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EFFECTS OF AN HERBICIDE AND AN INSECTICIDE ON POND COMMUNITY STRUCTURE AND PROCESSES

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Abstract. Virtually all species live within complex food webs, and many of these organisms are exposed to contaminants. However, we know little about how community processes, such as competition and predation, influence susceptibility to contaminants or how different types of contaminants shape communities. The objective of our study was to determine how realistic concentrations of the herbicide atrazine and the insecticide endosulfan influence the structure of a pond community when the presence of common community members was manipulated. We employed a factorial design in mesocosms to evaluate the effects of pesticide treatments (25 μg/L of atrazine, 10 μg/L of endosulfan, solvent control; two pulses separated by two weeks) and the presence or absence of wood frog tadpoles (*Rana sylvatica*), adult snails (*Planorbella trivolvis*), and caged dragonfly larvae (*Anax junius*) on a freshwater community.

Tadpoles, snails, and chironomid larvae, *Polypedilum* sp. (Dipterans), all competed for periphyton. As a result, tadpoles reduced the survival, mass, and reproduction of snails; snails reduced the growth, development, inactivity, and dragonfly avoidance of tadpoles; snails and tadpoles reduced the abundance of chironomid larvae; and chironomid larvae reduced snail mass. The adverse effect of snails on tadpole growth and behavior was greater in the presence of the caged tadpole predator, *A. junius*. Neither pesticide affected dragonfly survival, but endosulfan directly reduced zooplankton (*Daphnia*), and atrazine indirectly reduced chironomid abundance. Atrazine also directly decreased periphyton, and endosulfan decimated chironomid larvae, resulting in indirect increases and decreases in competition for both snails and tadpoles, respectively. Consequently, relative to endosulfan, atrazine tended to decrease snail mass and reproduction and reduce tadpole mass, development, inactivity, refuge use, and dragonfly avoidance. However, the indirect effects of pesticides depended upon the presence of heterospecifics. The indirect benefit of endosulfan on snail mass was greater in the presence of caged dragonfly larvae, and endosulfan's indirect benefit on tadpole mass was greater in the absence of snails. The effect of pesticides on tadpole activity depended on both caged dragonflies and snails. Thus, environmentally realistic concentrations of pesticides directly and indirectly shaped species responses and community composition, but the initial composition of the community influenced these pesticide effects. These results emphasize the importance of quantifying the effects of contaminants within complex natural communities.

Key words: amphibian declines; anurans; behavior; Daphnia; *food web; metamorphosis; periphyton; trematode; trophic cascade.*

INTRODUCTION

One of the central principles of community ecology is that changes in the density or behavior of organisms can have effects that proliferate across and within trophic levels of food webs, influencing these same population- and individual-level traits in other species of the community (Morin 1999). A fundamental principle of evolutionary ecology is that species experience different evolutionary histories and selection pressures, and consequently, can differ vastly in their tolerances to stressors (Futuyma 1998). What then syllogistically follows from these two tenets is that the susceptibility of a given species to a stressor can have trophic-mediated positive or negative effects on other species in the community, independent of these species susceptibility to the same stressor. For instance, exposure to a pesticide may have no detectable direct effects on a focal organism, but may directly reduce the density or alter the behavior of its prey, competitors, or predators, and thus may have profound detrimental or beneficial indirect effects that are only apparent in the context of its community.

Pesticides are one of many stressors which can threaten the natural functioning of communities. There

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are approximately 63 000 pesticides registered in the United States (Ramade 1988), and many can be toxic to nontarget organisms, which can then impact other community members. In fact, indirect effects of contaminants have been detected in nearly all aquatic systems where they have been studied and may be more common and more important than direct effects (Fleeger et al. 2003). Nevertheless, indirect effects of toxicants are one of the more understudied areas of ecotoxicology (Clements and Kiffney 1994, Boyle and Fairchild 1997, Clements and Newman 2002). Most toxicology studies are conducted in the laboratory on organisms that are isolated from the species and abiotic environment with which they naturally coexist, and thus these studies cannot reveal indirect effects (Kimball and Levin 1985, Kapustka et al. 1996). Consequently, it is difficult to extrapolate from single-species, laboratory studies to effects in nature (Cairns 1983, Joern and Hoagland 1996).

While it is common for community-level toxicology studies to focus on how contaminants directly and indirectly affect community composition, few studies have assessed how community composition affects responses to contaminants (Boyle and Fairchild 1997; but see Hanazato and Yasuno 1990, Hanazato 1991). This requires manipulating the presence of species within a community and determining how the remaining species respond to the contaminated environment. Food web manipulations also serve to experimentally distinguish between direct and indirect effects and to elucidate the trophic links propagating indirect effects. By revealing the mechanistic underpinnings of indirect effects, factorial food web manipulations can improve our limited understanding of toxicant effects on natural communities.

The likelihood of inaccurately estimating the effects of contaminants in nature is of great concern because the current rate of species extinction is estimated to be greater than any known in the last 100 000 years (Wilson 1992). These potential inaccuracies are especially disconcerting for the health of aquatic organisms because pesticides often accumulate and concentrate in water bodies (Streit 1992), and many aquatic organisms are in decline or already extinct. For example, amphibians are declining globally (Blaustein and Wake 1990), and declines in the western United States have been correlated with physiological signatures of insecticide exposure (i.e., reduced cholinesterase activity and increased insecticide concentrations in tissues; Sparling et al. 2001), and with greater amounts of upwind agriculture (Davidson et al. 2001, 2002). These findings suggest that pesticide exposure may be a contributing factor in some amphibian declines.

The objectives of our experiment were (1) to determine whether environmentally realistic concentrations of the commonly used herbicide, atrazine (Solomon et al. 1996), and the widely applied insecticide, endosulfan (Ernst et al. 1991, Broomhall and Shine 2003), have effects that propagate through a pond community, and (2) to better understand how variation in species composition influences the effects of pesticides. To accomplish these objectives we reared pond communities in mesocosms manipulating pesticide treatments and the presence of adult snails (*Planorbella trivolvis*), wood frog tadpoles (*Rana sylvatica*), and caged dragonfly larvae (*Anax junius*), the latter of which is a predator of larval wood frogs. We hypothesized that adult *P. trivolvis* and *R. sylvatica* tadpoles would compete for periphytic (adherent) algae, but that this competitive relationship would be influenced by pesticide and dragonfly treatments. We expected atrazine to have net negative effects on *P. trivolvis* and *R. sylvatica* by reducing periphytic algae, and endosulfan to have net positive effects on these species by reducing insect predators (dragonfly larvae) and competitors (chironomid larvae). The magnitude of pesticide-induced, indirect effects was expected to depend upon the composition of the community (i.e., competition and predation).

METHODS AND MATERIALS

Chemical contaminants

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine) is an inhibitor of photosynthesis that is used predominantly for weed control in corn and sorghum production. It is the most commonly applied herbicide in the United States and possibly the world, with use in over 80 countries. Atrazine is relatively mobile, washing into water bodies in the spring and early summer during the embryo and larval development of many amphibians (Solomon et al. 1996). In surface waters, atrazine seldom exceeds 50 μg/L (Solomon et al. 1996), but in water bodies near agricultural areas, atrazine levels have been reported at and above 500 μg/L (e.g., Kadoum and Mock 1978, de Noyelles et al. 1982). Atrazine is relatively persistent with reported half-lives in ponds and mesocosms ranging from 95 to 350 days (Diana et al. 2000).

