Ecological Applications, 15(2), 2005, pp. 618–627

THE IMPACT OF INSECTICIDES AND HERBICIDES ON THE BIODIVERSITY AND PRODUCTIVITY OF AQUATIC COMMUNITIES

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Abstract. Pesticides constitute a major anthropogenic addition to natural communities. In aquatic communities, a great majority of pesticide impacts are determined from single-species experiments conducted under laboratory conditions. Although this is an essential protocol to rapidly identify the direct impacts of pesticides on organisms, it prevents an assessment of direct and indirect pesticide effects on organisms embedded in their natural ecological contexts. In this study, I examined the impact of four globally common pesticides (two insecticides, carbaryl [Sevin] and malathion; two herbicides, glyphosate [Roundup] and 2,4-D) on the biodiversity of aquatic communities containing algae and 25 species of animals.

Species richness was reduced by 15% with Sevin, 30% with malathion, and 22% with Roundup, whereas 2,4-D had no effect. Both insecticides reduced zooplankton diversity by eliminating cladocerans but not copepods (the latter increased in abundance). The insecticides also reduced the diversity and biomass of predatory insects and had an apparent indirect positive effect on several species of tadpoles, but had no effect on snails. The two herbicides had no effects on zooplankton, insect predators, or snails. Moreover, the herbicide 2,4-D had no effect on tadpoles. However, Roundup completely eliminated two species of tadpoles and nearly exterminated a third species, resulting in a 70% decline in the species richness of tadpoles. This study represents one of the most extensive experimental investigations of pesticide effects on aquatic communities and offers a comprehensive perspective on the impacts of pesticides when nontarget organisms are examined under ecologically relevant conditions.

Key words: *amphibian decline;* Anax junius; Bufo americanus; Daphnia; Dytiscus; *frogs;* Hyla versicolor; Lestes; Pseudacris crucifer; Rana pipiens; Rana sylvatica; Tramea.

INTRODUCTION

A central goal of ecology is to understand patterns of species abundance and diversity in communities and ecosystems. A great deal of research has documented the patterns of biodiversity and productivity using relatively pristine systems or experimental mesocosms that approximate natural systems (Tilman et al. 2001, Chase and Leibold 2002, Downing and Leibold 2002, Naeem 2002). However, many ecosystems are far from pristine due to a variety of anthropogenic influences, including exposure to a plethora of pesticides (Harris et al. 1998, McConnell et al. 1998, LeNoir et al. 1999, Sparling et al. 2001, Davidson et al. 2002). Herbicides and insecticides have the potential to cause dramatic changes in natural communities, yet our knowledge of pesticide effects on natural communities is largely limited to cases in which pesticides have been intentionally or accidentally applied to natural sites with subsequent floral and faunal surveys (e.g., reptiles and amphibians, Lambert [1997]; macroinvertebrates, Leonard et al. [1999]; plankton and fish, Favari et al. [2002]). In contrast, experimental efforts to understand community effects have primarily used single pesticides and have focused on a narrow range of taxonomic groups including zooplankton (Hanazato and Yasuno 1987, 1989, 1990, Havens 1994, 1995) and larval amphibians (e.g., Boone and Semlitsch 2001, 2002; but see Boone and James 2003). The challenge is to combine the best of both approaches by examining the impact of different pesticides on a broad diversity of taxa while taking advantage of the power that comes from experimental replication.

Aquatic communities are particularly well suited to experimental investigations of pesticide effects. There is a long history of using outdoor aquatic mesocosms to create experimental communities that can be replicated and manipulated (Morin 1981, Werner and Anholt 1996, Relyea and Yurewicz 2002, Downing and Leibold 2002). Mesocosms offer the potential to assemble diverse communities of predators, herbivores, and producers and make testable predictions about the impact of pesticides based on single-species laboratory tests (i.e., LC50 tests that estimate the lethal concentration necessary to kill 50% of a test population). For example, in pond communities, one would predict that the application of insecticides at realistic concentrations should have a direct lethal impact on aquatic in-

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sect predators, but no direct impact on herbivores or producers. However, insecticides may cause trophic cascades including indirect positive effects on the herbivores and indirect negative effects on the resources. In contrast, herbicides might have a direct negative impact on producers but no direct impact on herbivores or predators. However, herbicides may cause trophic cascades including indirect negative effects on herbivore biomass and predator biomass (Diana et al. 2000). In summary, mesocosms allow investigators to examine the impacts of relevant pesticide concentrations using realistic population densities, reasonable time scales, and relatively natural conditions.

In this study, I assembled diverse communities in outdoor aquatic mesocosms and then examined the impact of two insecticides and two herbicides (applied separately) on the diversity of the communities as well as the survival and biomass of each taxon in the community. Based on the known impact of these pesticides, I tested the following hypotheses. (1) All of the pesticides will reduce overall biodiversity. (2) The insecticides will reduce the diversity and abundance of insects (USDI [U.S. Fish and Wildlife Service] 1980) and zooplankton (Havens 1994, 1995), but will have no direct impact on the snails and tadpoles (Relyea 2003*b*, 2004). (3) Because of the reduction of insect predators, the insecticides will have an indirect positive effect on the biomass of herbivores and an indirect negative effect on the biomass of producers (i.e., periphyton). (4) The herbicides will reduce the biomass of producers but will have no direct impact on the snails and tadpoles. (5) Because of the reduction of producers, the herbicides will have an indirect negative effect on the biomass of herbivores and predators.

Pesticide background

The four pesticides used in the experiment were two insecticides (carbaryl and malathion) and two herbicides (2,4-D and glyphosate). Carbaryl and malathion are both broad-spectrum insecticides that kill by inhibiting acetylcholine esterase. In the United States, 1–2 $\times 10^6$ kg of carbaryl (commercial name: Sevin) are applied to rangelands, forests, oceans, homes, gardens, and 1.3 $\times 10^6$ ha of crops (Donaldson et al. 2002); see the online National Pesticide Use Database.[2] The half-life for carbaryl depends on pH and ranges from 0.1 days to 4 years (Aly and El-Dib 1971, Wauchope and Haque 1973). Malathion is applied to >800 000 ha of cropland including fruits, vegetables, and cotton at an annual amount of 14–16 $\times 10^6$ kg (Donaldson et al. 2002, National Pesticide Use Database [footnote 2]), and is a preferred insecticide for combating the mosquitoes that carry malaria and West Nile virus. The half-life of malathion is 2–26 days, depending on pH (Guerrant et al. 1970, Wang 1991). Glyphosate (commercial names: Roundup, Rodeo) is a broad-spectrum herbicide that kills plants by inhibiting the synthesis of essential amino acids. The most popular formulation, Roundup, actually is a combination of the active ingredient (glyphosate) and a surfactant that helps the herbicide to penetrate plant leaves (polyehtoxylated tallowamine; POEA). It is the second most commonly applied herbicide in the United States, with 38–43 $\times 10^6$ kg of active ingredient applied to homes, gardens, forests, wetlands, and 8.2 $\times 10^6$ ha of cropland in the United States (Donaldson et al. 2002, National Pesticide Use Database). The half-life of roundup is 7–70 days (Giesy et al. 2000). The herbicide 2,4-D is a broadleaf herbicide that operates as a growth regulator by altering proper cell division in plants. It is widely used in agriculture, with 24–28 $\times 10^6$ kg applied to nearly 33 $\times 10^6$ ha (Donaldson et al. 2002, National Pesticide Use Database). The half-life of 2,4-D is from 10 to >50 days, according to NIH data (*available online*).[3] These four pesticides are among the top 10 pesticides used in the United States for agriculture and home use (Donaldson et al. 2002), and all of them are either applied directly to aquatic habitats or can make their way into aquatic habitats via unintentional overspray, aerial drift, or runoff.

