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AN UNFORESEEN CHAIN OF EVENTS: LETHAL EFFECTS OF PESTICIDES ON FROGS AT SUBLETHAL CONCENTRATIONS

Rick A. Relyea[1] and Nicole Diecks

Department of Biological Sciences, University of Pittsburgh, Pittsburgh, Pennsylvania 15260 USA

Abstract. The field of toxicology has traditionally assessed the risk of contaminants by using laboratory experiments and a range of pesticide concentrations that are held constant for short periods of time (1–4 days). From these experiments, one can estimate the concentration that causes no effect on survival. However, organisms in nature frequently experience multiple applications of pesticides over time rather than a single constant concentration. In addition, organisms are embedded in ecological communities that can propagate indirect effects through a food web. Using outdoor mesocosms, we examined how low concentrations (10–250 μg/L) of a globally common insecticide (malathion) applied at various amounts, times, and frequencies affected aquatic communities containing zooplankton, phytoplankton, periphyton, and larval amphibians (reared at two densities) for 79 days. All application regimes caused a decline in zooplankton, which initiated a trophic cascade in which there was a bloom in phytoplankton and, in several treatments, a subsequent decline in the competing periphyton. The reduced periphyton had little effect on wood frogs (*Rana sylvatica*), which have a short time to metamorphosis. However, leopard frogs (*Rana pipiens*) have a longer time to metamorphosis, and they experienced large reductions in growth and development, which led to subsequent mortality as the environment dried. Hence, malathion (which rapidly breaks down) did not directly kill amphibians, but initiated a trophic cascade that indirectly resulted in substantial amphibian mortality. Importantly, repeated applications of the lowest concentration (a "press treatment" consisting of seven weekly applications of 10 μg/L) caused larger impacts on many of the response variables than single "pulse" applications that were 25 times as great in concentration. These results are not only important because malathion is the most commonly applied insecticide and is found in wetlands, but also because the mechanism underlying the trophic cascade is common to a wide range of insecticides, offering the possibility of general predictions for the way in which many insecticides impact aquatic communities and the populations of larval amphibians.

Key words: acetylcholine esterase inhibitor; amphibian decline; ecotoxicology; leopard frog (Rana pipiens); *pesticide pulse; wood frog* (Rana sylvatica).

Introduction

Understanding and predicting the impacts of anthropogenic chemicals on nontarget organisms is a challenging proposition for ecologists and toxicologists. Traditionally, toxicologists have taken a tiered approach that begins with short-term (1–4 days), single-species laboratory tests to determine which constant concentrations of a contaminant cause 50% lethality (LC50), and which concentrations cause no observable effect (NOEC). If these studies indicate sufficient risk relative to expected exposure concentrations, subsequent tests include longer-term, single-species experiments (e.g., life cycle tests) in the laboratory (EPA 1998). In this way, the tiered approach is designed to incorporate greater ecological realism.

One limitation of this approach is that these laboratory tests tell us how constant concentrations of a contaminant affect a species. The situation contrasts with real-world pesticide applications in aquatic systems, in which pesticides can not only vary in concentration, but also in the timing of the application, the rate of breakdown, and the frequency of repeated applications (McConnell et al. 1998, Viant et al. 2006). This is important because the sensitivity to contaminants may vary over ontogeny, and multiple applications cause a species to be exposed to a greater amount of pesticide over ontogeny than a single application (e.g., if the contaminant bioaccumulates or if the effects are cumulative). Although chronic, whole-life-cycle studies expose animals throughout ontogeny, fewer studies have examined the importance of specific application timing over ontogeny (Forget et al. 1998, Howe et al. 1998, Bridges 2000), and even fewer have directly compared the impact of single vs. multiple applications on aquatic communities (but see Hanazato and Yasuno [1990] for effects on zooplankton and Boone et al. [2001] for effects on tadpoles). In the latter two cases, both studies have found that multiple applications of the insecticide carbaryl had effects

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[1] E-mail: relyea@pitt.edu

similar to single applications (although tadpole development was more advanced with multiple applications to tadpoles at high densities). However, the concentrations used in these experiments (500 and 3500 μg/L, respectively) were relatively high relative to each taxon's LC50 (the concentration expected to kill 50% of a population), and a single application was generally capable of causing the maximal effect on the taxa. Therefore, additional applications could cause no further impacts. However, under lower and more commonly observed concentrations (e.g., ~1–10% of LC50 values), one might expect to observe substantial differences between single and multiple applications. Hence, there is a strong need to investigate how a range of application times, amounts, and frequencies affect nontarget organisms.

A second limitation with the tiered approach is that single-species tests exclude interspecific interactions among taxa that live with the focal organism, while short-term tests (i.e., 1–4 days) preclude observations of trophic cascades that could indirectly affect the focal organism but require more time to appear. A number of studies have demonstrated that density-mediated indirect effects initiated by contaminants might be quite common. In general, most of these studies have documented relatively simple indirect effects in which a contaminant causes a sensitive taxon to decline in abundance (predators, herbivores, competitors), which allows an insensitive, interacting taxon to increase in abundance (reviewed in deNoyelles et al. 1994, Brock et al. 2000*a*, *b*, Fleeger et al. 2003, Relyea and Hoverman 2006). This type of indirect effect appears to be common and is observable in a matter of days or weeks. However, indirect effects have the potential to be more complex and to take considerably longer (i.e., months) to appear.

If we combine the two issues of single vs. multiple applications and the importance of interspecific interactions in a community-oriented approach, we arrive at a number of interesting insights. For example, there is a long history in ecological studies of examining community impacts either as "pulse" experiments (i.e., a single disturbance) or "press" experiments (i.e., multiple disturbances [Bender et al. 1984, Paine et al. 1998, Clements and Newman 2002]). A number of community toxicology studies have identified important indirect effects by conducting pulse experiments, typically consisting of a pesticide addition at the beginning of the experiment (Havens 1994, Boone et al. 2004, Mills and Semlitsch 2004, Relyea 2005). Additional studies have focused on a community's ability to rebound from pulse treatments and found that the resiliency depends on pesticide breakdown rate, organism generation time, dispersal rates, and the regional species pool (Wallace et al. 1996, Spawn et al. 1997, Woin 1998, Brock et al. 2000*a*, *b*). In the case of press experiments, we might predict that multiple applications of a pesticide could hold the community in a constant state of disturbance, thereby preventing resilience. In this case, we might observe much lower concentrations of a pesticide having larger impacts on the community than single pulses of much greater magnitude. Surprisingly, few studies have directly compared the importance of single vs. multiple pesticide applications on aquatic communities (Hanazato and Yasuno 1990, Boone et al. 2001).

Indirect effects of pesticides are not simply of interest to basic ecology, but also can have important applications for conservation. For example, amphibians are a group of serious conservation concern due to ongoing global population declines (Alford and Richards 1999, Stuart et al. 2004), and some of these declines have been correlated with insecticide use (Davidson et al. 2001, 2002, Davidson 2004). However, observed insecticide concentrations in natural wetlands are frequently lower than concentrations known to have direct lethal effects on amphibians (McConnell et al. 1998, LeNoir et al. 1999, Sparling et al. 2001). Hence, there is a disconnect between correlative regional patterns of declines with pesticides and the most common explanation for the mechanism(s) underlying these declines (i.e., direct toxicity). In this study, we addressed whether low concentrations of an insecticide (at variable concentrations, times, and frequencies) can initiate a chain of indirect effects that ultimately affects amphibians.

