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East Coast vs West Coast: effects of an insecticide in communities containing different amphibian assemblages

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Abstract. The importance of understanding the mechanisms underlying insecticide disturbances in natural systems is growing because of increasing global insecticide use. Despite the prevalence of pesticides and the vulnerability of aquatic systems to insecticides, little is understood about the effect of lower concentrations of insecticides (<1 ppm) on aquatic community interactions. Whether insecticide effects are generalizable across different aquatic assemblages and trophic levels also is unclear. Furthermore, few investigators have examined the indirect consequences of insecticides beyond the primary consumer level. We examined how a single application of malathion at 3 concentrations (0, 6, or 40 μg/L) and the presence or absence of zooplankton predators (larval salamanders) affected aquatic communities composed of zooplankton, phytoplankton, periphyton, and 2 geographically distinct amphibian assemblages from Oregon and Pennsylvania. At these concentrations, malathion was directly lethal to certain zooplankton species, causing a shift from cladoceran-dominated assemblages to copepod-dominated assemblages. The decrease in cladoceran abundance released top-down pressure on phytoplankton, allowing an increase in phytoplankton abundance. The increase in phytoplankton was associated with a decrease in periphyton (the major food source of anurans) because of competition between phytoplankton and periphyton. We did not find direct mortality in anurans or salamanders, but the insecticide-mediated reduction in zooplankton indirectly caused a decrease in larval salamander mass. In contrast, anurans exposed to malathion were heavier at metamorphosis. Overall, these results demonstrate that low concentrations of insecticides have indirect consequences on nontarget members of the community across multiple trophic levels, and the indirect insecticide-mediated effects are generalizable across 2 geographically distinct amphibian assemblages.

Key words: trophic cascade, acetylcholine esterase inhibitor, community ecotoxicology, Pennsylvania amphibian assemblage, Oregon amphibian assemblage.

Understanding the patterns of species abundance and diversity is a central goal in ecology. An established way to assess these intricate interactions is to examine how a perturbation alters species interactions (Bender et al. 1984). Ecologists can better understand the underlying mechanisms shaping abundance and diversity by perturbing communities and tracking their recovery (Pickett et al. 1989, Rohr et al. 2006).

The role of anthropogenic perturbations in disrupting these patterns is becoming increasingly apparent. Pesticide use is of particular interest because agricultural development is projected to increase drastically in the next few decades (Laurance 2001, Tilman et al. 2001). Aquatic systems are especially susceptible to contamination by pesticides via direct application and accidental drift or runoff. Investigators estimate that 30 to 60% of shallow ground water and 60 to 95% of streams are contaminated by ≥1 pesticide. Insecticide contamination is especially common because insecticides are applied for a number of reasons including agricultural use and disease prevention (Frank et al. 1990, LeNoir et al. 1999, Aston and Seiber 1997, McConnell et al. 1998, Kiely et al. 2004, Meester et al. 2005, Gilliom et al. 2007). Despite the high frequency of insecticide exposure in natural aquatic communities, our understanding of insecticides is largely derived from artificial, single-species, short-term laboratory studies (Moore et al. 1998). These laboratory studies are critical to understanding the direct toxicity of insecticides to individual species, but they do not consider indirect effects. To understand the direct and the indirect impact of insecticides, more

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realistic scenarios must be included in toxicological studies.

Insecticides at high concentrations can be directly lethal to many taxa including zooplankton, insects, and amphibians (Boone et al. 2004, Mills and Semlitsch 2004, Metts et al. 2005). However, the consequences that arise indirectly at lower concentrations are less intuitive. Low insecticide concentrations (<1 mg/L) that are not directly lethal to more-tolerant taxa, such as amphibians, can still have lethal indirect effects via the food web (Relyea and Diecks 2008, Relyea 2009). In these studies, low concentrations of insecticides in aquatic mesocosms caused high zooplankton mortality, which led to a decline in grazing pressure on phytoplankton and a phytoplankton bloom. Subsequent decreases in light availability caused declines in benthic periphyton growth, resulting in decreased growth and development of periphyton grazers, such as tadpoles (Relyea and Diecks 2008, Relyea and Hoverman 2008). Thus, low concentrations of insecticides not directly toxic to certain taxa can have indirect cascading consequences.

Despite our growing understanding that low levels of insecticides can initiate lethal trophic cascades, the mechanisms underlying these indirect effects are still poorly understood. Aquatic communities worldwide vary greatly in species composition and trophic complexity, so generalizations about the underlying mechanisms shaping the effects of insecticides are critically needed. For instance, whether communities containing different assemblages react similarly to insecticide contamination is unclear. Few investigators have examined the indirect consequences of insecticides beyond the level of primary consumer. For example, predators can have large effects on the abundance and behavior of prey (Fretwell 1977, Lawler 1989), yet the combined effect of predators and contaminants have been addressed in only a few studies. To address these issues, we applied a low concentration of a globally common insecticide (malathion) and assessed the responses of aquatic communities containing 2 geographically separated amphibian assemblages (from Pennsylvania [PA] or Oregon [OR]) with similar life histories. We also examined whether the addition of a zooplankton predator (salamanders) altered the effect of an insecticide perturbation on the community in an additive or synergistic fashion (Holomuzki et al. 1994, Davic and Welsh 2004). We hypothesized that communities containing amphibians with similar life histories would exhibit similar responses to the insecticide and that the addition of larval salamanders would interact to reduce zooplankton abundance and cause more severe trophic cascades through the community.