Methods

The experiment was a completely randomized design with five pesticide treatments that were each replicated six times for a total of 30 experimental units. The experimental units were 1200-L polyethylene tanks that were filled with 1000 L of well water during 26–28 April 2002. On 6 May, I added 300 g of dry leaves (*Quercus* spp.) and 25 g of rabbit chow to serve as habitat structure and an initial nutrient source. I also added an aliquot of zooplankton and phytoplankton that was a mixture from six local ponds. On 23 May, I placed two 10 × 10 cm ceramic tiles in each tank (oriented vertically) to serve as future estimates of periphyton growth in each tank.

Five days later, I began adding macro-organisms that I collected from natural habitats, either as mixtures of ≥10 egg masses that were previously hatched in wading pools (four of the five tadpole species), or as larvae and adults dip-netted from ponds and wetlands (Table 1). On 28 May, I added five species of larval anurans, two species of snails, and one species of larval damselfly (predators on zooplankton). The following day, I added a third snail species. On 30 May, I added the remaining predators: larval *Anax* and *Tramea* dragonflies (predators on both tadpoles and snails), larval *Dytiscus* and *Acilius* beetles (predators on tadpoles and zooplankton, respectively), *Notonecta* and *Belostoma* hemipterans (predators on both tadpoles and snails), and recently hatched spotted salamander larvae (predators on zooplankton). All of these species naturally coexist and, for each species, I used densities that were

[2] ⟨http://www.ncfap.org⟩

[3] ⟨http://toxnet.nlm.nih.gov⟩

TABLE 1. A list of the taxa used in the experiment.

Common name	Species	Size	Density†	Trophic level
Spotted salamander‡	*Ambystoma maculatum*	49 ± 3 mg	2	predator
Diving beetle‡	*Dytiscus* sp.	28 ± 1.1 mm	1	predator
Diving beetle‡	*Acilius semisulcatus*	21 ± 0.4 mm	1	predator
Dragonfly‡	*Anax junius*	39 ± 0.9 mm	1	predator
Dragonfly‡	*Tramea* sp.	23 ± 0.6 mm	1	predator
Damselfly‡	*Lestes* sp.	15 ± 0.3 mm	1	predator
Backswimmer	*Notonecta undulata*	10 ± 0.3 mm	3	predator
Water bug	*Belostoma flumineum*	20 ± 0.2 mm	1	predator
Wood frog‡	*Rana sylvatica*	104 ± 10 mg	10	herbivore
Leopard frog‡	*Rana pipiens*	42 ± 8 mg	10	herbivore
American toad‡	*Bufo americanus*	45 ± 5 mg	10	herbivore
Gray tree frog‡	*Hyla versicolor*	4 ± 0 mg	10	herbivore
Spring peeper‡	*Pseudacris crucifer*	214 ± 16 mg	10	herbivore
Snail	*Physa integra*	62 ± 4 mg	10	herbivore
Snail	*Helisoma trivolvis*	434 ± 31 mg	10	herbivore
Snail	*Stagnicola elodes*	177 ± 20 mg	10	herbivore
Cladoceran	*Daphnia pulex*	···	···	zooplankton
Cladoceran	*Daphnia ambigua*	···	···	zooplankton
Cladoceran	*Daphnia longiremis*	···	···	zooplankton
Cladoceran	*Ceriodaphnia* sp.	···	···	zooplankton
Cladoceran	*Scapholebris* sp.	···	···	zooplankton
Copepod	*Eurytemora affinis*	···	···	zooplankton
Copepod	*Eurycyclops agilis*	···	···	zooplankton
Copepod	*Mesocyclops edax*	···	···	zooplankton
Copepod	*Leptochaptumorus siciloides*	···	···	zooplankton

Notes: Standard length was used as an initial size measure for the aquatic insects, whereas mass was used as an initial size measure for amphibians and snails. Values are means ± 1 SE. The tadpoles and snails are herbivores on periphyton, whereas the zooplankton are herbivores on phytoplankton.

† Density is the number of individuals per 1000-L experimental tank.

‡ Larval stages were used in the experiment.

within the range of natural densities based on seven years of quantitative surveys of natural aquatic habitats (R. A. Relyea, E. E. Werner, D. K. Skelly, and K. L. Yurewicz, *unpublished data*).

There were five pesticide treatments: controls (250 mL of water added), carbaryl, malathion, glyphosate, or 2,4-D. For all four chemicals, I wanted to simulate the impact of a direct overspray on a wetland. Thus, I purchased commercial forms of each chemical and had the concentrations of each chemical's active ingredient independently confirmed by the Mississippi State Laboratory (Mississippi State, Mississippi, USA) using high-pressure liquid chromatography (carbaryl, 22.3%; malathion, 50.6%; 2,4-D, 44.5%; glyphosate, 25.2%). Based on the surface area of the cattle tanks (2.41 m^2), I applied each chemical at the manufacturer's recommended maximum application rates (Sevin, 0.955 mL/m^2; malathion, 0.234 mL/m^2; 2,4-D, 0.117 mL/m^2; Roundup, 6.4 mL/m^2). Thus, I added 2.3 mL of Sevin, 0.6 mL of malathion, 0.3 mL of 2,4-D, and 15.3 mL of Roundup. Because the tanks contained 1000 L of water, these application rates translated to 0.51 mg carbaryl/L, 0.32 mg malathion/L, 0.12 mg 2,4-D/L, and 3.8 mg glyphosate/L. The pesticides were added immediately after all taxa had been added to the tanks (30 May).

On 12 June, the experiment was terminated. I began by first sampling the zooplankton using a 0.2-L tube sampler that was plunged into the tanks in the center and at each of the four cardinal directions. The five samples were combined and filtered through 62-μm Nitex screening (Small Parts, Miami, Florida, USA). All zooplankton were preserved in 70% ethanol and subsequently counted and identified to species. Next, the ceramic tiles were removed and the periphyton was scrubbed (using toothbrushes) onto oven-dried, preweighed filter paper. The algae-covered filters were oven-dried again for 15 h at 80°C and then weighed to determine the dry mass of algae on each tile. Finally, the tanks were drained and all macro-organisms were sorted from the leaves, counted, and weighed. Amphibians were preserved in 10% formalin and invertebrates were preserved in 70% ethanol.

