most other prey taxa) reduce their foraging activity in the presence of predators (Lawler

1989, Tejedo 1993, Relyea and Werner 1999, Relyea 2001, Relyea 2002a). While reduced activity decreases the likelihood of being killed by predators (Kats and Dill 1998), it comes at the cost of reduced growth (Skelly 1992, Relyea and Werner 1999). Hence, the leopard frogs in the current study suffered direct mortality via predation, and the subsequent generation of chemical cues (emitted when prey are consumed) likely induced decreased foraging and an indirect reduction in growth. Reduced growth is important to the long-term fitness of amphibians as slower growth is correlated with increased susceptibility to pond drying, lower post-metamorphic survival, and smaller size at first reproduction (Berven and Gill 1983, Newman 1988, Semlitsch et al. 1988). The stress of these chemical cues can also cause synergistic interactions with pesticides (Relyea and Mills 2001, Relyea 2003, 2004). However, because the predators were free ranging and not caged, one cannot determine how much of the predator-associated death arose from direct predation vs. synergistic interactions between the pesticides and the stress of chemical cues emitted by predators.

The final indirect effect in the experiment was the impact of malathion on newt growth. If the pesticides reduced the survival of the newts' prey, then newt growth and survival should also be affected. While malathion had no apparent effect on tadpole growth and survival in this experiment, malathion (and several other insecticides) can have a major impact on zooplankton in aquatic communities. At high concentrations (>1 mg/L), insecticides can eliminate all zooplankton; however, at lower concentrations (<1 mg/L), insecticides eliminate cladocerans but not copepods (Hanazato and Yasuno 1990, Hanazato 1991, Havens and Hanazato 1993, Havens 1994, 1995, Wong et al. 1995). In a previous mesocosm experiment using the same concentration of malathion (0.3 mg/L), we found a 34% reduction in zooplankton (Relyea 2005a). Given that salamanders consume both tadpoles and zooplankton, one would predict that malathion's negative impact on zooplankton abundance would have an indirect negative impact of newt growth and this was what we observed (however, reduced growth could have also occurred due to the newt's metabolic costs of detoxification). Similar results have been found with the insecticide carbaryl and two species of salamanders (*Ambystoma maculatum* and *A. texanum*). At high concentrations of carbaryl (5 mg/L), zooplankton were completely eliminated from mesocosms and caused a nearly complete elimination of the salamanders via starvation (Boone and James 2003). Hence, taxa-specific pesticide effects can have important cascading effects throughout a community.

Conclusions

Pesticides have the potential to have dramatic effects on ecological communities. Some effects on aquatic communities can be predicted from the direct toxic

effects observed in single-species laboratory experiments. However, other effects are mediated through other species in the community and can only be observed when we return the taxa to their natural ecological context. When we add pesticides to aquatic communities, the impact can depend on both the type of pesticide applied and the type of predator present. Thus, although pesticides are found in amphibian habitats (Harris et al. 1998, McConnell et al. 1998, LeNoir et al. 1999, Sparling et al. 2001) and amphibian declines are associated with upwind agriculture (Davidson et al. 2001, 2002), the role that pesticides may play in amphibian population declines will clearly be a complex mélange of direct and indirect effects.

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