Endosulfan(6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide) is an organochlorine insect neurotoxin used extensively worldwide on an assortment of crops, including cotton and a variety of fruits, vegetables, and grains. Concentrations of endosulfan near agricultural fields have been reported as high as 700 μg/L (Ernst et al. 1991). Transport of the parent compound is mainly through aerial drift, but its primary toxic degradate, endosulfan sulfate, enters water bodies with surface run-off (Leonard et al. 1999, Broomhall 2002). Like atrazine, endosulfan peaks in water bodies during the spring, when many amphibians breed and their embryos and larvae are developing. But, unlike atrazine, endosulfan is relatively short lived. The toxic metabolite, endosulfan sulfate, has a reported half-life of a few weeks in freshwater (Miles and Moy 1979).

Animal collection and maintenance

In late February 2003, we collected 20 *R. sylvatica* clutches from Gray's Arch Pond in Stanton, Kentucky. We hatched and reared these tadpoles at the University of Kentucky's Ecological Research Facility in outdoor wading pools containing aged tap water and covered with 60% shade cloth. Tadpoles were fed rabbit chow ad libitum and clutches were mixed thoroughly before their use in the experiment to homogenize genetic variation. Adult *P. trivolvis* were collected in early March 2003 from a pond at the University of Kentucky's Ecological Research Facility and were raised in pond water in the laboratory until their use in the experiment. Late-instar *A. junius* were collected in early March 2003 from various ponds at the University of Kentucky's Ecological Research Facility. Before the experiment, they were held individually in the laboratory and fed aquatic invertebrates and *R. sylvatica* tadpoles.

Experimental design and treatments

To quantify the effects of atrazine and endosulfan on a typical pond community, we employed a 3 × 2 × 2 × 2 completely randomized factorial design with three pesticides treatments (solvent control, 25 μg/L of atrazine, 10 μg/L of endosulfan), two tadpole treatments (presence or absence of 10 *R. sylvatica* tadpoles [Gosner stage 23–26; Gosner 1960]), two snail treatments (presence or absence of 10 *P. trivolvis*), and two dragonfly treatments (presence or absence of one caged, late-instar *A. junius*). The chosen densities of tadpoles, snails, and dragonflies are at the high end of regional densities (J. R. Rohr and P. W. Crumrine, *personal observation*). The experiment lasted for four weeks and the entire design was replicated four times in four spatial blocks for a total of 96 experimental units. A water control was not included in our design because it would have required an additional 32 mesocosms. However, the concentration of acetone used in this study is not expected to be harmful to any of the fauna, especially in light of its highly ephemeral nature.

Experimental units were plastic tubs (polyethylene, 11.3 L, 40.6 × 28.5 × 17.5 cm) containing a perforated, transparent plastic cup (0.296 L) secured to one end of the tub with monofilament line and five microscope slides attached to the side of the tub with silicone. The tubs were located on gravel at the University of Kentucky's Ecological Research Facility. On 31 March 2003, each tub received 9.0 L of aged tap water, 1.0 L of water from the pond from which snails were collected, 15 g of leaves from the mixed deciduous forest surrounding the pond from which the wood frogs were collected, and 3 g of rabbit chow. The pond water supplied an inoculation of phytoplankton, zooplankton, and insect eggs and larvae, and the rabbit chow added nutrients to accelerate the growth of periphyton (attached algae, protozoa, rotifers, and filamentous bacteria), the food source for *R. sylvatica* and *P. trivolvis*. The leaves simulated pond benthos and provided surface area for periphyton growth. The tubs were covered with 60% shade cloth to prevent unwanted predator and amphibian colonization and to simulate tree cover. We allowed two weeks for periphyton establishment before the pesticide, tadpole, snail, and dragonfly treatments were added.

Treatments were applied to the tubs on 14 April 2003. Pesticide stock solutions were first prepared by dissolving 8.984 mg of atrazine and 0.406 mg of endosulfan (both were 99% pure and purchased from ChemService, Westchester, Pennsylvania, USA) per mL of acetone. Concentrations of these stock solutions were verified using flame ionization detection gas chromatography. We added 27.8 μL of atrazine stock solution, 246 μL of endosulfan stock solution, or 246 μL of acetone to the appropriate tubs, producing nominal concentrations of 25 μg/L of atrazine and 10 μg/L of endosulfan. We redosed the tubs two weeks later, at the midpoint of the experiment, to simulate a second runoff event washing pesticides into these mesocosms. Thus, the maximum nominal concentrations were 50 μg/L of atrazine and 20 μg/L of endosulfan. These pesticide concentrations were chosen because they are sublethal to wood frogs (Berrill et al. 1998, Allran and Karasov 2001) and because they are likely to be encountered in ponds (see *Methods and Materials: Chemical contaminants*).

Actual pesticide concentrations were not monitored, but the maximum nominal concentrations were certainly higher than actual concentrations because of heavy rain inputs. Thus, our nominal concentrations represent conservative estimates of what was in each mesocosm. Atrazine is relatively stable and thus degradation probably did not substantially further reduce its concentration. However, endosulfan is much less persistent and thus both dilution and degradation should have reduced concentrations substantially (see *Methods and Materials: Chemical contaminants*).

Tadpole, snail, and dragonfly masses were obtained before they were added to each tub (0.219 ± 0.002, 0.692 ± 0.007, 0.960 ± 0.032 g/individual, respectively[mean ± 1 SE]). Dragonflies were placed into the transparent, perforated cup in each tub so that they were nonlethal to the tadpoles outside the cup but could be visually and chemically detected. Dragonflies were fed two arbitrarily chosen *R. sylvatica* tadpoles from the wading pool stocks three times per week throughout the experiment, and empty cups were picked up and gently dropped to control for feeding disturbance across tubs. Although *A. junius* may consume juvenile *P. trivolvis* (Corbett 1999), they are less likely to consume the larger adults and are known to be one of the predominant predators on *R. sylvatica* tadpoles (Relyea 2002). Thus, we focused strictly on *A. junius* predation on wood frog tadpoles. Dragonfly mortality was assessed during these feedings.

Periphytic algae in each mesocosm was estimated by removing a microscope slide, scraping the periphyton into a vial containing ethanol, and quantifying chlorophyll *a* concentration in these samples using American Public Health Association standard protocols (American Public Health Association 1995). Periphyton samples were taken from all 96 mesocosms immediately before the initial pesticide application to estimate starting values. Samples were then taken from half the spatial blocks every other week throughout the experiment. Dissolved oxygen (DO) and pH were measured weekly for each mesocosm using meters (ICM Model 31200, ± 0.01 mg/L [Industrial Chemical Measurement, Hillsboro, Oregon, USA] and Corning Model 320, ± 0.01 [Corning International, Corning, New York, USA], respectively).