Statistical analyses

I analyzed the data using ANOVAs. The first analysis examined the impact of the pesticides on total species richness of the animals in the community using a one-way ANOVA. The second analysis examined species richness and biomass of the four major functional groups: predators (insects and salamanders), large herbivores (snails and tadpoles), zooplankton, and periphyton algae (algae was not separated into species). The third set of analyses examined the abundance of individual species within each of the three animal groups (predators, large herbivores, and zooplankton). Because much of these latter data contained heterogeneous errors (some treatments had 0% survival), I first ranked the data and then conducted a MANOVA on the ranked values. When I found a significant multivariate effect,

I conducted univariate analyses. When I found significant univariate effects, I conducted mean comparison tests using Fisher's test. I weighed all animals coming out of the tanks at the end of the experiment and found no significant treatment effects on mass for any of the taxa ($P > 0.05$), so I chose to not include the mass data in the analysis. Thus any differences in biomass among treatments simply reflect differential survival across treatments. Two of the tanks developed an unusual red periphyton that was not present in any other tanks in the experiment (and had not been observed in dozens of previous mesocosm experiments). Both tanks had been randomly assigned the control treatment and both were removed from the analysis.

Results

The first analysis examined the impact of the pesticides on the species richness of all animal taxa in the communities (Fig. 1). There was a significant impact of pesticides on total animal richness ($F_{4,23} = 10.1$, $P < 0.001$). Compared to the control tanks, species richness was 15% lower with Sevin ($P = 0.041$), 30% lower with malathion ($P < 0.001$), and 22% lower with Roundup ($P = 0.005$). The addition of 2,4-D had no effect ($P = 0.543$).

The analysis of species richness and biomass by functional group produced a significant multivariate effect (Wilks' $F_{28,63} = 5.5$, $P < 0.001$; Fig. 1). The richness of predators, large herbivores (tadpoles and snails), and zooplankton were all affected by the treatments ($P < 0.001$). Predator richness declined with Sevin and malathion ($P < 0.03$), but not with 2,4-D or Roundup ($P > 0.35$). Large-herbivore richness decreased with Roundup ($P < 0.001$), but was not affected by the other three pesticides ($P > 0.7$). The richness of zooplankton declined significantly with Sevin ($P = 0.044$) and malathion ($P = 0.008$), but not with 2,4-D or Roundup ($P > 0.3$).

The biomass of predators, large herbivores, zooplankton, and periphyton also differed among treatments (univariate tests; $P < 0.03$; Fig. 2). Predator biomass was lower with Sevin, malathion, and Roundup ($P < 0.001$), but not with 2,4-D ($P = 0.406$). The biomass of the large herbivores was higher with Sevin ($P = 0.039$), unaffected by malathion and 2,4-D ($P > 0.25$), and lower with Roundup ($P = 0.024$). The abundance of zooplankton was not different between the control tanks and the four pesticide treatments ($P > 0.09$). Periphyton biomass was unaffected by Sevin, malathion, and 2,4-D ($P, > 0.15$), but was 40% greater with Roundup ($P = 0.028$).

In the remaining analyses, I examined the impact of pesticides on the survival of each species in the three functional groups. In the MANOVA on predator species, I found a significant multivariate effect of the pesticides (Wilks' $F_{28,63} = 2.5$, $P = 0.002$; Fig. 3). There were no pesticide effects on the survival of *Anax junius* dragonflies, water bugs (*Belostoma flumineum*), or damselflies (*Lestes* sp.) (univariate test, $P > 0.25$); marginally significant effects on the survival of *Dytiscus* beetles (univariate test, $P = 0.081$); and significant effects on the survival of *Tramea* dragonflies, backswimmers (*Neonecta undulata*), and spotted salamanders (*Ambystoma maculatum*) (univariate test, $P \leq 0.03$). *Dytiscus* beetles were eliminated with Sevin and malathion ($P = 0.054$), whereas *Tramea* dragonfly survival was reduced with malathion ($P = 0.016$) and nearly reduced with 2,4-D ($P = 0.065$). Backswimmer survival was increased with 2,4-D ($P = 0.035$), whereas spotted salamander survival was marginally higher with Sevin ($P = 0.075$) and significantly higher with 2,4-D ($P = 0.011$). No diving beetle (*Acilius semisulcatus*) larvae survived in any of the tanks.

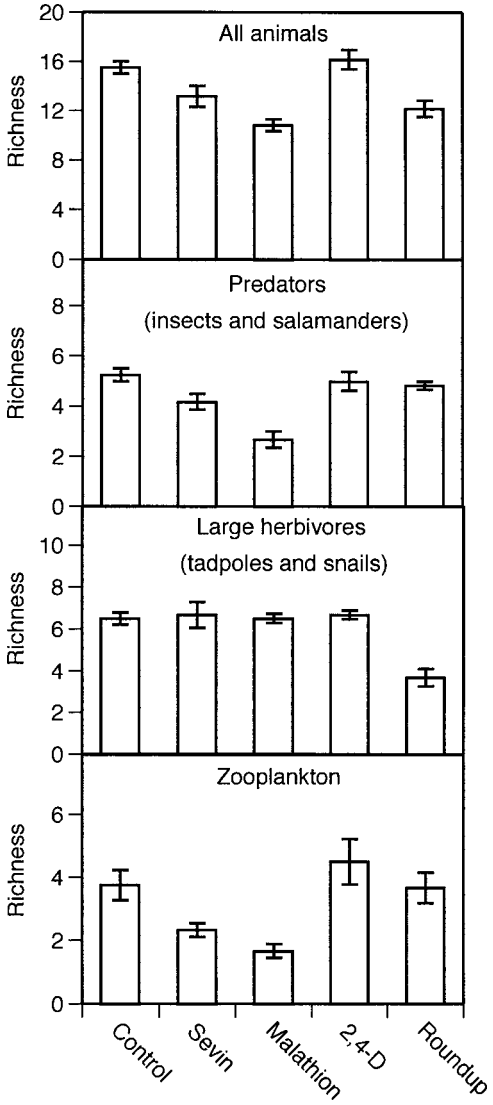

FIG. 1. The impact of four different pesticides on the species richness of predators (insects and spotted salamanders), large herbivores (tadpoles and snails), and zooplankton in aquatic mesocosm communities. Data are means ± 1 SE.