We addressed this question using tadpoles living at low and high densities in communities containing zooplankton, phytoplankton, and periphyton. In this community, zooplankton feed primarily on planktonic algae (i.e., phytoplankton), whereas tadpoles feed primarily on attached algae (i.e., periphyton; Fig. 1A). Using this community, we can make a priori predictions about the impacts of insecticides at low concentrations (~1–10% of LC50 values for tadpoles). At these concentrations, one would expect no direct lethal effects on the amphibians but substantial direct lethal effects on the zooplankton, because aquatic invertebrates are highly sensitive to insecticides (deNoyelles et al. 1994, Brock et al. 2000*b*, Fleeger et al. 2003). If much of the zooplankton assemblage is eliminated by the insecticide, there should be a subsequent increase in phytoplankton (providing that phytoplankton is limited by herbivory and not by nutrients). The increase in phytoplankton should then reduce the amount of light reaching the periphyton on the bottom of the pond and thereby cause a reduction in periphyton. Because tadpoles rely on periphyton as their food source, this trophic cascade should cause a reduction in growth and development, thereby causing a change in the age and size at metamorphosis (Fig. 1B). In short, low concentrations of an insecticide (<LC50) that rapidly degrade should negatively impact amphibians via a cascade of indirect effects that occurs long after the pesticide has left the system. Moreover, this negative effect on the tadpoles should be most pronounced under four conditions: (1) when tadpoles are resource limited, (2) when a species of tadpole has an inherently long larval period that would subject it to a longer exposure of the cascade, (3) when

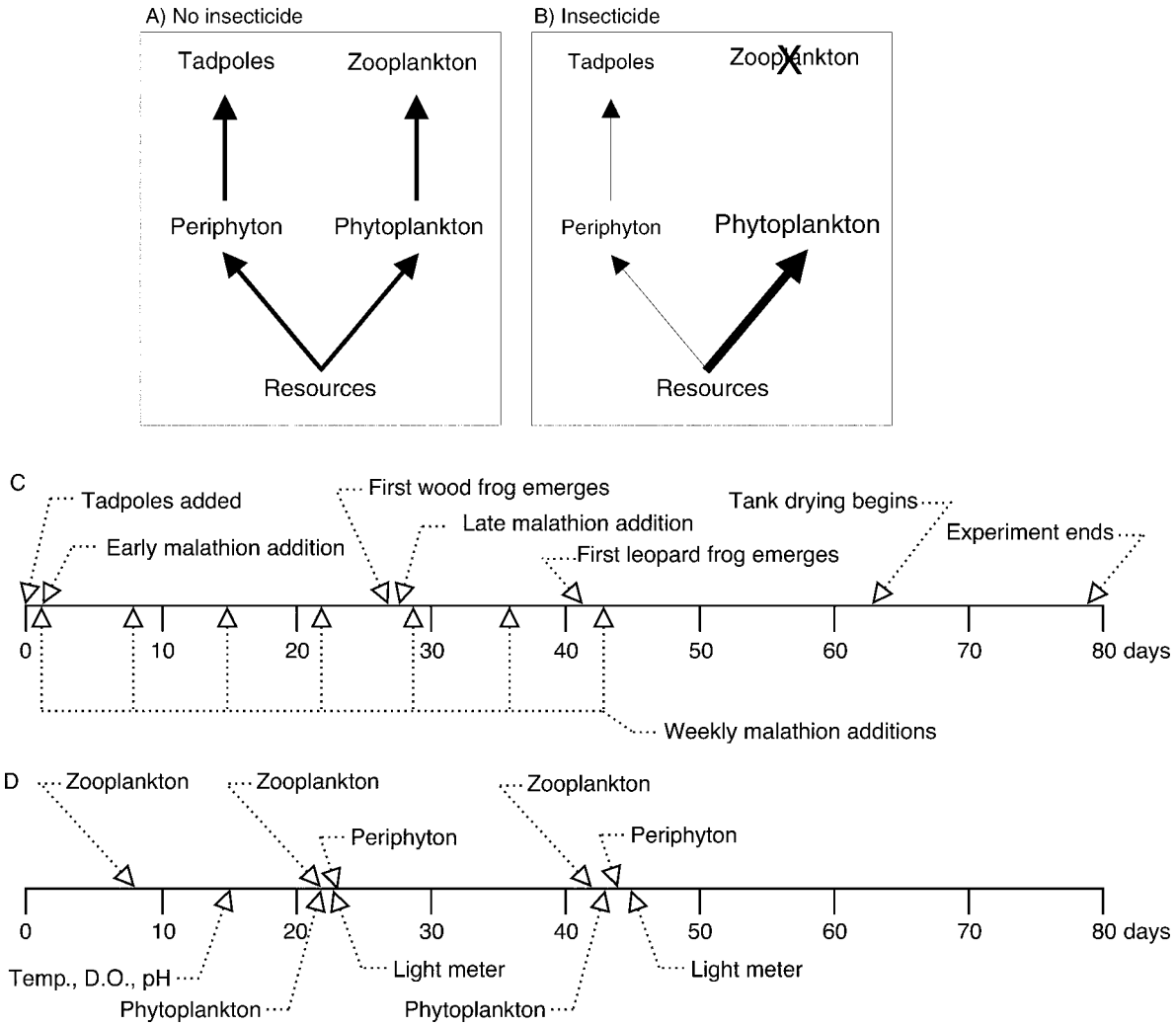

FIG. 1. Hypothesized indirect effects in a simple aquatic food web that contains either (A) no pesticide or (B) contamination by a low concentration of a pesticide that is only directly toxic to zooplankton. A time line is also provided for (C) when the pesticide additions occurred and (D) when each response variable was measured. D.O. is dissolved oxygen. Malathion was the only pesticide used. "Light meter" indicates when water transparency was measured.

multiple applications of the pesticide continue to reinforce the trophic cascade and prevent the zooplankton community from recovering, and (4) when the pesticide is applied early rather than late in the amphibian's larval period.

Insecticide background

Of the large number of insecticides currently applied around the world, we chose to work with the insecticide malathion because it is the most common insecticide used in the United States, and can be found in natural water bodies (Kiely et al. 2004). Malathion is a broad-spectrum insecticide that kills a range of invertebrates including the mosquitoes that serve as vectors for West Nile virus and malaria (Gratz and Jany 1994, Walker 2000). Annual applications of malathion amount to 10–14 million kg of active ingredient applied to nearly 10^6 ha of cropland as well as home, garden, government, and industrial use (Kiely et al. 2004; National Pesticide Use Database, *available online*).[2] In general, malathion is highly toxic to most aquatic invertebrates (including zooplankton) and moderately toxic to larval amphibians (USEPA Ecotox Database, *available online*).[3]

Expected concentrations for malathion in water range from 0 to 1600 μg/L, and wetland surveys have found up to 600 μg/L (California Department of Fish and Game 1982, USDA 1997, McConnell et al. 1998, LeNoir et al. 1999, Relyea 2004). Evaluating typical concentrations in standing water is difficult because major aquatic surveys have only examined either streams and rivers or well water. For example, stream surveys indicate that

[2] ⟨www.ncfap.org/database/default.htm⟩
[3] ⟨http://cfpub.epa.gov/ecotox⟩

malathion is most common in urban streams with up to 43% of samples detecting the chemical (USGS 2000). Only recently has the USEPA determined the estimated environmental concentrations (EEC) for malathion in aquatic habitats as part of a risk assessment of malathion on the California red-legged frog (*R. aurora draytonii* [Odenkirchen and Wente 2007]). Based on application frequencies (every 2–14 days), application rates, and the proportion of expected drift, the EEC for malathion in water (based on >50 categories of terrestrial crops) is 9 ± 27 μg/L (mean and 95% CI). Moreover, direct applications to control mosquitoes produces an EEC of 539 μg/L, and applications as pest control for aquatic crops (e.g., rice and watercress) produces an EEC of 1404–1797 μg/L. These peak levels typically drop off rapidly over time given malathion's half-life of 2 days at pH = 8 and 26 days at pH = 6 (Guerrant et al. 1970, Wang 1991); the pH of natural wetlands typically ranges from 5 to 8 (Mitsch and Gosselink 1986).

Methods

We conducted a mesocosm experiment at the University of Pittsburgh's Pymatuning Laboratory of Ecology. The experiment was a completely randomized design employing a factorial combination of two densities of tadpoles (low and high) crossed with six nominal pesticide application regimes (control; 50 or 250 μg/L applied at the start of the experiment; 50 or 250 μg/L applied later in the experiment; and 10 μg/L applied once per week for 7 weeks). While these concentrations are sublethal to amphibians (Relyea 2004), they are lethal to many invertebrates. Moreover, our lowest concentration (10 μg/L) is similar to the average EEC resulting from spraying >50 categories of crops (9 μg/L), whereas our highest concentration (250 μg/L) is well below the EEC for direct wetland oversprays (539–1797 μg/L [Odenkirchen and Wente 2007]).

The 12 treatment combinations were replicated four times for a total of 48 experimental units. The experimental units were 1200-L cattle tanks filled with ~1000 L of well water (pH = 8) on 1–4 May 2006. To each tank, we added 300 g of dry leaf litter (primarily *Quercus* spp.) and 25 g of rabbit chow on 4 May to provide an initial nutrient source and additional surfaces upon which periphyton could grow. On 4–5 May, we collected water from four nearby ponds, visually screened the samples and removed any tadpoles or zooplankton predators, mixed the water, and added an aliquot to each tank to serve as a natural source of algae and zooplankton. A week later, we added two unglazed tiles (10 × 10 cm) to each tank to serve as periphyton samplers. All tiles were placed in the tanks vertically (leaning up against the tank side wall) and in the same position relative to the sun.