Twice a week and once during the last week, we conducted four observations on each mesocosm, recording the number of tadpoles observed (those not hiding under leaves) and the proportion observed avoiding the cup (on the opposite half of the mesocosm) and moving during a 15-s interval. Metamorphs (Gosner stage 42–46; Gosner 1960) were observed and removed during only the last week of the experiment. The experiment was terminated on 12 May for blocks 1 and 2 and on 13 May for blocks 3 and 4, at which time we counted and weighed surviving snails and tadpoles and counted snail egg masses deposited on each mesocosm. Surviving tadpoles were euthanized with a 0.01% solution of benzocaine and preserved in 10% neutral buffered formalin for later determination of Gosner development stage (Gosner 1960). Water clarity in each mesocosm was independently quantified by the experimenters using a scale of 1–5. Chironomid larvae (dipterans) of the genus *Polypedilum* (periphytivores and detritivores; Coffman and Ferrington 1984) and cladoceran zooplankton of the genus *Daphnia* (primarily *Daphnia pulex*; phytoplanktivores) were the most common invertebrates in the mesocosms, and thus we quantified their abundance on a scale of 1–5. Chironomids and zooplankton likely entered our mesocosms via the pond water aliquots. The presence of insects and crustaceans in genera other than *Polypedilum* and *Daphnia* were rare. So, we recorded the presence or absence of ''other insects and crustaceans'' and lumped them into a single category for statistical analysis.

All statistical analyses were conducted with Statistica 5.5a (Statsoft, Inc., Tulsa, Oklahoma, USA), using the mesocosm as the experimental unit. Proportion data were angularly transformed, and covariates and block effects were included when significant. Because some response variables were correlated, we conducted three separate multivariate analyses of variance (MANOVA) to analyze tadpole behavior (percent hiding, percent observed avoiding cup, percent observed moving), tadpole growth and development (mass/tadpole and Gosner stage), and snail growth and reproduction (mass per snail and egg masses per snail), and then followed these tests with univariate analyses of variance (ANOVA). To assess how DO and pH affected tadpole growth and development (mass per tadpole and Gosner stage), and snail growth and reproduction (mass per snail and egg masses per snail), we regressed DO and pH against these two sets of response variables. The influence of pesticide treatments on the duration of dragonfly survival was analyzed with a one-way ANOVA. The effect of treatments on chlorophyll *a* in the first and second half of the experiment was analyzed using a repeated-measures ANOVA. Treatment effects on the number of snails that died and the number of tadpoles that reached metamorphosis were evaluated using Poisson regression (PR; with a log link). Water clarity and the abundance of *Daphnia* and *Polypedilum* were analyzed using logistic regression for an ordinal multinomial distribution (OMR; with a logit link), while the presence of other insects or crustaceans and dragonfly survival were evaluated using logistic regression for a binomial distribution (BR; with a logit link). Where the main effect of pesticide was significant ($P < 0.05$) in ANOVAs, we examined all pairwise comparisons using a Tukey's honestly significant difference multiple comparison test (Tukey's hsd). Where the main effect of pesticide was significant ($P < 0.05$) using logistic regression (PR, OMR, BR), we examined all pairwise comparisons using Bonferroni-adjusted linear contrasts.

RESULTS

Effects on periphytic algae

Starting values of periphytic algae ($F_{1,68} = 7.40$, $P = 0.008$) and spatial block ($F_{3,68} = 25.58$, $P < 0.001$) were controlled for when evaluating the effects of treatments on periphytic algal abundance through time. Periphytic algae, estimated using chlorophyll *a* measurements, increased from the first half to the second half of the experiment (effect of time, $F_{1,68} = 6.76$, $P = 0.011$), predominantly due to an algal increase in mesocosms containing a dragonfly (time × dragonfly, $F_{1,68} = 5.44$, $P = 0.023$). There were no other significant treatment-by-time interactions ($F < 1.95$, $P > 0.149$). Atrazine (mean ± SE, 5.575 ± 0.292 mg/m^2) significantly reduced periphytic algae relative to endosulfan (6.937 ± 0.330 mg/m^2) and solvent (6.575 ± 0.411 mg/m^2; $F_{2,68} = 11.27$, $P < 0.001$; Tukey's hsd, $P < 0.001$), which were not significantly different from one another (Tukey's hsd, $P = 0.492$). Periphytic algae was reduced by tadpoles ($F_{1,68} = 11.12$, $P = 0.001$) and snails ($F_{1,68} = 5.71$, $P = 0.020$). Mesocosms with a dragonfly had more periphytic algae than those without a dragonfly ($F_{1,68} = 5.71$, $P = 0.020$; Fig. 1), presumably due to nutrient inputs associated with dragonfly feeding. The increase in periphytic algae associated with dragonflies was reduced by the presence of tadpoles and snails (tadpole × dragonfly, $F_{1,68} = 5.86$,

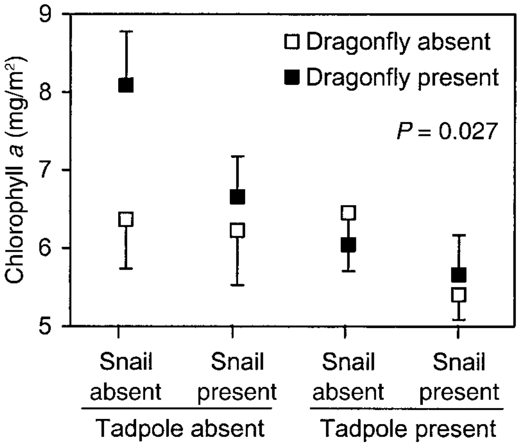

FIG. 1. Interaction among tadpole, snail, and dragonfly treatments (presence and absence) on the concentration of chlorophyll *a* in periphyton. Values represent means and SE of 12 mesocosms; the probability value is for the tadpole × snail × dragonfly interaction.

$P = 0.018$; tadpole × snail × dragonfly, $F_{1,68} = 5.08$, $P = 0.027$; Fig. 1), suggesting consumption of this algae by these two species. There were no other significant interactions among treatments (all $F < 0.72$, all $P > 0.492$).

Effects on water clarity

Water clarity was greater in mesocosms with atrazine, (OMR, df = 2, 71, $\chi^2 = 6.19$, $P = 0.045$), snails (OMR, df = 1, 71, $\chi^2 = 3.79$, $P = 0.050$), and greater quantities of *Daphnia* (OMR, df = 1, 71, $\chi^2 = 31.99$, $P < 0.001$), and lower in mesocosms with a dragonfly larva (OMR, df = 1, 71, $\chi^2 = 6.70$, $P = 0.010$). All other effects were nonsignificant (OMR, $\chi^2 < 3.48$, $P > 0.176$).