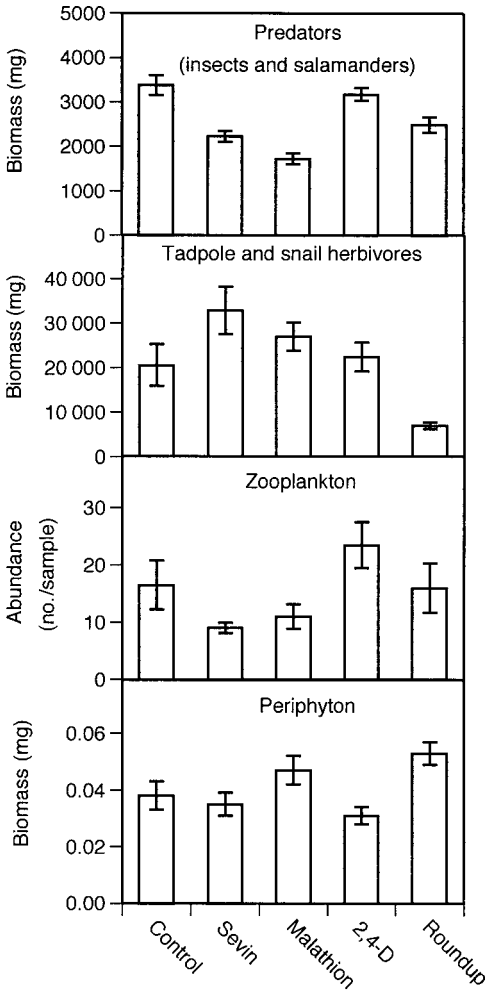

FIG. 2. The impact of four different pesticides on the biomass (or abundance) of predators (insects and spotted salamanders), large herbivores (tadpoles and snails), zooplankton, and periphyton in aquatic mesocosm communities. Data are means ± 1 SE.

In the MANOVA on zooplankton species, there was a significant multivariate effect of pesticides (Wilks' $F_{36,58} = 3.4$, $P < 0.001$). In univariate tests, there was no effect of the pesticides on *Daphnia longiremis*, *Ceriodaphnia* sp., *Scapholebris* sp., *Eurycyclops* sp., or *Leptochaptumorus* sp. ($P > 0.1$). However, there were significant impacts on *Daphnia pulex*, *Daphnia ambigua*, *Eurytemora* sp., and *Mesocyclops* sp. ($P \leq 0.02$; Fig. 4). *Daphnia pulex* was completely absent from tanks with Sevin or malathion ($P < 0.001$). *Daphnia ambigua* showed a similar pattern, although the effects of Sevin and malathion were not significantly different from the controls ($P = 0.063$ and $P = 0.136$, respectively). *Eurytemora* was more abundant with Sevin and malathion ($P \leq 0.03$), but nearly absent with Roundup ($P = 0.028$). *Mesocyclops* was more abundant with Sevin ($P = 0.021$), but was unaffected by the other pesticides.

In the MANOVA on the large herbivores, I found a significant multivariate effect of the pesticides (Wilks' $F_{32,61} = 2.9$, $P < 0.001$). There was no effect of pesticides on any of the three snail species (univariate tests, $P > 0.1$). Across all treatments, the mean survival (±1 SE) was 3 ± 1% for *Physa integra*, 24 ± 4% for *Stagnicola elodes*, and 61 ± 3% for *Helisoma trivolvis*. Among the tadpoles, there were significant impacts of pesticides on leopard frogs (*Rana pipiens*), wood frogs

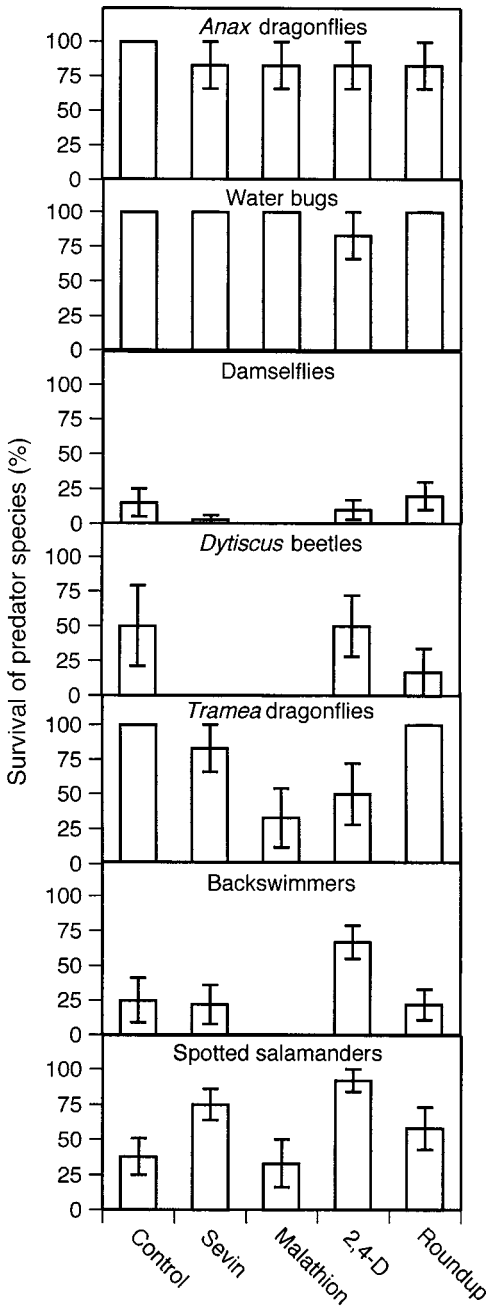

FIG. 3. The impact of four different pesticides on the survival of individual species of predators (insects and spotted salamanders). Data are means ± 1 SE.

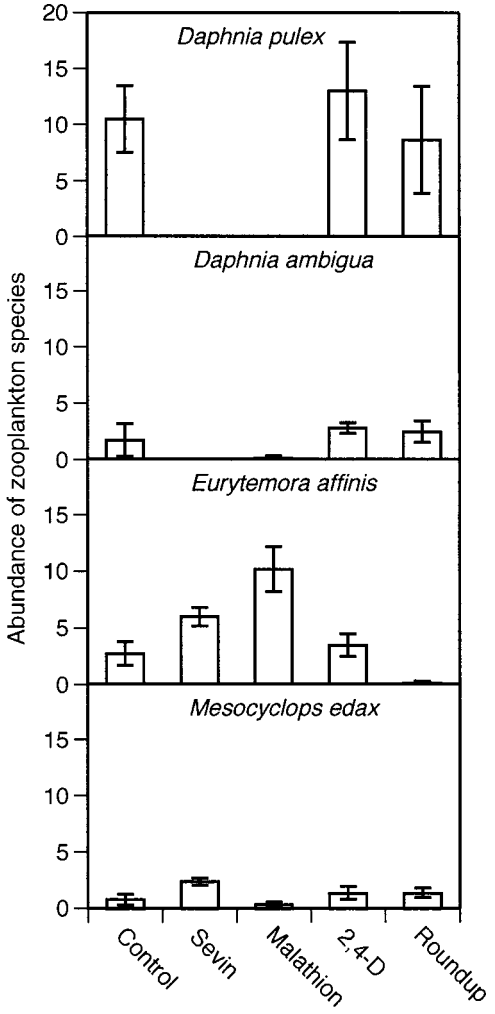

FIG. 4. The impact of four different pesticides on the abundance of individual species of zooplankton. Data are means ± 1 SE.