Methods

Pesticide background

We used the insecticide malathion to examine these questions because of its common use worldwide. Malathion is an organophosphate insecticide used in the agricultural sector and for residential and public pest control. It inhibits acetylcholine esterase activity, which negatively affects nerve transmissions. Up to 11.3 million kg of active ingredient is sprayed annually in the USA (Kiely et al. 2004). Malathion reaches water bodies indirectly via runoff and aerial transport and directly via application to water bodies to control mosquito vectors of West Nile virus and malaria and pests of aquatic crops, such as rice and watercress (Schiff and Sutula 2004, Odenkirchen and Wente 2007). Malathion has a half-life of 1.4 d at pH = 8 and 147 d at pH = 6 and was detected in 7% of ponds sampled between 1992 and 1996 during the US Geological Survey's National Water Quality Assessment (NAWQA) (Newhart 2006). The US Environmental Protection Agency's expected environmental concentrations for malathion in surface water in California are 9 to 27 μg/L when sprayed on terrestrial crops and 1404 to 1797 μg/L when sprayed on rice and watercress (Odenkirchen and Wente 2007). Malathion is highly toxic to most aquatic invertebrates at concentrations as low as 0.5 μg/L. It is also moderately toxic to larval amphibians. The concentration that kills 50% of a sample population (LC50) for larval anurans ranges between 1.3 and 5.9 ppm (Relyea 2004, Newhart 2006, USEPA 2007).

Experimental design

We conducted a mesocosm experiment at the University of Pittsburgh's Pymatuning Laboratory of Ecology. Using mesocosms allowed us to use substantial replication while assessing the risk of contaminants to diverse communities (Fig. 1) (Touart 1988, Lozano et al. 1992, Boone and Bridges-Britton 2006, Relyea and Diecks 2008). A limitation of mesocosms is that they often represent simplified communities, but this reductionist approach is critical to developing our broader understanding of the underlying mechanisms shaping the effects of insecticides in aquatic communities. We included many of the strong interactors that would be present in a wetland community, and subsequent work building on our results has added many more interactors including predatory insects.

The experimental design was a factorial combination of 2 geographically distinct amphibian assemblages (*Rana sylvatica* [PA] or *Rana cascadae* [OR]), the

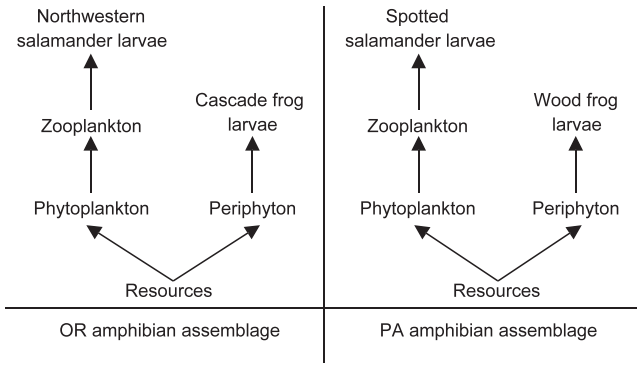

FIG. 1. Simplified wetland food webs used in the experiment. Arrows indicate the direction of energy flow. PA = Pennsylvania, OR = Oregon.

presence or absence of a zooplankton predator (larval *Ambystoma gracile* [PA] or *Ambystoma laterale* [OR]), and 3 nominal insecticide concentrations of malathion (originally 0, 1, or 10 μg/L; later increased to 0, 5, or 50 μg/L) (Fig. 1). The 12 treatment combinations were replicated 4 times for a total of 48 experimental units.

The experimental units were 800-L cattle-tank outdoor mesocosms filled with ~545 L of well water on 14 to 17 May 2007. On 17 May, we added 15 g of rabbit chow to provide a source of nutrients. On 21 May, we added 200 g of dry leaves (primarily *Quercus* spp.) to provide nutrients and substrate for periphyton growth. On 18 and 23 to 24 May, we collected pond water from 6 nearby ponds and visually screened it for invertebrate predators. After removing the predators, we combined the pond water and added equal aliquots to each tank to provide a natural source of algae and bacteria to all 48 cattle tanks. On 27 May, we positioned 2 unglazed ceramic tiles (15 × 15 cm) vertically against the north wall of each tank to provide a standardized surface for periphyton sampling. We allowed algae and zooplankton communities to develop for 15 d before adding amphibians and applying the insecticide treatments.