Effects on insects and crustaceans

Relative to mesocosms containing solvent control, mesocosms with endosulfan had lower densities of *Polypedilum* larvae, *Daphnia*, and other insects and crustaceans, which included predominantly mosquito larvae and calanoid copepods (OMR and BR, $P < 0.014$), while mesocosms with atrazine had lower densities of only chironomid larvae (OMR, $P = 0.004$; Fig. 2). The abundance of *Polypedilum* larvae was also reduced by tadpoles (OMR, df = 1, 72, $\chi^2 = 14.27$, $P < 0.001$) and snails (OMR, df = 1, 72, $\chi^2 = 4.00$, $P = 0.046$; Fig. 3). There were no other significant main effects or interactions for the abundance of *Polypedilum* larvae (OMR, $\chi^2 < 3.93$, $P > 0.140$), *Daphnia* (OMR, $\chi^2 < 5.83$, $P > 0.054$), or other insects and crustaceans (BR, $\chi^2 < 5.82$, $P > 0.054$).

Pesticide treatments did not significantly affect the percent of dragonfly larvae that survived until the end of the experiment (Atrazine, 12.5%; endosulfan, 25%; solvent; 25%; BR, df = 2, 45, $\chi^2 = 1.08$, $P = 0.582$) or the mean number of days that dragonfly larvae were alive (means ± SE; atrazine, 17.50 ± 2.43; endosulfan, 21.56 ± 1.96; solvent, 22.13 ± 1.72; ANOVA, $F_{2,45} = 1.60$, $P = 0.213$).

Effects on snails

When controlling for spatial block, *Polypedilum* abundance, and initial snail mass, MANCOVA revealed that snail growth and reproduction were influenced by pesticide and tadpole treatments (Table 1). Snail final mass was reduced by atrazine (relative to endosulfan and solvent; Tukey's hsd, $P < 0.001$; Fig. 4A), tadpoles (Fig. 5A), and *Polypedilum* larvae, and was greater in mesocosms with, than without, a dragonfly larva (mean ± SE; 0.675 ± 0.013 vs. 0.643 ±

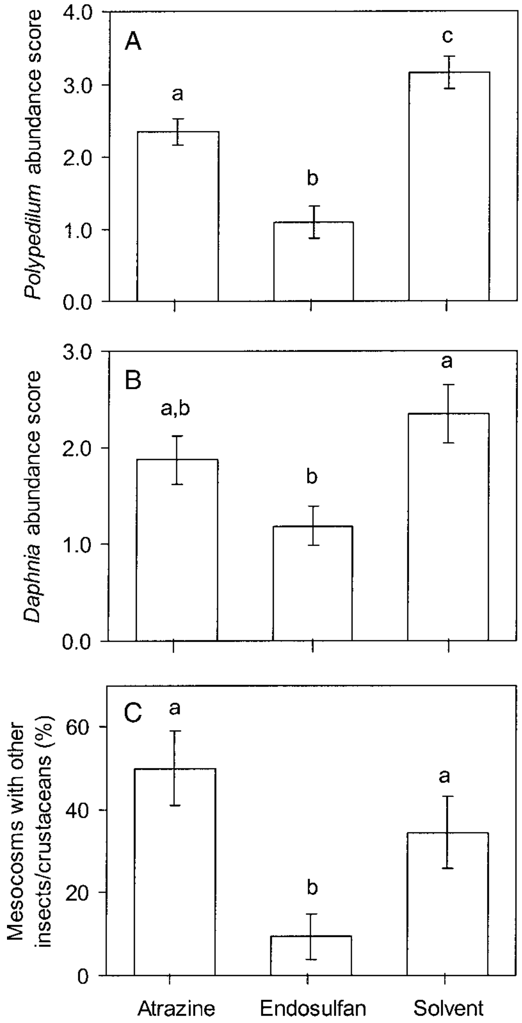

FIG. 2. Effects of pesticide treatments (atrazine, endosulfan, solvent control) on (A) *Polypedilum* (chironomid) larvae abundance, (B) *Daphnia* abundance, and (C) the percentage of mesocosms containing insects or crustaceans other than *Polypedilum* and *Daphnia*. Bars represent means (± SE) of 32 mesocosms. Different lowercase letters above bars reflect significant differences at $P < 0.05$ among treatments according to Tukey's honestly significant difference multiple comparison tests.

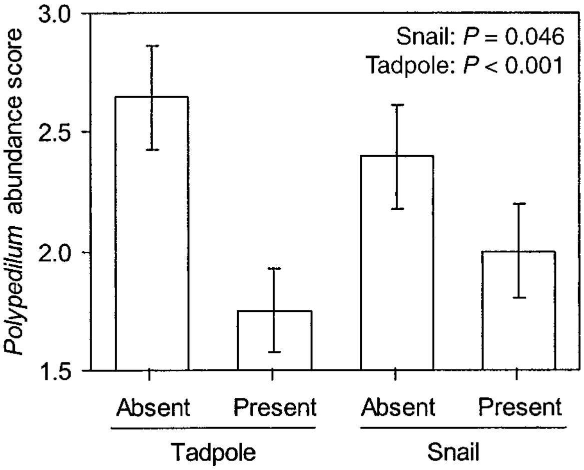

FIG. 3. Effects of tadpole and snail presence and absence on *Polypedilum* chironomid abundance. Bars represent means (±SE) of 48 mesocosms, and probability values are for the main effects of each treatment.

0.011; Table 1). The increase in snail mass associated with a dragonfly larva was greater in the presence of endosulfan than the other pesticide treatments (Fig. 4A, Table 1). When controlling for final mass, fewer egg masses were deposited per surviving snail in mesocosms with atrazine (relative to endosulfan; Tukey's hsd, $P = 0.020$; Fig. 6A) and with tadpoles (Fig. 5B, Table 1). Effects on growth and reproduction could not be attributed to differences in DO (regression; Wilk's $F_{2,45} = 0.94$, $P = 0.399$) or pH (regression: Wilk's $F_{2,45} = 1.98$, $P = 0.151$) among mesocosms.

Because of low snail mortality, we excluded interaction terms from our analyses on snail survival to increase statistical power. When controlling for the abundance of chironomid competitors (PR, df = 1, 42, $\chi^2 = 2.85$, $P = 0.091$), the presence of tadpoles significantly increased snail mortality (13 vs. five dead; PR, df = 1, 42, $\chi^2 = 5.40$, $P = 0.020$). Snail survival was not significantly affected by pesticide (PR, df = 2, 42, $\chi^2 = 2.19$, $P = 0.335$) or dragonfly treatments (PR: df = 1, 42, $\chi^2 = 0.01$, $P = 0.912$).