(*R. sylvatica*), and gray tree frogs (*Hyla versicolor*) (univariate tests, $P < 0.01$; Fig. 5) but no impacts on toads (*Bufo americanus*) or spring peepers (*Pseudacris crucifer*) ($P \geq 0.09$). Leopard frog survival improved from 28% to 58% with Sevin ($P = 0.037$) and 28% to 43% with malathion, but the latter effect was not significant ($P = 0.391$). Leopard frogs were completely exterminated with Roundup ($P = 0.004$). Gray tree frog survival was unaffected by the insecticides, but gray tree frogs were eliminated with Roundup ($P = 0.003$). Wood frog survival improved from 50% to 72% with Sevin ($P = 0.054$) and 50% to 67% with malathion, although the latter effect was not significant ($P = 0.194$). Wood frog survival was reduced to only 2% with Roundup ($P = 0.012$). None of the species was affected by 2,4-D ($P > 0.5$).

DISCUSSION

The results of this study indicate that pesticides can have profound impacts on the diversity and productivity of aquatic communities over relatively short time scales (two weeks). However, the impacts on the communities were very pesticide specific. As expected, the two insecticides reduced the diversity and biomass of the insect predators, completely exterminating *Dytiscus* beetles and reducing the abundance of *Tramea* and backswimmers (the latter was only reduced with malathion). This effect was predictable from the large literature on the susceptibility of aquatic insects and crustaceans to carbaryl and malathion. The $LC50_{72\text{–}96\,h}$ values range from 0.005 to 0.026 mg/L for carbaryl (USDI 1980) and 0.005 to 0.18 mg/L for malathion (USDI 1980, Key et al. 1998, Leight and Van Dolah 1999).

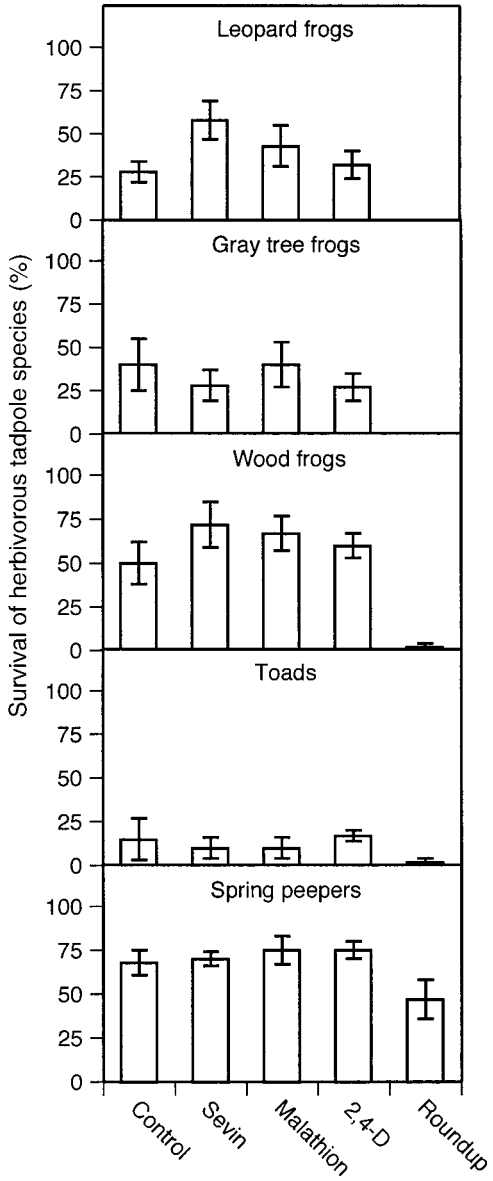

FIG. 5. The impact of four different pesticides on the survival of individual species of herbivorous tadpoles. Data are means ± 1 SE.

Interestingly, the two insecticides had no effect on two of the insect species (*Anax* dragonflies and water bugs; few damselflies survived in any of the treatments, making it difficult to draw any firm conclusions), suggesting that insects vary in their susceptibility to the insecticides (when applied at these recommended rates). In other words, the insecticides did not eliminate the entire insect community. Thus, although predation from aquatic insects can be reduced with the application of insecticides, major predators such as *Anax* dragonflies (Relyea 2001, 2003*a*) will continue to consume prey (although pesticide effects on the foraging behavior of these predators are unknown).

In addition to the effects on insects, the insecticides also affected the zooplankton by eliminating cladocerans while favoring copepods. The change in zooplankton community composition with acetylcholine esterase-inhibiting insecticides is in accord with a number of previous studies. At higher concentrations (>1 mg/L), carbaryl (the active ingredient of Sevin) can completely wipe out nearly all species of zooplankton. However, under lower concentrations, such as those used in the current study, carbaryl only eliminates cladoceran zooplankton. As a result, the phytoplankton resource can increase and provide an indirect, positive effect on the abundance of grazing copepods (copepod body size also may have increased, but this was not measured). However, copepods typically cannot graze the smallest algae that are consumed by the cladocerans; hence, the copepod populations are unable to completely compensate for the decrease in cladoceran abundance (Hanazato and Yasuno 1990, Hanazato 1991, Havens and Hanazato 1993, Havens 1994, 1995, Wong et al. 1995). Thus, it appears that these two insecticides can have both direct and indirect effects on zooplankton.

At the concentrations used, the insecticides were predicted to have no direct negative effects on the survival of the large herbivores (snails and tadpoles). There appear to be very few comparative data addressing the impacts of carbaryl and malathion on snails (Martinez-Tabche et al. 2002), but the current study suggests minimal impacts. In contrast, we have a large number of studies on the impacts of carbaryl and malathion on tadpoles. The LC50 for carbaryl ranges from 1 to 18 mg/L (Marchal-Segault 1976, Marian et al. 1983, Bridges 1997, Zaga et al. 1998, Relyea and Mills 2001, Relyea 2003*b*) for all amphibians and from 1.2 to 3.4 mg/L for the populations of wood frogs, leopard frogs, toads, and gray tree frogs used in the current study (Relyea [2003b]; including LC50 estimates when Sevin is combined with predator chemical cues). The LC50 values for malathion range from 1.2 to 5.9 across all amphibians, including the populations of wood frogs, leopard frogs, toads, and gray tree frogs used in the current study (Fordham et al. 2001, Relyea 2004). Because the current study used concentrations of carbaryl and malathion that were well under these LC50 values (0.51 and 0.32 mg/L, respectively), there should have been minor negative impacts of insecticides on tadpole survival and this is what I observed.

Interestingly, the survival of tadpoles actually increased with the addition of insecticides; this was probably an indirect effect of high predator mortality. The addition of Sevin reduced the biomass of the insect predators by 44%, increased tadpole (wood frog and leopard frog) survival by 22–30%, and increased total tadpole biomass by 85%. Similarly, the salamander larvae (which were small and susceptible to insect predation) experienced a 37% increase in survival when Sevin was added. While the addition of malathion reduced the biomass of the insect predators by a similar amount as Sevin (48%), the 15–17% increase in wood frog and leopard frog survival was not significant and the salamander survival was unchanged. Thus, changes in predator biomass do not completely explain changes in herbivore survival, suggesting that we also need to examine how the different pesticides affect the foraging behavior of the surviving predators. For example, Boone and Semlitsch (2001, 2002) found that carbaryl (in the absence of insect predators) can have both positive and negative effects on tadpole survival. In contrast to the tadpoles, snails did not experience a positive indirect effect on their biomass because the specialist snail predator (*Belostoma*) was not killed by the insecticides. This suggests that although higher concentrations of carbaryl and malathion certainly can kill many amphibians (>5 mg/L; Boone and Semlitsch 2001, 2002, Relyea 2003*b*, 2004), under lower concentrations these insecticides, and perhaps other insecticides that share the same mode of action, actually can have positive indirect effects on the survival and biomass of tadpoles. Thus, in assessing the impacts of insecticides on amphibians, it is critical that we consider both relevant concentrations and relevant ecological contexts.