After waiting 18 days for the tanks to develop algal and zooplankton communities, we added tadpoles to the tanks (defined as day 0 of the experiment). Each tank received both wood frogs (*Rana sylvatica*) and leopard frogs (*R. pipiens*) at densities of either 20 or 40 tadpoles for each species. These densities (9–18 tadpoles of each species/m^2) are well within natural densities (R. A. Relyea, E. E. Werner, D. K. Skelly, and K. L. Yurewicz, *unpublished data*). The tadpoles were originally collected as newly oviposited eggs on 31 March 2006 (12 masses of wood frogs, 10 masses of leopard frogs) and hatched in 200-L wading pools containing aged well water and fed rabbit chow ad libitum. The tadpoles were selected from a mixture of all egg masses and added to the experiment while they were still early in development (wood frogs = 68 ± 4 mg; leopard frogs = 91 ± 7 mg; initial mean mass ± 1 SE).

We treated the tanks with a commercial formulation of malathion that was reported to contain 50% active ingredient (Malathion Plus; Ortho Corporation, Marysville, Ohio, USA). Our objective was to examine how two relatively low concentrations of malathion (50 or 250 μg/L of active ingredient) impacted the food web when applied either at the start of the experiment, or later in the experiment when the wood frogs began to metamorphose but the leopard frogs still had several more weeks of development. In addition, we wanted to examine how an even lower concentration of malathion (10 μg/L) impacted the food web when applied weekly from the start of the experiment through the time of the first emergence of the leopard frog metamorphs (i.e., seven weekly applications). To achieve these manipulations, we applied 0.02 mL, 0.10 mL, and 0.50 mL of the commercial malathion on day 1 of the experiment (24 May) to tanks assigned the 10 μg/L weekly, 50 μg/L initial, and 250 μg/L initial treatments, respectively. For the treatment involving multiple pulses (10 μg/L weekly), we continued to add 0.002 mL of malathion each week from day 1 through day 43. After each pesticide application, the water was well mixed in all tanks. After approximately four weeks (i.e., day 27; one-third of the experiment's duration), the wood frogs began to emerge; the following day we applied the appropriate amounts of malathion to the 50 μg/L later and 250 μg/L later treatments (Fig. 1C, D).

Within one hour after each dosing, we collected ~10 mL of water from the tanks and pooled the water from each pesticide treatment into a single sample that was frozen (−29°C) and subsequently analyzed by an independent laboratory using high-pressure liquid chromatography (Mississippi State Chemical Laboratory, Mississippi State, Mississippi, USA; lower detection limit = 0.2 parts per billion). For the 10 μg/L weekly treatment, we had the first and last samples analyzed, and the measured concentrations were 10 and 9 μg/L, respectively. For the 50 μg/L initial and 50 μg/L later treatments, the measured concentrations were 40 and 32 μg/L, respectively (mean = 36 μg/L). For the 250 μg/L initial and 250 μg/L later treatments, the measured concentrations were 300 and 190 μg/L, respectively (mean = 245 μg/L). Hence, the concentrations in the

tanks were close to the nominal concentrations, and importantly, the concentrations in the tanks receiving weekly pulses did not increase over time (i.e., the malathion was breaking down before the next weekly pulse).

To add ecological reality to the experiment, we added a pond-drying component to simulate the ephemeral ponds that are frequented by both of the tadpole species used in the experiment. A commonly used protocol in amphibian ecology studies (e.g., Semlitsch 1987, Chase and Knight 2003), this placed a realistic time constraint on the tadpoles such that individuals that had not completed metamorphosis by day 79 were considered dead due to pond drying. Because tadpoles detect pond drying primarily via reductions in water volume (as opposed to increased solute concentration) and can increase their developmental rate to metamorphose before a water body dries (Denver et al. 1998), it would be inappropriate to simply terminate the experiment on a particular day and determine that day to signify a dried pond. Hence, we began a gradual drying of the tanks on 24 July (day 62; after all wood frogs had metamorphosed) and continued until 10 August (day 79). On each day during this dry-down period, we removed ~60 L of tank water. If there had been considerable rainfall during the previous 24 hours, we removed an additional amount of water to compensate for the rain. Given the rapid breakdown rates of malathion, the water removed would no longer have contained malathion.

Response variables

During the experiment, we quantified a variety of abiotic and biotic parameters at several time points to understand how the communities were responding to the treatments (Fig. 1C, D). For the abiotic variables, we measured temperature, pH, and dissolved oxygen in each tank on day 15 using a calibrated digital water meter (WTW, Woburn, Massachusetts, USA). This was done to determine the abiotic conditions of the experiment and to estimate how rapidly the malathion would break down. For the biotic variables, we positioned the time points relative to the timing of the initial pesticide applications (day 2), the later pesticide applications (day 29), the final weekly application of 10 μg/L (day 44), and the final day of the experiment (day 79).

The abundance of zooplankton (cladocerans and copepods) was assessed on day 8, day 22, and day 43 (prior to the weekly applications of malathion in all cases). We used a 0.2-L tube sampler that was plunged into the water column at five locations per tank. These five samples were pooled into a single 1-L sample and filtered through a 62-μm Nitex screen (Nitex, Sofia, Bulgaria). The zooplankton were then preserved in 70% ethanol and subsequently identified to species. We identified six species of cladocerans (*Daphnia pulex*, *D. schodleri*, *D. ambigua*, *D. galeata*, *Simocephalus* sp., and *Scapholebris* sp.), with *D. pulex* and *D. schodleri* dominating the assemblage (92% and 5% of all cladocerans, respectively). We identified nine species of copepods (*Acanthocyclops vernalis*, *Senecella calanoides*, *Skistodiaptomus pallidus*, *Eucyclops agilis*, *Leptodiaptomus sicilis*, *L. minutus*, *Skistodiaptomus oregonensis*, *Diacyclops thomasi*, and *Eurytemore affinis*) with *L. minutus* and *S. oregonensis* dominating the assemblage (56% and 41% of all copepods, respectively). Because we were interested in potential trophic cascades caused by the treatments, and because the two dominant species within each zooplankton assemblage both responded to the treatments in similar ways, we simply analyzed the total abundance of cladocerans and copepods.

Phytoplankton abundance was assessed on days 22 and 43 (prior to the weekly doses of malathion). For each tank, we collected 500 mL of water from the middle of the water column and vacuum-filtered the phytoplankton onto a Whatman GF/C filter (Whatman, Incorporated, Florham Park, New Jersey, USA). The filters were wrapped in foil and frozen. The frozen samples were later analyzed for chlorophyll *a* by following the protocols of Arar and Collins (1997), including the recommended correction by acidification. Chlorophyll *a* concentrations were assayed using a fluorometer (Model TD-700, Turner Designs, Sunnyvale, California, USA).

The abundance of periphyton was determined by removing one of the clay tiles on day 23 and day 44. On each date, we scrubbed all periphyton from one side of the tile and into a tub of filtered well water. The resulting algal slurry was then filtered through a Whatman GF/C filter that was previously dried at 80°C for 24 hours and then weighed. After collecting the periphyton, the filter was dried again at 80°C for 24 hours, and then reweighed to determine the biomass of periphyton that had grown on the tile.

To quantify the magnitude of the shading effect that phytoplankton blooms had on the growth of periphyton on the bottom of the tanks, we measured the rate of sunlight decay with depth on days 23 and 45. Using an underwater quantum sensor (LI-COR, Lincoln, Nebraska, USA), we measured the amount of photosynthetically active radiation at 10 cm and 30 cm below the water's surface. Using these two measurements, one can determine the decay rate of light with increased water depth (K) via the following formula:

$$K = \frac{\ln(L_{10}/L_{30})}{d}$$

where L_{10} is the intensity of sunlight at a depth of 10 cm, L_{30} is the intensity of sunlight at a depth of 30 cm, and d is the difference in depth between the two intensity measurements.

As the tadpoles metamorphosed, we quantified survival, time to metamorphosis, and size at metamorphosis. We conducted frequent checks of the tanks as the wood frogs approached metamorphosis. The wood

frogs began emerging on day 27, and we conducted daily checks for metamorphs in every tank through day 79 (leopard frogs began emerging on day 41). Metamorphs were collected when their tails were visually estimated to be <3 cm and the collected animals were held in 1-L tubs containing moist sphagnum moss until tail resorption was complete (Gosner stage 46 [Gosner 1960]). An individual's time to metamorphosis was defined as the number of days from the time when the amphibians were added to the experiment, to the date of achieving stage 46 (i.e., excluding the number of days from oviposition to the start of the experiment). Animals reaching stage 46 were euthanized in 2% MS-222 (tricaine methane sulfonate) and then preserved in 10% formalin. At the end of the experiment, all preserved metamorphs were weighed to determine each individual's mass at metamorphosis. For both amphibian species, our response variables for each tank were the percentage survival, mean time to metamorphosis, and mean mass at metamorphosis.