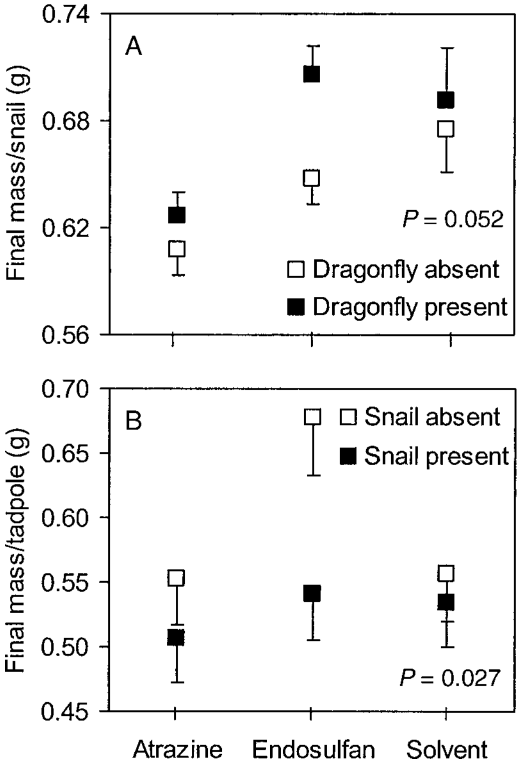

FIG. 4. (A) Effects of pesticide (atrazine, endosulfan, solvent control) and dragonfly treatments (presence and absence) on snail mass and (B) effects of pesticide and snail treatments (presence and absence) on tadpole mass. Values represent means and SE of eight mesocosms, and probability values are for the interaction between displayed treatments when controlling for significant predictors (see Tables 1 and 3 for predictors).

TABLE 1. Results of multivariate analysis of covariance (MANCOVA) and univariate analyses of covariance (ANCOVA) for effects of pesticide (atrazine, endosulfan, or solvent control), tadpole (present or absent), dragonfly (present or absent), and interactions on final mass per snail and egg masses deposited per snail when controlling for significant predictors.

	MANCOVA			ANCOVA				
					Final mass/snail		Egg masses/snail	
Factor	df	Wilks' *F*	*P*	df	*F*	*P*	*F*	*P*
Block	6, 60	4.80	<0.001	3	6.33	0.002	···	···
Polypedilum	2, 30	2.64	0.088	1	10.80	0.003	···	···
Initial mass	2, 30	8.05	0.002	1	5.43	0.026	···	···
Final mass	···	···	···	1	···	···	11.43	0.002
Pesticide	4, 60	5.35	0.001	2	6.78	0.004	7.67	0.002
Tadpole	2, 30	101.50	<0.001	1	34.89	<0.001	147.76	<0.001
Dragonfly	2, 30	2.67	0.086	1	5.01	0.032	1.46	0.235
Pesticide × tadpole	4, 60	1.37	0.256	2	1.18	0.322	1.12	0.339
Pesticide × dragonfly	4, 60	1.61	0.183	2	3.26	0.052	0.10	0.904
Tadpole × dragonfly	2, 30	0.94	0.401	1	0.28	0.603	1.36	0.251
Pesticide × tadpole × dragonfly	4, 60	0.41	0.800	2	0.26	0.775	1.27	0.292

Notes: Ellipses represent nonsignificant predictors that were removed from the statistical model. There were no other significant predictors. The error degrees of freedom are 31 for final mass per snail and 35 for egg masses per snail.

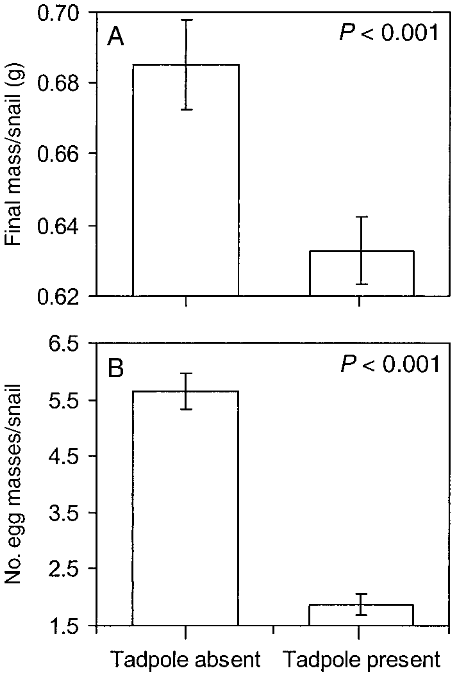

FIG. 5. Effects of tadpole presence and absence on (A) the final mass per surviving snail and (B) the number of egg masses deposited per surviving snail. Bars represent means (±SE) of 24 mesocosms. Probability values are for the main effect of treatment when controlling for significant predictors (see Table 1 for predictors).

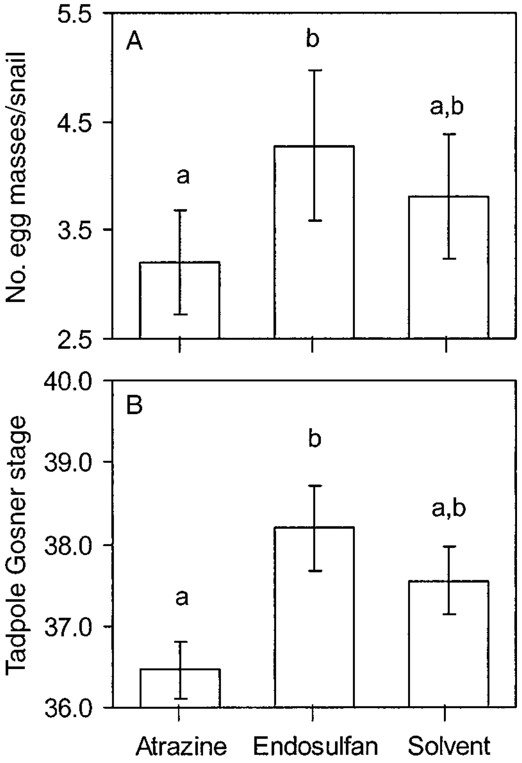

FIG. 6. Effects of pesticide treatments (atrazine, endosulfan, solvent control) on (A) the number of egg masses deposited per surviving snail and (B) tadpole Gosner development stage. Bars represent means (±SE) of 16 mesocosms. Different lowercase letters above bars reflect significant differences at $P < 0.05$ among treatments according to Tukey's honestly significant difference multiple comparison tests when controlling for significant predictors (see Tables 1 and 3 for predictors).

Effects on wood frogs

We first examined wood frog behavioral responses through time and discovered that significant temporal changes were primarily due to dragonfly mortality. Because there were no significant treatment differences in percent dragonfly mortality or in the timing of dragonfly mortality, we took the mean of the seven days of behavioral observations and used this in subsequent analyses. MANCOVA showed that tadpole behavior (hiding, avoiding, and moving) depended on *Daphnia* abundance and pesticide, snail, and dragonfly treatments (Table 2). More specifically, a greater percentage of tadpoles were hiding in the presence of endosulfan and solvent than atrazine (Tukey's hsd, $P < 0.001$ and $P = 0.028$, respectively; Fig. 7A) and in the presence,

TABLE 2. Results of MANCOVA, ANCOVA, and ANOVA for the effects of pesticide (atrazine, endosulfan, or solvent control), snail (present or absent), dragonfly (present or absent), and interactions on *Rana sylvatica* tadpole behavioral responses (percentages hiding, observed avoiding cage, and observed moving in 15 s).