The two herbicides had very different effects on the community than the two insecticides. Although glyphosate and 2,4-D are designed to kill plants, they did not reduce the biomass of periphyton in the experiment. In fact, 2,4-D had few effects on any species or trophic group in the entire community during the 14-day experiment (only backswimmers and spotted salamanders increased survival with 2,4-D, although the causes are unclear). This general lack of impact from 2,4-D is consistent with past toxicity studies that have found relatively high $LC50_{96\text{-}h}$ values for 2,4-D, including 45 mg/L for lake trout (*Salvelinus namaycush*), 301 mg/L for American eels (*Anguilla rostrata*), and 363–389 mg/L for cladocerans (*Daphnia magna*; USDI 1980, Verschueren 1983). In this system, 2,4-D appeared to have no substantial impact on a diverse aquatic community.

In stark contrast, Roundup had a major effect on the community. Roundup reduced tadpole richness by 70% by completely exterminating two species (leopard frogs

and gray tree frogs) and nearly exterminated a third species (wood frogs). Roundup did not have a significant effect on toads, spring peepers, and the spotted salamanders, although few toads survived even in the control treatments, making it difficult to assess the effects of Roundup on survival. These reductions in tadpole survival were concomitant with a decrease in predator biomass, suggesting that Roundup also caused a trophic cascade from the herbivores to the predators. In comparison to the 3.8 mg/L of glyphosate used in the mesocosm study (based on the manufacturer's recommended application rate; AI = active ingredient), concentrations of glyphosate in nature have been observed up to 2.3 mg AI/L and are capable of being as high as 3.7 mg AI/L (Giesy et al. 2000).

Giesy et al. (2000) recently reviewed the toxicity of glyphosate and found that its toxicity (expressed as mg of active ingredient per liter) to invertebrates can be quite high, ranging from 3.5 mg AI/L in crayfish (*Orconectes nais*; $LC50_{96\text{-h}}$) to 5600 mg AI/L in midge larvae (*Chironomus riparius*; $LC50_{48\text{-h}}$). As expected from these previous studies, glyphosate had no effect on the insect predators and snails in the mesocosm experiment. Glyphosate also has a wide range of toxic effects on fish, ranging from 3.5 mg AI/L in *Tilapia* sp. ($LC50_{96\text{-h}}$) to >1300 mg AI/L in sheepshead minnows (*Cyprinodon variegatus*; $LC50_{96\text{-h}}$). Prior tests of glyphosate on amphibians have been rare. In four species of Australian tadpoles (*Crinia insignifera*, *Heleioporus eyrei*, *Limnodynastes dorsalis*, and *Litoria moorei*), Mann and Bidwell (1999) found that $LC50_{48\text{-h}}$ values in the laboratory ranged from 3.9 to 15.5 mg AI/L for Roundup (glyphosate plus POEA surfactant), 108 to 161 mg AI/L for technical grade glyphosate acid, and >450 mg AI/L for glyphosate isopropylamine salt (the latter two formulations lack the POEA surfactant). Perkins et al. (2000) conducted laboratory experiments on *Xenopus laevis* tadpoles and found $LC50_{96\text{-h}}$ values of 12.4 mg AI/L for Roundup, 6.8 mg/L for the POEA surfactant alone, and 9729 mg AI/L for Rodeo (an aquatic form of glyphosate that lacks the POEA surfactant). Smith (2001) examined the impact of Kleeraway (another form of glyphosate that includes the POEA surfactant) and found that nearly half of western chorus frog tadpoles (*Pseudacris triseriata*) died at 0.75 mg AI/L; plains leopard frog larvae (*Rana blairi*) experienced 0% and 100% survival at 0.75 mg AI/L in two separate experiments. All tadpoles of both species died at higher concentrations (7.5, 750, and 7500 mg AI/L). These studies suggest that the high mortality associated with commercial forms of Roundup is actually due to the POEA surfactant and not to glyphosate itself.

The high mortality rates of tadpoles associated with Roundup are in agreement with those of several other experiments that I have recently completed on tadpole species from the midwestern United States. Using static exposure experiments in the laboratory, I reared six different species of tadpoles under a range of Roundup concentrations to estimate the LC50 values. The estimated $LC50_{16\text{-d}}$ values for these North American species were lower than previously observed for most amphibian species (Mann and Bidwell 1999, Perkins et al. 2000), ranging from 0.5 to 2.5 mg AI/L (Relyea, *in press*). This suggests that a direct overspray at the manufacturer's recommended rate (a realized pond concentration of 3.8 mg/L) should be highly lethal to these amphibians. The current study is consistent with this prediction.

I have also conducted a second outdoor mesocosm experiment in the absence of predators (to eliminate this source of mortality) and with the addition of either no soil, sand, or loam (because soil is known to absorb the two components of Roundup (glyphosate and the POEA surfactant) and remove them from the water column; Giesy et al. 2000). I exposed communities of three tadpoles species to 3.8 mg AI/L of glyphosate (in the form of Roundup, similar to the current experiment) and found that it reduced tree frog tadpole survival from 75% to 2%, toad tadpole survival from 97% to 0%, and leopard frog tadpole survival from 98% to 4% (R. A. Relyea, *unpublished manuscript*). Moreover, the addition of soil did not diminish the toxic effect. Collectively, the available data indicate that, contrary to conventional wisdom, current application rates of Roundup can be highly lethal to many species of amphibians. This result is of particular interest in light of the global decline of amphibians (Wake 1998, Alford and Richards 1999, Houlihan et al. 2001, Blaustein and Kiesecker 2002) which, in some cases, is correlated with a proximity to agricultural areas that use pesticides (Bishop et al. 1999, Davidson et al. 2001, 2002, Sparling et al. 2001).

Although Roundup is an herbicide, two lines of evidence suggest that the widespread tadpole mortality was directly due to toxicity and not to the destruction of the tadpoles' algal food source. First, much of the mortality occurred within the first 24 hours (*personal observations*). This mortality rate was much faster than would be expected to occur with a lack of food (Audo et al. 1995) and was consistent with our single-species laboratory experiments that did not use algal food sources (Relyea, *in press*). Second, the biomass of periphyton did not decrease with Roundup. Roundup actually caused a 40% increase in periphyton by removing a large fraction of the herbivores and allowing periphyton to attain a higher standing crop. Thus, there was a positive, indirect effect of Roundup on periphyton. This indicates that Roundup directly kills amphibians rather than indirectly causing amphibians to starve to death.