Because of the drying regime imposed during late July through mid-August, not all tadpoles achieved metamorphosis. On the final day of the experiment, the water depth in the tanks was down to ~10 cm, and this condition was treated as a dried pond. All remaining animals were removed from the tanks, and any tadpole that possessed at least one emerged forelimb was considered a successful metamorph and held until complete tail resorption. All tadpoles lacking at least one emerged forelimb were considered nonsurvivors. We counted the number of these tadpoles remaining in each tank to determine whether decreases in survival were caused by direct mortality or due to retardation of growth and development that prevented metamorphosis prior to the pond drying.

Statistical analyses

We analyzed the data using analyses of variance. The measurements of pH, temperature, and dissolved oxygen were all taken on day 15, allowing us to conduct a single multivariate analysis of variance (MANOVA) on these data. For the remaining response variables, we began assessing overall treatment effects by conducting a MANOVA on the final measurements of cladocerans, copepods, phytoplankton, periphyton, and light decay rate, as well as the response variables for the wood frogs and leopard frogs (survival, time to metamorphosis, and size at metamorphosis). In this way, we can control the overall experiment-wise error when conducting subsequent univariate analyses of variance (ANOVA) on each response variable, including repeated-measures ANOVAs on those variables for which we took multiple samples over time (cladocerans, copepods, phytoplankton, periphyton, and light decay rate). Data were log-transformed where needed. Whenever we detected significant main effects, we conducted mean comparison tests using Fisher's LSD test.

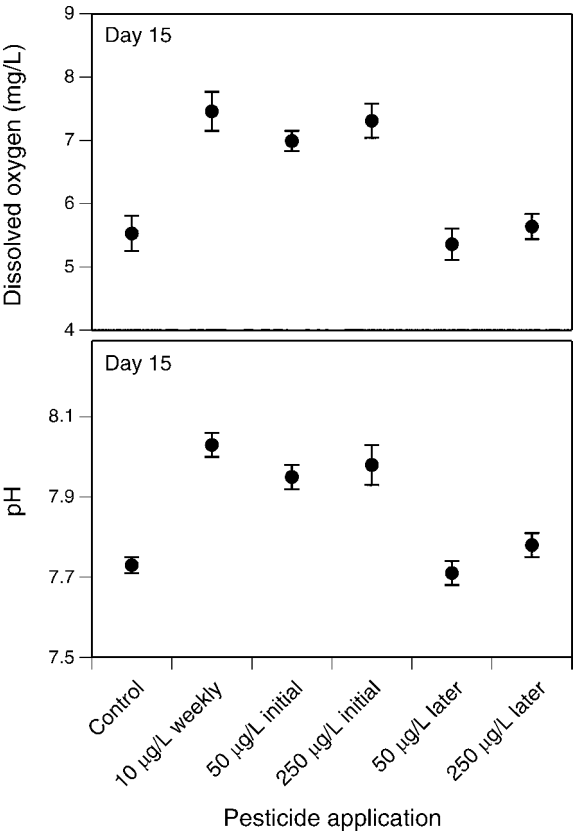

FIG. 2. The effect of different pesticide application regimes on the dissolved oxygen and pH in outdoor pond mesocosms. There was no effect of tadpole density nor any treatment interaction, so values are averaged across density treatments. Data are means ± SE.

RESULTS

The MANOVA on pH, temperature, and dissolved oxygen revealed a multivariate effect of pesticide (Wilks' λ, $F_{15,94} = 4.3$, $P < 0.001$) but no effect of tadpole density (Wilks' λ, $F_{3,34} = 1.0$, $P = 0.415$) or the interaction (Wilks' λ, $F_{15,94} = 0.8$, $P = 0.649$). The multivariate pesticide effect was caused by univariate effect of pH ($F_{5,36} = 17.6$, $P < 0.001$) and dissolved oxygen ($F_{5,36} = 14.9$, $P < 0.001$; Fig. 2), but there was no effect on temperature ($F_{5,36} = 0.9$, $P = 0.436$). Mean comparisons indicated that the three treatments involving initial applications of malathion had higher pH and dissolved oxygen than the controls ($P < 0.001$), but they did not differ from one another ($P > 0.1$). As one would expect when measuring these variables on day 15, tanks assigned the later applications of malathion (on day 28) did not differ from the controls ($P \geq 0.3$).

The MANOVA on all final measurements (cladocerans, copepods, phytoplankton, light decay rate, periphyton, and the three traits of each amphibian species [survival, time to metamorphosis, and size at metamorphosis]) indicated multivariate effects of pesticide (Wilks' λ, $F_{55,124} = 5.4$, $P < 0.001$), tadpole density

TABLE 1. Results of repeated-measures ANOVAs (F values followed by P in parentheses) to determine the effects of experimental manipulations on the abundance of aquatic organisms.

Factor	Cladocerans	Copepods	df	Phytoplankton	Light decay (K)	Periphyton	df
Tadpole density	2.4 (0.131)	0.7 (0.422)	1, 36	0.4 (0.532)	0.0 (0.962)	1.5 (0.235)	1, 36
Pesticide	6.0 (**<0.001**)	4.9 (**0.002**)	5, 36	3.6 (**0.010**)	2.9 (**0.028**)	10.4 (**<0.001**)	5, 36
Density × pesticide	0.9 (0.515)	0.6 (0.674)	5, 36	1.8 (0.137)	1.3 (0.270)	2.8 (**0.029**)	5, 36
Time	11.3 (**<0.001**)	56.5 (**<0.001**)	2, 72	13.0 (**<0.001**)	57.0 (**<0.001**)	117.4 (**<0.001**)	1, 36
Time × density	0.2 (0.851)	0.4 (0.697)	2, 72	0.2 (0.625)	0.3 (0.585)	1.6 (0.217)	1, 36
Time × pesticide	10.4 (**<0.001**)	5.1 (**<0.001**)	10, 72	9.7 (**<0.001**)	6.2 (**<0.001**)	1.1 (0.371)	5, 36
Time × density × pesticide	1.2 (0.291)	0.7 (0.729)	10, 72	1.5 (0.217)	1.9 (0.124)	2.6 (**0.041**)	5, 36

Note: Significant effects are in boldface type.

(Wilks' λ, $F_{11,26} = 19.5$, $P < 0.001$), and a pesticide-by-density interaction (Wilks' λ, $F_{55,124} = 1.5$, $P = 0.023$). To understand which response variables were causing these multivariate effects, we examined each univariate effect by conducting ANOVAs on those variables that were measured only at the end of the experiment (amphibian survival, time to metamorphosis, and size at metamorphosis), and repeated-measures ANOVAs on those variables that were measured more than once during the experiment (zooplankton, phytoplankton, light decay, and periphyton).

Zooplankton

The repeated-measures ANOVA on cladocerans revealed effects of pesticide, time, and a pesticide-by-time interaction (Table 1, Fig. 3). As a result, we analyzed the effects of the pesticide treatments within each sample date. On the first sample date, there was an effect of pesticide ($F_{5,42} = 71.9$, $P < 0.001$); compared to the control treatment, the three treatments receiving weekly or initial pesticide applications had fewer cladocerans ($P \leq 0.001$). On the second sample date, there also was an effect of pesticide ($F_{5,42} = 44.2$, $P < 0.001$); compared to the control treatment, the three treatments receiving weekly or initial pesticide applications still had fewer cladocerans ($P \leq 0.001$). On the third sample date (~2 weeks after the later applications of malathion), there was a different pattern of cladoceran abundance ($F_{5,36} = 43.2$, $P < 0.001$); compared to the control, treatments receiving weekly or later applications of malathion had fewer cladocerans ($P = 0.019$), whereas treatments receiving initial applications of 50 or 250 μg/L had rebounded to be similar to the control treatment ($P > 0.8$). In summary, cladocerans experienced a decline following the application of malathion, the declines persisted in the multiple-pulse treatment, and the populations rebounded in the early, single-pulse treatments.