				ANOVA and ANCOVA						
	MANCOVA				Hiding		Avoiding		Moving	
Factor	df	Wilks' *F*	*P*	df	*F*	*P*	*F*	*P*	*F*	*P*
Daphnia	3, 33	3.27	0.033	1	···	···	···	···	9.73	0.004
Pesticide	6, 66	4.42	<0.001	2	8.64	<0.001	4.41	0.019	9.44	<0.001
Snail	3, 33	6.76	0.001	1	2.17	0.150	9.94	0.003	15.23	<0.001
Dragonfly	3, 33	53.11	<0.001	1	43.04	<0.001	36.88	<0.001	141.75	<0.001
Pesticide × snail	6, 66	1.93	0.089	2	0.26	0.771	1.16	0.326	4.84	0.014
Pesticide × dragonfly	6, 66	1.48	0.200	2	1.74	0.191	1.76	0.186	3.28	0.049
Snail × dragonfly	3, 33	3.82	0.019	1	1.02	0.318	7.20	0.011	7.41	0.010
Pesticide × snail × dragonfly	6, 66	0.47	0.828	2	0.03	0.969	0.14	0.870	1.32	0.281

Notes: Ellipses represent nonsignificant predictors that were removed from the statistical model. There were no other significant covariates. The error degrees of freedom are 36 for hiding and avoiding and 35 for moving.

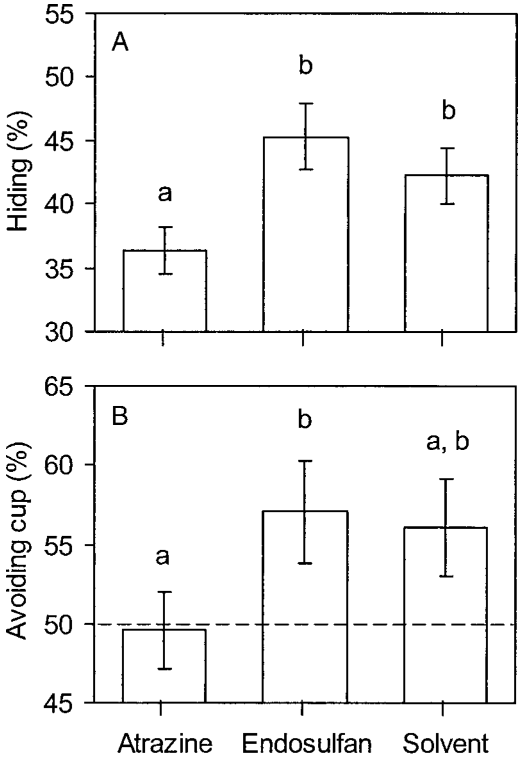

FIG. 7. Effects of pesticide treatments (atrazine, endosulfan, solvent control) on (A) the percentage of tadpoles hiding (measured as the percentage of total individuals that were not observed) and (B) the percentage of individuals observed that were avoiding the cup. Bars represent means (±SE) of 16 mesocosms. Different lowercase letters above bars reflect significant differences at $P < 0.05$ among treatments according to Tukey's honestly significant difference multiple comparison tests when controlling for significant predictors (see Table 2 for predictors).

than absence, of a dragonfly (Fig. 8A, Table 2). Tadpoles were more likely to avoid the cup when it contained a dragonfly, but this avoidance was significantly reduced in the presence of snails (Fig. 8B, Table 2). A greater percent of tadpoles exhibited cup avoidance in the presence of endosulfan than atrazine (Tukey's hsd, $P = 0.025$; Fig. 7B, Table 2), due primarily to atrazine reducing dragonfly avoidance. Tadpole activity was elevated in mesocosms containing atrazine (relative to endosulfan, Tukey's hsd, $P = 0.007$) and snails, and lower in mesocosms with abundant *Daphnia* and dragonflies (relative to solvent control). This reduction in tadpole activity associated with dragonflies was greater when reared in endosulfan and solvent than atrazine (pesticide × dragonfly) and when reared in the absence, than presence, of snails (snail × dragonfly). Furthermore, the increase in tadpole activity associated with snails was greater in the presence of endosulfan and solvent than in the presence of atrazine (pesticide × snail; Fig. 9, Table 2).

One solvent control mesocosm was a significant and influential outlier (determined using leverage, DFFITS, and DFBETAS tests; Neter et al. 1996) for tadpole mass and development and thus was removed from the analyses. No tadpoles were found dead at anytime during the experiment and none had any obvious physical deformities. When controlling for initial tadpole mass and spatial block, MANCOVA revealed that tadpole growth and development were affected by *Daphnia* abundance, and pesticide, snail, and dragonfly treatments (Table 3). At the end of the experiment, tadpoles weighed

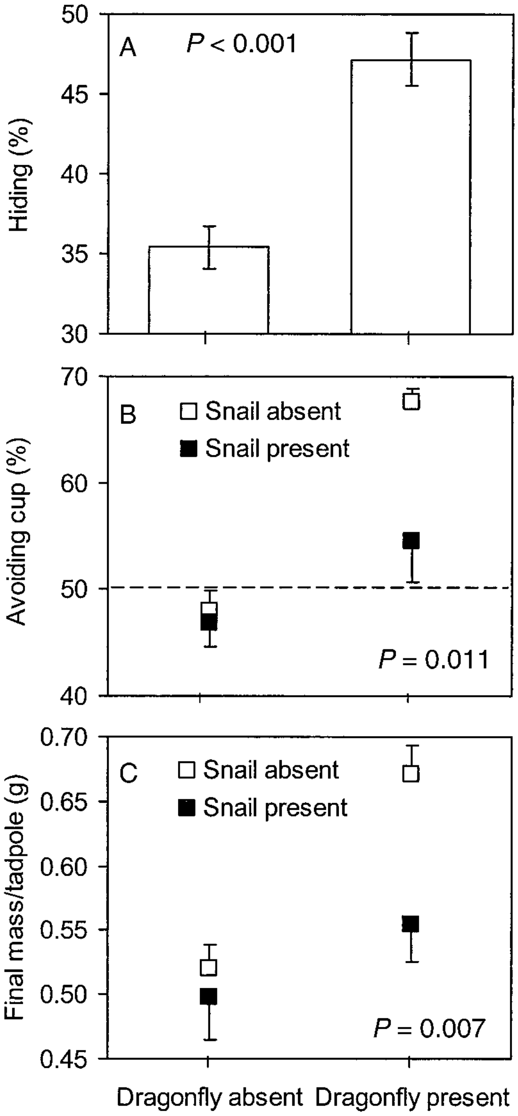

FIG. 8. (A) Effects of dragonfly treatments (presence and absence) on the percentage of tadpoles hiding (measured as the percentage of total individuals that were not observed); (B) the interaction between dragonfly and snail treatments (presence and absence) on the percentage of tadpoles observed that were avoiding the cup, and (C) tadpole final mass. Values in (A) represent means (±SE) of 24 mesocosms and values in (B) and (C) represent means (±SE) of 12 mesocosms. The probability values in (B) and (C) are for the snail × dragonfly interaction.