Conclusions

This study highlights the importance of examining the impact of pesticides within the natural ecological context in which the taxa live. Single-species toxicity

studies are invaluable to assess the relative lethality of different chemicals on both target and nontarget species. However, when toxicity studies are embedded in the nexus of interactions that compose natural food webs, we can arrive at very different interpretations due to the prevalence of both direct and indirect effects. At realistic concentrations, the two insecticides had substantial negative effects on the predatory insects and cladocerans, but they had substantial indirect positive effects on the copepods and tadpoles. The two herbicides had quite contrasting effects; 2,4-D had no impact on the community, whereas Roundup caused a major reduction in amphibian diversity, an indirect, positive impact on the periphyton that the tadpoles consume, and an indirect, negative effect on the biomass of insect predators. It is important to note that these impacts occurred over relatively short time scales (two weeks). Over longer time scales (months to years, depending on the species), many of the taxa have the potential to recover their population sizes, provided that the pesticide exposure is not a recurring event.

Although there is currently a strong empirical and theoretical push to understand the factors that determine species diversity and abundance in relatively pristine systems (Tilman et al. 2001, Chase and Leibold 2002, Downing and Leibold 2002, Naeem 2002), few habitats are untouched by anthropogenic effects, including the direct application or drift of pesticides (Lambert 1997, LeNoir et al. 1999, Leonard et al. 1999, Favari et al. 2002). We need to understand how these effects impact natural systems and whether they contribute to the global decline in biodiversity.

Acknowledgments

My thanks to Josh Auld, Jason Hoverman, Laura Howell, Adam Marko, and Nancy Schoeppner for assisting with the experiments. I thank Josh Auld, Jason Hoverman, April Randle, and Nancy Schoeppner for reviewing the manuscript. The research was supported by the National Science Foundation.

Literature Cited

Alford, R. A., and S. J. Richards. 1999. Global amphibian declines: a problem in applied ecology. Annual Review of Ecology and Systematics **30**:133–165.

Aly, O. M., and M. A. El-Dib. 1971. Studies on the persistence of some carbamate insecticides in the aquatic environment–I: hydrolysis of sevin, baygon, pyrolan and dimetilan in waters. Water Research **5**:1191–1205.

Audo, M. C., T. M. Mann, T. L. Polk, C. M. Loudenslager, W. J. Diehl, and R. Altig. 1995. Food deprivation during different periods of tadpole (*Hyla chrysoscelis*) ontogeny affects metamorphic performance differently. Oecologia **103**:518–522.

Bishop, C. A., N. A. Mahony, J. Struger, P. Ng, and K. E. Pettit. 1999. Anuran development, density and diversity in relation to agricultural activity in the Holland River watershed, Ontario, Canada (1990–1992). Environmental Monitoring and Assessment **57**:21–43.

Blaustein, A. R., and J. M. Kiesecker. 2002. Complexity in conservation: lessons from the global decline of amphibian populations. Ecology Letters **5**:597–608.

Boone, M. D., and S. M. James. 2003. Interactions of an insecticide, herbicide, and natural stressors in amphibian community mesocosms. Ecological Applications **13**:829–841.

Boone, M. D., and R. D. Semlitsch. 2001. Interactions of an insecticide with larval density and predation in experimental amphibian communities. Conservation Biology **15**: 228–238.

Boone, M. D., and R. D. Semlitsch. 2002. Interactions of an insecticide with competition and pond drying in amphibian communities. Ecological Applications **12**:307–316.

Bridges, C. M. 1997. Tadpole swimming performance and activity affected by acute exposure to sublethal levels of carbaryl. Environmental Toxicology and Chemistry **16**: 1935–1939.

Chase, J. M., and M. A. Leibold. 2002. Spatial scale dictates the productivity–biodiversity relationship. Nature **416**: 427–430.

Davidson, C., H. B. Shafer, and M. R. Jennings. 2001. Declines of the California red-legged frog: climate, UV-B, habitat, and pesticides hypotheses. Ecological Applications **11**:464–479.

Davidson, C., H. B. Shafer, and M. R. Jennings. 2002. Spatial tests of the pesticide drift, habitat destruction, UV-B, and climate-change hypotheses for California amphibian declines. Conservation Biology **16**:1588–1601.

Diana, S. G., W. J. Resetarits, Jr., D. J. Shaeffer, K. B. Beckman, and V. R. Beasley. 2000. Effects of atrazine on amphibian growth and survival in artificial aquatic communities. Environmental Toxicology and Chemistry **19**:2961–2967.

Donaldson, D., T. Kiely, and A. Grube. 2002. Pesticides industry sales and usage: 1998 and 1999 market estimates. USEPA Report Number 733-R-02-001, U.S. Environmental Protection Agency, Washington, D.C., USA.

Downing, A. L., and M. A. Leibold. 2002. Ecosystem consequences of species richness and composition in pond food webs. Nature **416**:837–841.

Favari, L., E. Lopez, L. Martinez-Tabche, and E. Diaz-Pardo. 2002. Effect of insecticides on plankton and fish of Ignacio Ramirez reservoir (Mexico): a biochemical and biomagnification study. Ecotoxicology and Environmental Safety **51**:177–186.

Fordham, C. L., J. D. Tessari, H. S. Ramsdell, and T. J. Keefe. 2001. Effects of malathion on survival, growth, development, and equilibrium posture on bullfrog tadpoles (*Rana catesbeiana*). Environmental Toxicology and Chemistry **20**:179–184.

Giesy, J. P., S. Dobson, and K. R. Solomon. 2000. Ecotoxicological risk assessment for Roundup® herbicide. Reviews of Environmental Contamination and Toxicology **167**:35–120.

Guerrant, G. O., L. E. Fetzer, Jr., and J. W. Miles. 1970. Pesticide residues in Hale County, Texas, before and after ultra-low volume aerial application of malathion. Pesticide Monitoring Journal **4**:14–20.

Hanazato, T. 1991. Effects of long- and short-term exposure to carbaryl on survival, growth, and reproduction of *Daphnia ambigua*. Environmental Pollution **74**:139–148.

Hanazato, T., and M. Yasuno. 1987. Effects of a carbamate insecticide, carbaryl, on summer phyto- and zooplankton communities in ponds. Environmental Pollution **48**:145–159.

Hanazato, T., and M. Yasuno. 1989. Effects of carbaryl on spring zooplankton communities in ponds. Environmental Pollution **56**:1–10.

Hanazato, T., and M. Yasuno. 1990. Influence of time of application of an insecticide on recovery patterns of a zooplankton community in experimental ponds. Archives of Environmental Contamination and Toxicology **19**:77–83.