The analysis of copepod abundance also indicated effects of pesticide, time, and a pesticide-by-time interaction (Table 1, Fig. 3). On the first sample date, copepod abundance was very low across all treatments, but there was an effect of pesticide ($F_{5,42} = 2.8$, $P = 0.027$); compared to the control, tanks receiving initial applications of 50 or 250 μg/L of malathion had fewer copepods ($P < 0.02$), whereas the other three treatments did not differ. On the second sample date, there was no effect of pesticide ($F_{5,42} = 2.3$, $P = 0.063$). On the third sample date, there was again an effect of the pesticide treatments on copepod abundance ($F_{5,42} = 7.3$, $P < 0.001$); compared to the control treatment, tanks receiving malathion at any time or frequency had more copepods ($P \leq 0.02$). In summary, malathion initially only caused small declines in copepods, followed by rapid increases in copepods that were associated with decreased cladocerans.

Phytoplankton, light decay rate, and periphyton

The analysis of phytoplankton revealed effects of pesticide, time, and the pesticide-by-time interaction (Table 1, Fig. 4). On the first sample date, there was an effect of pesticide ($F_{5,42} = 7.3$, $P < 0.001$); compared to the control, tanks receiving weekly or initial applications of malathion had more phytoplankton ($P \leq 0.008$). On the second sample date, there also was an effect of pesticide ($F_{5,42} = 5.9$, $P = 0.001$); compared to the control, weekly applications were not different ($P = 0.312$), initial applications of 50 or 250 μg/L and later applications of 50 μg/L had less phytoplankton ($P < 0.04$), and later applications of 250 μg/L did not differ ($P = 0.130$).

The analysis on the rate of light decay detected effects of pesticide, time, and the pesticide-by-time interaction (Table 1, Fig. 4). On the first sample date, there was no effect of pesticide ($F_{5,42} = 1.0$, $P = 0.404$). However, on the second sample date (~2 weeks after the later application of malathion), there was an effect ($F_{5,42} = 6.5$, $P < 0.001$). Compared to the control, tanks receiving the weekly pulses of 10 μg/L of malathion had greater rates of light decay (i.e., lower light transmission; $P < 0.001$).

The analysis of periphyton detected effects of pesticides, time, a density-by-pesticide interaction, and a three-way interaction (Table 1, Fig. 5). On the first sample date, there was an effect of pesticide ($F_{5,36} = 4.5$, $P = 0.003$), but no effect of tadpole density ($F_{1,36} = 2.6$, $P = 0.115$) or the interaction ($F_{5,36} = 0.4$, $P = 0.814$). Compared to the control treatment, only tanks receiving an initial pulse 50 μg/L of malathion had reduced periphyton ($P = 0.046$). On the second sample date, there was no effect of tadpole density ($F_{1,36} = 0.007$, $P = 0.934$), but there was an effect of pesticides ($F_{5,36} = 8.9$,

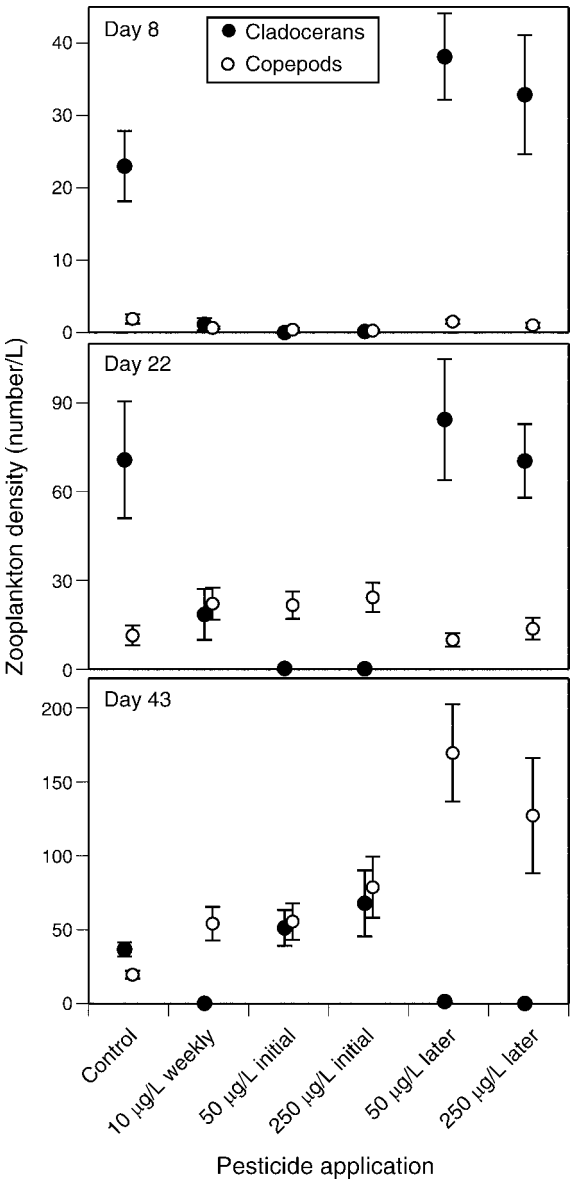

FIG. 3. The effect of different pesticide application regimes (pooled across tadpole competition treatments) on the density of cladoceran and copepod zooplankton in outdoor pond mesocosms over time. Data are means ± SE. Note that different scales are used on each panel to show detail. "Light meter" indicates that water transparency was measured at this point.

$P < 0.001$) and a density-by-pesticide interaction ($F_{5,36} = 5.9$, $P < 0.001$). Under low tadpole density, tanks containing malathion had similar periphyton abundances as the control tanks ($P > 0.12$). Under high tadpole density, however, tanks receiving an initial pulse of malathion (50 or 250 μg/L) or weekly pulses of 10 μg/L of malathion had 44–79% less periphyton ($P < 0.035$). In summary, the application of malathion (either as single pulses early in the experiment or as multiple pulses for the first 7 weeks of the experiment) was associated with increases in phytoplankton and decreases in periphyton.

Amphibians

The univariate analyses of wood frogs and leopard frogs examined survival, time to metamorphosis, and size at metamorphosis (Table 2, Fig. 6). Wood frog survival was reduced by tadpole density but was unaffected by the pesticide or the interaction. On average, wood frogs had 17% lower survival when faced with high density. Given that the last wood frog emerged on day 62, this lower survival is not a reflection of retarded development preventing metamorphosis prior to pond drying (which occurred on day 79).

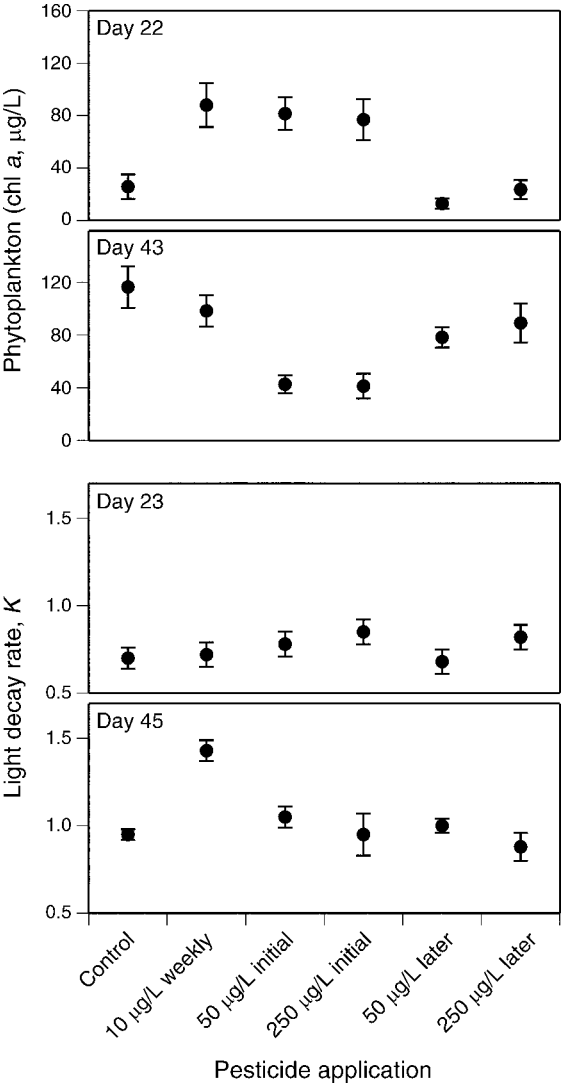

FIG. 4. The effect of different pesticide application regimes (pooled across both tadpole densities) on the amount of phytoplankton and the rate of light decay with water depth in outdoor pond mesocosms over time. Light decay rate was determined by measuring light intensity at two depths in the mesocosm. Data are means ± SE.