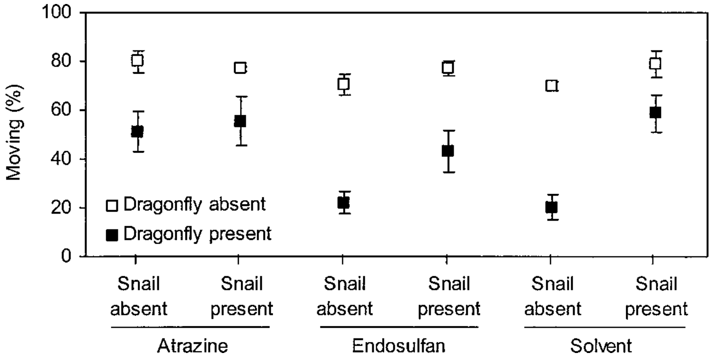

FIG. 9. Interaction among pesticide (atrazine, endosulfan, solvent control), snail, and dragonfly treatments (presence and absence) on the percentage of tadpoles observed that were moving during a 15-s observation period. Values represent means (±SE) of four mesocosms. All main effects and two-way interactions were significant ($P < 0.05$) when controlling for *Daphnia* abundance (see Table 2).

more in mesocosms with abundant *Daphnia*, endosulfan (Tukey's hsd; atrazine, $P = 0.004$; solvent, $P = 0.025$; Fig. 4B), and a dragonfly, and weighed less in mesocosms with snails (Fig. 4B, Table 3). This decrease in tadpole mass in the presence of snails (relative to their absence) was significantly greater when tadpoles were reared in the presence of endosulfan (vs. atrazine and solvent, pesticide × snail; Fig. 4B) or a dragonfly (snail × dragonfly; Fig. 8C, Table 3). Development was slowed by atrazine (Tukey's hsd; endosulfan, $P = 0.002$; solvent, $P = 0.067$; Fig. 6B) and snails (Fig. 10A), and there were fewer wood frog metamorphs in mesocosms with, than without, snails (Fig. 10B, Table 3). Effects on wood frog growth and development could not be attributed to differences in DO (regression, Wilk's $F_{2,44} = 0.87$, $P = 0.425$) or pH (regression, Wilk's $F_{2,44} = 0.98$, $P = 0.385$) among mesocosms.

DISCUSSION

This study demonstrated that adult *P. trivolvis*, *R. sylvatica* tadpoles, and *Polypedilum* chironomid larvae are competitors; that predation, competition, and pesticides can have synergistic effects on aquatic organisms; and that low, ecologically relevant concentrations of pesticides can have both positive and negative effects on species that are mediated through indirect and direct trophic interactions. Below we discuss the supporting evidence for these claims and their consequences for ecotoxicology and conservation biology.

Various results support a competitive relationship among adult *P. trivolvis*, larval *R. sylvatica*, and larval *Polypedilum* chironomids. Both *P. trivolvis* and *R. sylvatica* reduced adherent algae, and when coexisting, they significantly reduced the growth, development, and/or abundance of the other species. The snails and tadpoles also consumed the increased periphyton associated with mesocosms containing a dragonfly, which elevated both of their growth rates. The presence of *P. trivolvis* reduced tadpole refuge use, predator avoidance, and inactivity, responses which are typically associated with increased feeding rates (e.g., Horat and Semlitsch 1994, Relyea and Werner 1999). While these responses might increase periphyton intake, they should increase predation risk as well, a common consequence of competition (Chase et al. 2002). Although there is evidence for some species of snails to facilitate the growth of tadpoles (Bronmark et al. 1991), our

TABLE 3. Results of MANCOVA, ANCOVA, ANOVA, and Poisson regression for effects of pesticide (atrazine, endosulfan, or solvent control), snail (present or absent), dragonfly (present or absent), and interactions on final mass per tadpole, tadpole Gosner stage, and number of metamorphs.

				ANCOVA and ANOVA					Poisson regression	
	MANCOVA				Final mass/tadpole		Gosner stage		No. metamorphs	
Factor	df	Wilks' F	P	df	F	P	F	P	χ^2	P
Block	6, 58	3.18	0.009	3	4.43	0.011	⋯	⋯	⋯	⋯
Daphnia	2, 29	3.40	0.047	1	6.64	0.015	⋯	⋯	⋯	⋯
Initial mass	2, 29	7.61	0.002	1	⋯	⋯	9.62	0.004	⋯	⋯
Pesticide	4, 58	5.31	0.001	2	9.65	<0.001	6.80	0.003	2.27	0.322
Snail	2, 29	12.05	<0.001	1	11.44	0.002	23.83	<0.001	8.16	0.004
Dragonfly	2, 29	14.84	<0.001	1	32.50	<0.001	1.23	0.275	0.50	0.479
Pesticide × snail	4, 58	1.99	0.108	2	4.08	0.027	1.33	0.278	0.19	0.909
Pesticide × dragonfly	4, 58	1.02	0.404	2	1.95	0.159	0.38	0.684	1.02	0.600
Snail × dragonfly	2, 29	6.02	0.006	1	8.49	0.007	0.69	0.412	<0.01	0.961
Pesticide × snail × dragonfly	4, 58	1.46	0.226	2	0.87	0.428	1.01	0.374	2.70	0.260

Notes: Ellipses represent nonsignificant predictors, which were removed from the statistical model. There were no other significant predictors. The error degrees of freedom are 31 for final mass per tadpole, 34 for tadpole Gosner stage, and 35 for number of metamorphs.

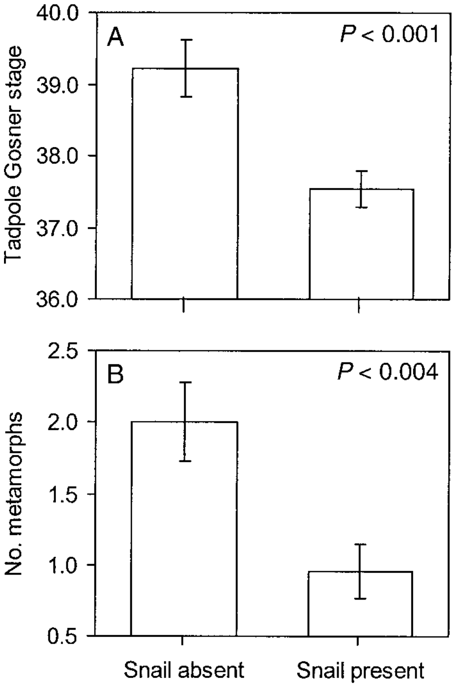

FIG. 10. Effects of snail presence and absence on (A) tadpole Gosner stage and (B) the number of tadpoles reaching metamorphosis. Bars represent the means (±SE) of 24 mesocosms.

results, like those of Holomuzki and Hemphill (1996) and Lefcort et al. (1999), support a strict competitive relationship between these two species. This competition may be asymmetric because wood frog tadpoles reduced the survival and reproduction of the snails with no evidence of reciprocal effects. Snails and tadpoles also reduced the density of the periphytivorous *Polypedilum* larvae, and in turn, these larvae reduced the growth of snails and tadpoles. However, growth reductions for tadpoles were not significant, suggesting that *Polypedilum* larvae may be less effective competitors with *R. sylvatica* than with *P. trivolvis*.

Unlike *Polypedilum* larvae and snails, the phytoplanktivorous *Daphnia* had a positive effect on the growth of tadpoles. We suspect that *Daphnia* may facilitate tadpole growth by increasing water clarity, which would then allow more light to reach surfaces on which periphyton can grow (see Underwood et al. 1992, Thomas and Daldorph 1994). However, a more specific test of this hypothesis is necessary.