Harris, M. L., C. A. Bishop, J. Struger, B. Ripley, and J. P. Bogart. 1998. The functional integrity of northern leopard

frog (*Rana pipiens*) and green frog (*Rana clamitans*) populations in orchard wetlands. II. Genetics, physiology, and biochemistry of breeding adults and young-of-the-year. Environmental Toxicology and Chemistry **17**:1338–1350.
Havens, K. E. 1994. An experimental comparison of effects of two chemical stressors on a freshwater zooplankton assemblage. Environmental Pollution **84**:245–251.
Havens, K. E. 1995. Insecticide (carbaryl, 1-napthyl-n-methylcarbamate) effects on a freshwater plankton community: zooplankton size, biomass, and algal abundance. Water Air and Soil Pollution **84**:1–10.
Havens, K. E., and T. Hanazato. 1993. Zooplankton community responses to chemical stressors: a comparison of results from acidification and pesticide contamination research. Environmental Pollution **82**:277–288.
Houlihan, J. E., C. S. Findlay, B. R. Schmidt, A. H. Meyers, and S. L. Kuzmin. 2001. Quantitative evidence for global amphibian population declines. Nature **404**:752–755.
Key, P. B., M. H. Fulton, G. I. Scott, S. L. Layman, and E. F. Wirth. 1998. Lethal and sublethal effects of malathion on three life stages of the grass shrimp, *Palaemonetes pugio*. Aquatic Toxicology **40**:311–322.
Lambert, M. R. K. 1997. Environmental effects of heavy spillage from a destroyed pesticide store near Hargeisa (Somaliland) assessed during the dry season, using reptiles and amphibians as bioindicators. Archives of Environmental Contamination and Toxicology **32**:80–93.
Leight, A. K., and R. F. Van Dolah. 1999. Acute toxicity of the insecticides endosulfan, chlorpyrifos, and malathion to the epibenthic estuarine amphipod *Gammarus palustris* (Bousefield). Environmental Toxicology and Chemistry **18**: 958–964.
LeNoir, J. S., L. L. McConnell, G. M. Fellers, T. M. Cahill, and J. N. Seiber. 1999. Summertime transport of current-use pesticides from California's central valley to the Sierra Nevada Mountain range, USA. Environmental Toxicology and Chemistry **18**:2715–2722.
Leonard, A. W., R. V. Hyne, R. P. Lim, and J. C. Chapman. 1999. Effect of endosulfan runoff from cotton fields on macroinvertebrates in the Namoi River. Ecotoxicology and Environmental Safety **42**:125–134.
Mann, R. M., and J. R. Bidwell. 1999. The toxicity of glyphosate and several glyphosate formulations to four species of southwestern Australian frogs. Archives of Environmental Contamination and Toxicology **26**:193–199.
Marchal-Segault, D. 1976. Toxicité de quelques insecticides pour des têtards de *Bufo bufo* (Amphibiens, Anoures). Bulletin Ecology **7**:411–416.
Marian, M. P., V. Arul, and T. J. Pandian. 1983. Acute and chronic effects of carbaryl on survival, growth, and metamorphosis in the bullfrog (*Rana tigrina*). Archives of Environmental Contamination and Toxicology **12**:271–275.
Martinez-Tabche, L., M. M. Galar, H. E. Olvera, R. A. Chehue, E. L. Lopez, L. Gomez-Olivan, and O. T. Sierra. 2002. Toxic effect and bioavailability of malathion spiked in natural sediments from the Ignacio Ramirez dam on the snail *Stagnicola* sp. Ecotoxicology and Environmental Safety **52**: 232–237.
McConnell, L. L., J. S. LeNoir, S. Datta, and J. N. Seiber. 1998. Wet deposition of current-use pesticides in the Sierra Nevada Mountain range, California, USA. Environmental Toxicology and Chemistry **17**:1908–1916.
Morin, P. J. 1981. Predatory salamanders reverse outcome of competition among three species of anuran tadpoles. Science **212**:1284–1286.
Naeem, S. 2002. Ecosystem consequences of biodiversity loss: the evolution of a paradigm. Ecology **83**:1537–1552.
Perkins, P. J., H. J. Boermans, and G. R. Stephenson. 2000. Toxicity of glyphosate and triclopyr using the frog embryo teratogenesis assay—*Xenopus*. Environmental Toxicology and Chemistry **19**:940–945.
Relyea, R. A. 2001. The relationship between predation risk and anti-predator responses in larval anurans. Ecology **82**: 541–554.
Relyea, R. A. 2003*a*. How prey respond to combined predators: a review and an empirical test. Ecology **84**:1827–1839.
Relyea, R. A. 2003*b*. Predator cues and pesticides: a double dose of danger for amphibians. Ecological Applications **13**: 1515–1521.
Relyea, R. A. 2004. Synergistic impacts of malathion and predatory stress on six species of North American tadpoles. Environmental Toxicology and Chemistry **23**:1080–1084.
Relyea, R. A. *In press*. The lethal impacts of Roundup and predatory stress on six species of North American tadpoles. Archives of Environmental Contamination and Toxicology.
Relyea, R. A., and N. Mills. 2001. Predator-induced stress makes the pesticide carbaryl more deadly to grey treefrog tadpoles (*Hyla versicolor*). Proceedings of the National Academy of Sciences (USA) **98**:2491–2496.
Relyea, R. A., and K. L. Yurewicz. 2002. Predicting community outcomes from pairwise interactions: integrating density- and trait-mediated effects. Oecologia **131**:569–579.
Smith, G. R. 2001. Effects of acute exposure to a commercial formulation of glyphosate on the tadpoles of two species of anurans. Bulletin of Environmental Contamination and Toxicology **67**:483–488.
Sparling, D. W., G. M. Fellers, and L. S. McConnell. 2001. Pesticides and amphibian population declines in California, USA. Environmental Toxicology and Chemistry **20**:1591–1595.
Tilman, D., P. B. Reich, J. Knops, D. Wedin, T. Mielke, and C. Lehman. 2001. Diversity and productivity in a long-term grassland experiment. Science **294**:843–845.
USDI [U.S. Fish and Wildlife Service]. 1980. Handbook of acute toxicity of chemicals to fish and aquatic invertebrates. Resource Publication Number 137, U.S. Government Printing Office, Washington, D.C., USA.
Verschueren, K. 1983. Handbook of environmental data of organic chemicals. Second edition. Van Nostrand Reinhold, New York, New York, USA.
Wake, D. B. 1998. Action on amphibians. Trends in Ecology and Evolution **13**:379–380.
Wang, T. 1991. Assimilation of malathion in the Indian River estuary, Florida. Bulletin of Environmental Contamination and Toxicology **47**:238–243.
Wauchope, R. D., and R. Haque. 1973. Effects of pH, light and temperature on carbaryl in aqueous media. Bulletin of Environmental Contamination and Toxicology **9**:257–260.
Werner, E. E., and B. R. Anholt. 1996. Predator-induced behavioral indirect effects: consequences to competitive interactions in anuran larvae. Ecology **77**:157–169.
Wong, C. K., K. H. Chu, and F. F. Shum. 1995. Acute and chronic toxicity of malathion to the fresh-water cladoceran *Moina macrocarpa*. Water Air and Soil Pollution **84**:399–405.
Zaga, A., E. E. Little, C. F. Raben, and M. R. Ellersieck. 1998. Photoenhanced toxicity of a carbamate insecticide to early life stage anuran amphibians. Environmental Toxicology and Chemistry **17**:2543–2553.