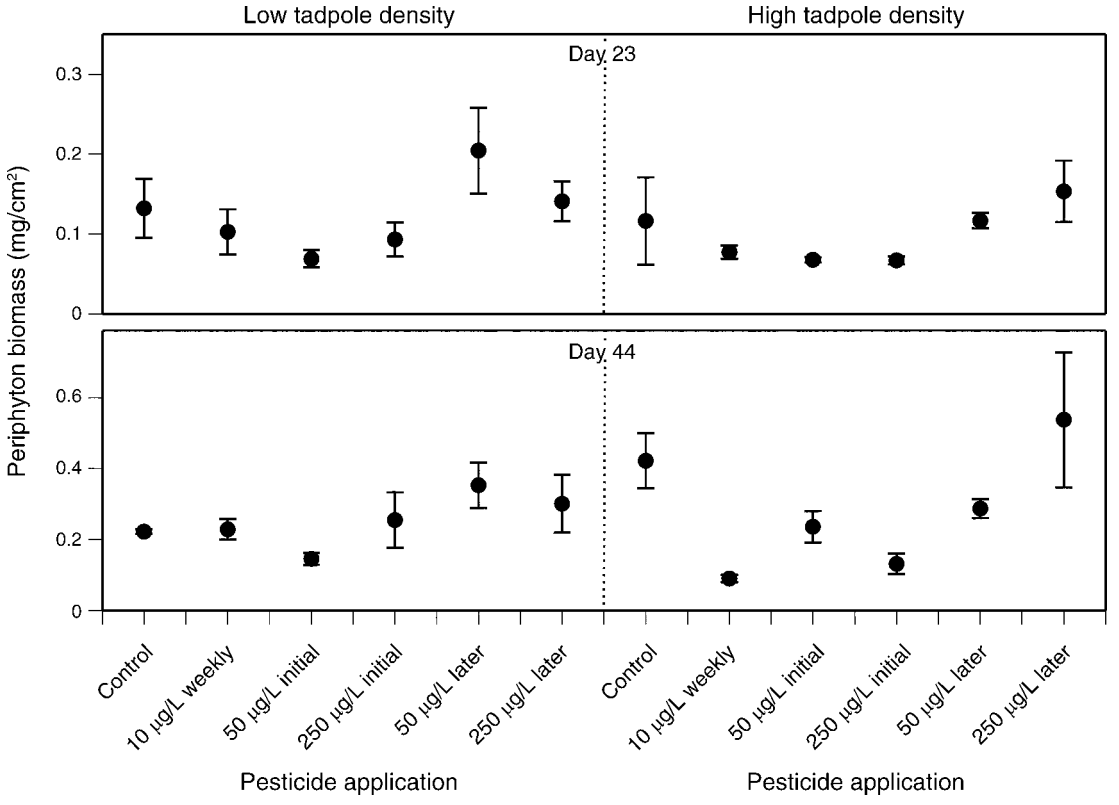

FIG. 5. The effect of tadpole density and different pesticide application regimes on periphyton abundance over time in outdoor pond mesocosms. Data are means ± SE. Note that top and bottom panels have different *y*-axis scales to show detail.

We then analyzed the wood frog life history traits. There were no significant main effects or interaction on time to metamorphosis (Table 2, Fig. 6). Mass at metamorphosis was affected by pesticide and tadpole density, but there was no interaction (Table 2, Fig. 6). High density caused a 22% reduction in mass. Mean comparisons among the pesticide treatments indicated that none of the malathion treatments differed from the control ($P > 0.1$). In summary, increased tadpole density had important negative effects on wood frog survival and growth, but the pesticide treatments had no effect on wood frogs compared to the control.

Leopard frog survival to metamorphosis was affected by tadpole density, pesticides, and their interaction (Table 2, Fig. 6). The interaction occurred because the application of malathion had no effect on leopard frog survival under low density, but weekly applications of 10 µg/L of malathion caused a 43% decrease in survival under high density (compared to the control). However, if we combined the number of metamorphs and the number of nonmetamorphosed tadpoles remaining when the tanks dried, we found no difference between control tanks and the 10 µg/L weekly tanks ($P = 0.541$). Thus, the lower percentage of metamorphs was not due to direct toxic effects, but due to the retarded growth and development of tadpoles exposed to this treatment. This retarded growth and development caused by the

TABLE 2. Results of ANOVAs showing the effects of tadpole density and pesticide application regimes on the growth and development of wood frogs and leopard frogs.

Response variable	Density (df = 6, 31)		Pesticides (df = 30, 126)		Density × pesticides (df = 30, 126)	
	F	*P*	*F*	*P*	*F*	*P*
Wood frog survival	16.6	**<0.001**	0.3	0.934	0.7	0.575
Wood frog time to metamorphosis	1.1	0.301	1.8	0.134	2.2	0.075
Wood frog size at metamorphosis	73.3	**<0.001**	2.7	**0.035**	1.8	0.139
Leopard frog survival	112.7	**<0.001**	2.3	0.059	2.7	**0.036**
Leopard frog time to metamorphosis	116.0	**<0.001**	3.3	**0.015**	0.5	0.797
Leopard frog size at metamorphosis	83.0	**<0.001**	4.4	**0.003**	0.7	0.638

Notes: *F* and *P* values from univariate tests are shown for both main effects and their interaction. Degrees of freedom for each factor are provided in parentheses. Significant effects are in boldface type.

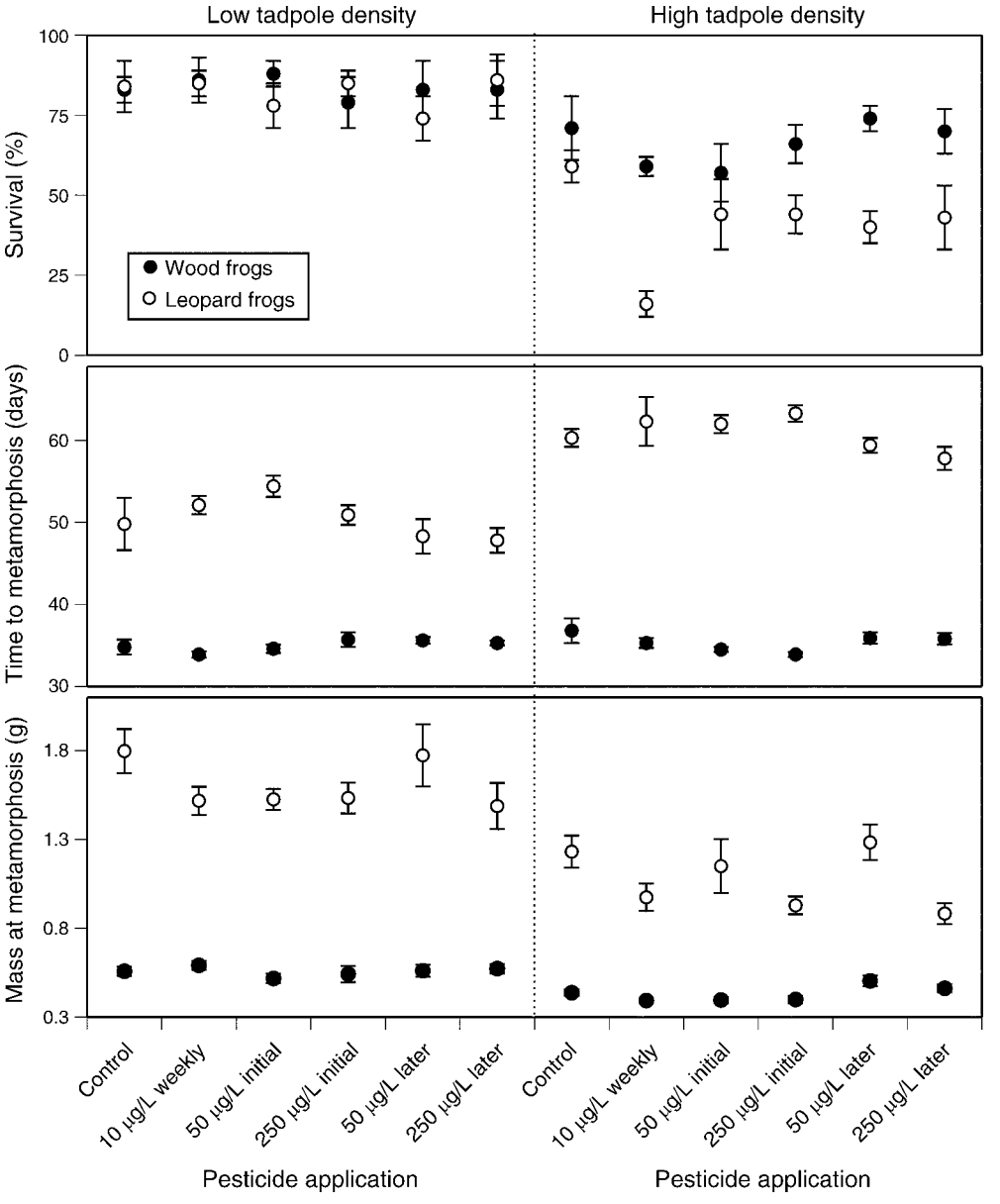

FIG. 6. The effect of tadpole density and different pesticide application regimes on the survival, time to metamorphosis, and size at metamorphosis of wood frogs and leopard frogs reared in outdoor pond mesocosms. Data are means ± SE.

malathion-induced trophic cascade caused the animals to not metamorphose prior to the pond drying.