Pesticide treatments had direct lethal effects on photosynthetic organisms, chironomid larvae, and crustaceans, but not on dragonfly larvae. Atrazine reduced algae, consistent with work examining the effects of similar levels of atrazine on periphyton and phytoplankton (see review by Solomon et al. 1996). Endosulfan decreased densities of cladoceran zooplankton and chironomids, as shown previously for comparable concentrations (Ernst et al. 1991, Barry and Logan 1998, Leonard et al. 1999, Barry 2000). Although endosulfan is designed to target insects, it surprisingly did not increase the mortality of *A. junius*. In addition to dragonfly larvae, tadpoles experience predation from hemipterans and beetle larvae (Relyea 2002), both of which have been shown to be susceptible to endosulfan (Ernst et al. 1991, Huang et al. 1994). Thus, by reducing other insect predators, endosulfan may reduce tadpole predation risk despite it having no detectable direct effect on *A. junius* survival.

As expected, the direct effects of atrazine and endosulfan proliferated through the food web resulting in indirect effects on snails, tadpoles, and chironomid larvae. Atrazine reduced snail reproduction, tadpole development, snail and tadpole growth, and chironomid survival, presumably by reducing periphyton, whereas endosulfan increased snail and tadpole growth through a reduction in chironomid competition. These effects on gastropods and amphibians are probably strictly indirect because the concentrations of the pesticides used in this study have rarely induced any detectable direct effects on these taxa (e.g., Baturo et al. 1995, Jonnalagadda and Rao 1996, Solomon et al. 1996, Berrill et al. 1998, Diana et al. 2000, Allran and Karasov 2001, Downs et al. 2001, Rohr et al. 2003, Rohr et al. 2004). The indirect effects on *P. trivolvis* and *R. sylvatica* were transmitted through a single trophic link—atrazine reducing their prey and endosulfan reducing their insect competitors. Thus, ecologically important effects of atrazine and endosulfan would have gone overlooked had we not conducted the study in the presence of periphytic algae or *Polypedilum* chironomid larvae, organisms commonly found in many freshwater habitats.

There were also at least three cases where these single-trophic-link effects would have been estimated inaccurately if other common, heterospecific community members were not included (i.e., pesticide × heterospecific interactions). First, the endosulfan-induced growth increase for tadpoles was smaller in the presence than absence of snails (pesticide × snail), most likely because the snails prevented the tadpoles from monopolizing the increased periphyton resulting from endosulfan reductions in chironomid larvae. Second, the endosulfan-induced growth increase for snails was synergistically modified by the presence of dragonflies (pesticide × dragonfly), presumably because dragonflies provided a release from tadpole competition (by reducing their foraging activity) on top of the release from chironomid competition imparted by endosulfan. Finally, the effect of atrazine on tadpole activity was dependent upon the presence of snails and dragonflies. This was apparently because periphyton was limiting in atrazine mesocosms encouraging tadpoles to sustain high foraging activity regardless of the presence of competitors or predators. Interestingly, this increase in foraging activity did not compensate for the reduced periphyton in atrazine mesocosms and, in combination with the concomitant reductions in refuge use and dragonfly avoidance, should increase encounter rates with predators. Although the presence of caged predators has been shown to increase the lethality of the insecticide carbaryl for several tadpole species (including *R. sylvatica*; Relyea and Mills 2001, Relyea 2003), we found no evidence of this extreme pesticide-heterospecific synergism for the selected concentration of atrazine or endosulfan. The multiple examples above of heterospecifics dictating the impacts of pesticides clearly demonstrate that community composition can play an important role in determining the ecological effects of contaminants.

The interaction between pesticides and heterospecifics has been an issue of concern for amphibian populations exposed to pathogens (e.g., Taylor et al. 1999, Christin et al. 2003). For instance, pesticides can reduce amphibian immunocompetence increasing the risk of trematode infections that cause malformations and mortality associated with amphibian population declines (Kiesecker 2002, Blaustein and Johnson 2003). By clarifying the relationship between the intermediate hosts of these trematodes (planorbid snails and tadpoles) within a community context and in the presence and absence of common pesticides, this study may facilitate understanding the epidemiology of this important amphibian disease.

Although the spatial scale of toxicology experiments conducted in mesocosms and microcosms typically does not match that of natural systems, numerous studies have documented the success of these experimental venues in predicting the effects of toxicants on higher levels of ecological organization (e.g., Larsen et al. 1986, Pontasch et al. 1989, Stay et al. 1989, Niederlehner et al. 1990, Pontasch and Cairns 1991, but see Skelly 2002). Thus, the results of this study, and other mesocosm and microcosm studies, may be useful for predicting the impacts of pesticides on populations in similarly structured, natural communities. For example, wood frog development and growth were reduced in mesocosms with atrazine and increased in mesocosms with endosulfan. These results may have negative and positive effects on anuran populations, respectively, because, for many amphibians, accelerated development reduces the risk of desiccation from water body drying and larger size at metamorphosis increases immunocompetence, terrestrial survival, and lifetime reproduction (Berven and Gill 1983, Smith 1987, Scott 1994, Carey et al. 1999).

Because anurans are presumably less susceptible to herbicides than their photosynthetic prey and more tolerant to insecticides than their insect competitors and predators, anuran populations may, in general, incur negative effects from herbicide exposure and positive effects from insecticide exposure (at environmentally common concentrations). Of the studies which have considered secondary effects of pesticides on freshwater herbivores (taken from Fleeger et al. 2003), we estimate that 92% (11 out of 12, with one study showing both positive and negative effects) showed adverse, indirect effects of herbicides on herbivores, and that 96% (23 out of 24, with three studies showing both positive and negative effects) showed that insecticides provide a competitive release for tolerant herbivores. Although these findings provide considerable support for the hypothesis that herbicides and insecticides have contrasting effects on freshwater herbivores, such as anurans, greater support may be needed to substantiate the hypothesis because of the great variety of pesticides (Ramade 1988), the vast differences in species susceptibilities (e.g., Bridges and Semlitsch 2000, Boone et al. 2001), and the enormous variation in species composition among ponds (e.g., Wellborn et al. 1996, Skelly et al. 1999, Relyea 2002). In fact, it is for these reasons, and others, that make it particularly challenging to identify and predict general responses to contaminants in nature. Nevertheless, research testing the generality of the contrasting effect of herbicides and insecticides on anurans should provide valuable insight into their global declines.

Perhaps most notably, this study underscores the importance of examining the effects of contaminants within natural or semi-natural, complex communities. Environmentally realistic concentrations of pesticides both directly and indirectly shaped community composition and community composition influenced the effects of pesticides. These ecologically important effects would have gone unnoticed in traditional toxicology studies where species are tested in the laboratory isolated from their natural communities. Because virtually all species live within complex food webs consisting of heterospecifics with different tolerances to contaminants, there is a pressing need for more toxicology studies conducted on communities where the presence of species are manipulated so we can better understand the effects of contaminants in nature.

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