The time to metamorphosis in leopard frogs was affected by pesticide and tadpole density, but not their interaction (Table 2, Fig. 6). Increased density caused a 20% (10-day) delay in metamorphosis. The various applications of malathion did not alter time to metamorphosis relative to the control ($P \geq 0.07$), but initial applications of 50 or 250 μg/L and weekly applications of 10 μg/L did cause a 5–7% (3–5 day) delay in metamorphosis compared to later application of 50 or 250 μg/L ($P = 0.013$, 0.058, and 0.051, respectively). Obviously, had the tanks not experienced drying in mid-August, the mean time to metamorphosis in the 10 μg/L weekly, high-competition treatment would have been longer.

The mass at metamorphosis in leopard frogs was affected by pesticides and tadpole density, but not their interaction (Table 2, Fig. 6). Averaged across pesticide treatments, increased density caused a 33% reduction in mass. Averaged across density treatments, weekly applications of 10 μg/L and applications of 250 μg/L (either initially or later) caused 18–22% reductions in mass ($P \leq 0.015$). In summary, increased tadpole density and pesticides both generally caused slower growth and development in leopard frogs; moreover, the

combination of higher density and frequent, low-concentration applications caused leopard frogs to be subjected to the lethal effects of pond drying.

Discussion

The results of this study indicated that low (and rapidly degrading) concentrations of malathion (10–250 μg/L), which have no direct lethal effects on amphibians (Relyea 2004), can indirectly cause reduced growth and development. When this disturbance to the community was reinforced by small weekly applications of the insecticide (10 μg/L), a substantial fraction of the leopard frogs failed to metamorphose before the pond dried. This indirect mortality appeared to occur via a pesticide-mediated trophic cascade that placed the amphibians in a vulnerable state as their environment dried. Thus, the proximate cause of death was pond drying, but the true cause of death was the apparent chain of events initiated by the application of malathion.

The trophic cascade was initiated by the direct toxic effect that malathion had on the assemblage of zooplankton in the community. Indeed, only nine days after the first applications of malathion, we observed a 97% decline in total zooplankton compared to the control. This sharp decline with 10–250 μg/L of malathion is consistent with other studies of zooplankton sensitivity in the laboratory ($LC50_{1-3d} = 2$–10 μg/L [Sanders and Cope 1966, Wong et al. 1995]) and in mesocosms (Relyea 2005). Importantly, the high sensitivity of zooplankton is not limited to malathion; a wide variety of insecticides are highly toxic to zooplankton, including carbaryl, diazinon, endosulfan, esfenvalerate, and pyridaben (Hanazato and Yasuno 1987, 1989, Fairchild et al. 1992, Lozano et al. 1992, Giddings et al. 1996, Barry and Logan 1998, Rand et al. 2000, Bridges and Boone 2003, Relyea 2005, Rohr and Crumrine 2005). Therefore, the trophic cascade initiated by the malathion-induced decline in zooplankton likely applies to a large number of insecticides.

The zooplankton exhibited population dynamics over time that demonstrated the relative impact of malathion on cladocerans vs. copepods, as well as the resilience of the zooplankton assemblage. When malathion was applied early in the experiment (day 1), the cladocerans experienced a sharp decline in numbers, while the copepods (which were initially rare) increased in abundance by day 22. When malathion was applied later in the experiment (day 28), we observed the same phenomenon in the zooplankton collected on day 43. These patterns of abundance reflect the generally lower sensitivity of copepods to insecticides compared to cladocerans. Given that cladocerans and copepods compete for some size classes of phytoplankton, this differential sensitivity allowed a competitive release of the copepods, a phenomenon that has been previously documented for higher concentrations of malathion and carbaryl (Hanazato and Yasuno 1987, 1989, 1990, Havens 1994, Mills and Semlitsch 2004, Relyea 2005; R. E. Relyea and J. T. Hoverman, *unpublished manuscript*). When there was only a single application of malathion at the start of the experiment (50 or 250 μg/L), the cladocerans began to recover to control levels by day 43. However, as one might expect from a "press" treatment that maintains a disturbance over time, the seven weekly applications of very low concentration (10 μg/L) prevented the cladocerans from recovering.

Given that zooplankton forage on phytoplankton, the pesticide-induced decline in zooplankton should allow an increase in the abundance of phytoplankton (if the phytoplankton are herbivore limited). Consistent with this expectation, the three treatments receiving applications of malathion on day 1, which all caused a sharp decline in cladocerans, experienced an increase in phytoplankton abundance by day 22. Phytoplankton blooms with insecticides have been observed in several past studies using several different insecticides, including malathion, carbaryl, and endosulfan (Havens 1994, 1995, Barry and Logan 1998, Bridges and Boone 2003, Fleeger et al. 2003, Boone et al. 2004, Mills and Semlitsch 2004). Additional signals of this phytoplankton bloom were seen in the increases in dissolved oxygen and pH on day 15, which are indicative of increased photosynthesis associated with a phytoplankton bloom. By day 43, the two initial exposure treatments (50 and 250 μg/L) had relatively less abundant phytoplankton, which may reflect either a reduction in available resources with time or the impacts of the rebounding cladoceran populations.

The bloom in phytoplankton often was associated with a decline in periphyton. On day 23, the reduction in periphyton was only significant in the 50 μg/L initial treatment. However, by day 44, the reduction in periphyton was clear under high tadpole density in tanks exposed to either of the two initial-pulse treatments or the multiple-pulse treatment. This suggests that the cascade took considerable time to develop as algal populations responded to the changing conditions. This is a key point, because it suggests that while insecticides such as malathion can break down within days, the cascade of indirect effects might not be observable for more than a month. For example, Relyea (2005) and Relyea et al. (2005) exposed tadpole communities to 500 μg/L of carbaryl or 315–320 μg/L of malathion in 2–3 week experiments (both chemicals share a common mode of action), and neither study found top-down effects on periphyton abundance. Hence, shorter-duration community assessments will miss many of these cascading effects. Although the treatment causing the strongest effects on the periphyton and amphibians (weekly pulses of 10 μg/L) was also associated with significant increase in light decay, the initial-pulse treatments did not show a similar association. This may simply reflect the fact that the maximum depth difference possible for measuring light transmission (20 cm) with our equipment did not permit a great deal of sensitivity in assessing differences in light

transmission from the surface to the actual bottom of the mesocosm (a depth of 46 cm).

Few longer-term studies have quantified periphyton abundance in tadpole communities exposed to insecticides. For example, using much higher concentrations of the insecticide carbaryl (2500 μg/L), Boone et al. (2005) and Boone and Bridges-Britton (2006) added the insecticide carbaryl after allowing the tadpoles to acclimate to the mesocosms for 8–15 d. Boone et al. (2005) found that periphyton abundance increased in the short term (7 days) but then rapidly converged to control levels, and Boone and Bridges-Britton (2006) found that periphyton was 2–3 times more abundant in carbaryl tanks (although the difference was not significant). In contrast, Mills and Semlitsch (2004) exposed aquatic communities to 2000 or 5000 μg/L of carbaryl and waited until the chemical had fully degraded before adding the tadpoles, to remove any direct effects of carbaryl on the tadpoles. They found that control tanks contained 3–4 times more periphyton than carbaryl-treated tanks. In the current study, we used much lower concentrations (10–250 μg/L) and also found a decline in periphyton with malathion. The most likely explanation for this diversity of outcomes is that low concentrations of insecticides applied with tadpoles present (this study) or higher concentrations that are applied and degraded before the tadpoles are added (Mills and Semlitsch 2004) will cause a trophic cascade through the zooplankton that negatively impacts periphyton abundance. However, when applying insecticides at much higher concentrations and after tadpoles are already in the system (Boone et al. 2005, Boone and Bridges-Britton 2006), there will be additional effects including a reduction in tadpole feeding when exposed to the acetylcholine esterase inhibitor until it breaks down, and this reduced foraging would permit an increase in periphyton. In the latter case, we would still expect a trophic cascade through the zooplankton, but the impact of this cascade could be countered by the positive effects of an early reduction in tadpole foraging.

The ultimate effect of the trophic cascade was observed on the amphibians. In general, the wood frogs exhibited little impact of the trophic cascade. The lack of large effects on wood frogs likely reflects the fact that they have relatively short larval periods (mean = 35 days), which allowed them to spend a large fraction of their larval period unaffected by the eventual trophic cascade that reduced the periphyton. However, wood frogs also possess the ability to adjust the relative size of their foraging morphology (e.g., mouthparts and gut length) under low-resource conditions to likely become more efficient herbivores, and this may contribute to the reduced impacts of the trophic cascade as well (Relyea and Auld 2004, 2005, Schiesari 2006). In contrast, leopard frogs experienced 18–22% reduced growth under three of the malathion treatments. The reductions in growth are important to the long-term fitness of amphibians, because reduced mass at metamorphosis is associated with reduced post-metamorphic survival, longer times to reproductive maturity, smaller sizes at reproductive maturity, decreased mating success, and the production of fewer eggs (Howard 1980, Berven 1981, 1982, Berven and Gill 1983, Smith 1987, Semlitsch et al. 1988, Gerhardt 1994, Altwegg and Reyer 2003). Moreover, the slower growth and slower development made the leopard frog tadpoles vulnerable to pond drying, especially when exposed to small weekly pulses of malathion, which resulted in 43% of the animals not metamorphosing before pond drying. Given that the mechanism for this effect was apparently the trophic cascade that caused reduced periphyton, it makes sense that the impacts on leopard frogs were much larger under high vs. low tadpole density.

A previous shorter-term mesocosm study (23-day duration) applied malathion to aquatic communities containing tadpoles and quantified growth (but not development). Using tadpoles of leopard frogs, American toads (*Bufo americanus*), and gray tree frogs (*Hyla versicolor*), Relyea et al. (2005) used a single initial concentration of malathion (320 μg/L) and found that leopard frogs experienced an 8% reduction in growth, but the effect was not significant. In this case, the initial biomass of the tadpoles was considerably lower than the current study (26–52%), which would pose a lower intensity of initial competition. As noted earlier, one would expect the effects of the trophic cascade to be the least intense under low competition. Thus, in shorter-term experiments, differences in initial biomass may explain the different growth outcomes of leopard frogs among studies. Previous studies have also been conducted on the insecticide carbaryl (which shares malathion's mode of action). The impacts of carbaryl on tadpole growth and development vary among studies, but the outcomes (in the absence of other factors such as predators or nitrates) generally follow one of two patterns; studies that apply carbaryl and allow complete degradation before adding the tadpoles cause a reduction in periphyton and subsequent slower growth and development of tadpoles (Mills and Semlitsch 2004), whereas studies that apply carbaryl after the tadpoles are already in the mesocosms cause a short-term increase in periphyton (likely via pesticide-induced reductions in tadpole foraging) and subsequent faster growth and development of tadpoles (Boone et al. 2005, Boone and Bridges-Britton 2006).

There was a larger effect of multiple small pulses compared with a single pulse that was 25 times greater in concentration. While numerous studies have examined the impact of multiple pesticide pulses (Kosinski and Merkle 1984, Jurgensen and Hoagland 1990, Pusey et al. 1994), few studies have directly compared the effects of single vs. multiple pesticide applications on aquatic communities (Hanazato and Yasuno 1990, Boone et al. 2001). For example, Hanazato and Yasuno (1990) examined communities of zooplankton and algae and found that multiple applications of 500 μg/L of an

insecticide (carbaryl) had no further effect on the community than a single application at the same concentration. Using the same insecticide, Boone et al. (2001) found no difference in the survival or growth of green frog tadpoles (*R. clamitans*) exposed to single and multiple applications of 3500 μg/L. In these studies, the single low concentrations were capable of eliminating the vast majority of the zooplankton, and subsequent applications could therefore not eliminate the zooplankton any further. This suggests that, when applications are relatively close in time, we may only be able to observe differences between single and multiple applications when the concentration of a single application is unable to cause a maximal effect on a given taxonomic group. This expectation changes, of course, when the applications are further apart in time and the system is capable of rebounding following pesticide breakdown. In the current study (using a lower range of malathion concentrations), the zooplankton populations were able to recover from the initial single applications and this would allow the initial trophic cascade to reverse itself. However, repeated applications of the lowest concentration (i.e., a press treatment) continued to reduce the zooplankton community (cladocerans in particular) and thereby continued to enforce the trophic cascade. One would predict that lower pH level would also accentuate this effect because malathion degrades at a slower rate under lower pH conditions (Guerrant et al. 1970, Wang 1991). This highlights the importance of examining a range of pesticide application regimes and demonstrates that multiple applications of an insecticide at quite low concentrations can have larger effects than single applications.

Although low concentrations of pesticides can have important sublethal effects on organisms via changes in behavior and physiology (Weis et al. 2001, Relyea and Hoverman 2006), two lines of evidence suggest that sublethal effects were not important in our experiment. First, any sublethal effects that could have occurred would have been restricted to very brief periods of time (i.e., a few days) given the rapid breakdown rate of malathion. Second, if there were important sublethal effects occurring during these brief time periods, one might expect similar impacts on both tadpole species, given that both species have similar $LC50_{16\text{–}d}$ values for malathion (2400 μg/L for leopard frog tadpoles and 1250 μg/L for wood frog tadpoles [Relyea 2004]). If anything, the wood frogs might be a bit more sensitive, but we observed larger effects on the leopard frogs in the current experiment. Hence, while sublethal pesticide effects can be important, they did not play an obvious role in the outcomes observed in our study.

Conclusions

Given the ubiquity of chemical contamination in nature, there is a compelling need to understand how such contaminants impact species and communities in nature. We found that a short-lived insecticide appeared to propagate a trophic cascade that began with an elimination of zooplankton and, in several treatment combinations, culminated with a decline in the growth and development of larval amphibians. By including both single and multiple applications of the pesticide, we were able to compare "pulse" and "press" disturbances from a pesticide on a wetland community. The single pulse treatments had a substantial but ephemeral impact on the zooplankton, which rebounded within the duration of the experiment, demonstrating the relatively high resiliency of zooplankton (Niemi et al. 1990, Brock et al. 2000*a*, *b*, Clements and Newman 2002). However, the pesticide disturbance had longer-lasting effects on the phytoplankton, periphyton, and amphibians, suggesting that being farther along the trophic cascade or having longer generation times can both cause longer-lasting effects of the disturbance (in this case up to 78 d after the initial pesticide pulse).

In contrast to the single pulses of pesticide-induced disturbance, the "press treatment" consisting of multiple pulses at low concentrations for 7 weeks held the community in a state of continued disturbance that continued to reinforce the trophic cascade. Under this condition, the pesticide had its largest impact on the food web and, hence, on the amphibians, causing up to 43% mortality when combined with the natural constraint of a drying pond. While the trophic cascade involving the zooplankton and algae has been previously described, the subsequent effect on amphibians has not. This discovery is particularly important because malathion is used globally to combat the adult mosquitoes that carry malaria and West Nile virus (Gratz and Jany 1994, Walker 2000), and is currently the most commonly applied insecticide in the United States for controlling a broad array of crop pests (Kiely et al. 2004). Thus, there is the potential to impact amphibian growth and survival across a wide geographic range. Although the current experiment examined only one insecticide (malathion), the large reduction in zooplankton that initiated the trophic cascade is a situation that is common to a wide range of insecticides including carbaryl, diazinon, endosulfan, esfenvalerate, and pyridaben (Hanazato and Yasuno 1987, 1989, Fairchild et al. 1992, Lozano et al. 1992, Giddings et al. 1996, Barry and Logan 1998, Rand et al. 2000, Relyea 2005, Rohr and Crumrine 2005). Therefore, it is likely that the mechanisms identified in this study can be generalized to many insecticides, and this offers the ability to understand how a large group of contaminants can potentially affect aquatic communities, including those communities containing amphibians.

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