We collected all amphibian species from natural ponds and wetlands. In PA, we collected 20 egg masses of wood frogs (*Rana sylvatica*) on 27 March and 24 egg masses of spotted salamanders (*Ambystoma maculatum*) on 30 to 31 March and 3 April from Mallard Pond. In OR, 5 egg masses of Cascades frog (*Rana cascadae*) were collected on 9 May from Parrish Pond, and 8 egg masses of northwestern salamanders (*Ambystoma gracile*) were collected on 24 March from Coast Pond. The OR egg masses were sent by overnight mail, and the eggs of all species were hatched in PA in 200-L culturing pools filled with well water. After they hatched, we fed rabbit chow to larval anurans and zooplankton to larval salamanders ad libitum.

We added all tadpoles and salamanders to mesocosms on 1 June (day 1). Mesocosms randomly assigned to the PA assemblage received 20 larval wood frogs, and mesocosms assigned to the OR assemblage received 20 larval Cascades frogs. Mesocosms assigned to the PA salamander predator treatment received 10 spotted salamanders, and mesocosms assigned to the OR predator treatment received 10 northwestern salamanders. We selected amphibians from a mixture of all egg masses and sorted them to ensure that the 2 species of tadpoles and the 2 species of salamanders were of similar mass (initial mass ± SE: wood frog = 208 ± 11 mg, Cascades frogs = 194 ± 12 mg; spotted salamander = 103 ± 8 mg; northwestern salamanders = 101 ± 9 mg). Salamanders were added at relatively smaller masses than tadpoles to ensure that the gape-limited salamander predators could not prey upon tadpoles. Use of animals in these size classes (rather than newly hatched animals) reflects a scenario in which animals have spent a substantial part of their early life under pesticide-free conditions and are then exposed to a pesticide. Survival of all amphibian species was 100% in the 24-h handling tests.

We exposed the mesocosms to 1 of 3 malathion concentrations. We added 0, 1.1, or 11 μL of commercial malathion (a formulation reported to contain 50% active ingredient; Malathion Plus; Ortho Corporation, Marysville, Ohio) to achieve nominal malathion concentrations of 0, 1, or 10 μg/L, respectively. We dosed control mesocosms with water and agitated all mesocosms similarly to equalize disturbance across all tanks. To verify actual malathion concentration, we collected 0.125 L of water from each of the tanks 4 h after dosing and pooled the samples by malathion treatment. We sent these samples to an independent laboratory for analysis using high-performance liquid chromatography (Mississippi State Chemical Laboratory, Mississippi State University, Mississippi, USA; lower detection limit = 0.2 μg/L). The 1-μg/L treatment was lost in transit but the nominal 10-μg/L treatment had an actual concentration of 1 μg/L. At this concentration, the zooplankton appeared to exhibit no response. Thus, on day 10, we re-applied malathion to the tanks to achieve higher nominal concentrations of 0, 5, and 50 μg/L. We collected samples from each treatment 3 h later and shipped them for testing. The actual concentrations of the 5 μg/L and 50 μg/L nominal concentrations were 6 μg/L and 40 μg/L, respectively. We did not test for malathion in the controls, but malathion was not detected in previous tests of the well water.

Abiotic response variables

We measured several abiotic response variables during the experiment to help us understand malathion's effect on the community. On day 22, we measured temperature, pH, and dissolved O_2 with a calibrated digital water meter (WTW, Woburn, Massachusetts). On day 22, we also measured light attenuation with an underwater light meter (LI-COR, Lincoln, Nebraska, USA). We measured photosynthetically active radiation at depths of 10 and 30 cm. The decay rate of light with increased water depth (k) was calculated as

$$k = \ln\left(\frac{L_{10}}{L_{30}}\right)/d$$

where L_{10} is the intensity of sunlight at depth = 10 cm from the surface, L_{30} is the intensity of sunlight at depth = 30 cm, and d is the difference in depth between the 2 intensity measurements.

Biotic response variables

We quantified several biotic response variables. We sampled zooplankton on day 22 with a 0.2-L tube sampler. We collected zooplankton at 5 different locations in the water column of each mesocosm. We pooled the 5 samples within a mesocosm and filtered the composite sample through a 62-μm Nitex screen into a 0.12-L glass jar and preserved it in 70% ethanol. Past studies with malathion have demonstrated important differences in sensitivity between cladocerans and copepod species, but relatively little variation in the sensitivity of species within each group (Relyea and Diecks 2008). Therefore, we classified zooplankton as cladocerans or copepods and used the total abundance of cladocerans and copepods sampled from each mesocosm as response variables.

We measured phytoplankton on day 22 by sampling 500 mL of water from each tank. We filtered phytoplankton samples through GF/C Whatman glass microfiber filters (Whatman Inc., Florham Park, New Jersey). Immediately after vacuum-filtering, we wrapped each individual sample in aluminum foil and placed it in the freezer (−20°C). We analyzed these samples later following the protocols developed by Arar and Collins (1997). We used a fluorometer (Model ED-700, Turner Designs, Sunnyvale, California) to measure chlorophyll *a* concentrations.

We measured periphyton abundance by removing one of the clay tiles from each mesocosm on day 22. We scrubbed all periphyton on the tiles with a toothbrush and rinsed the tiles with filtered well water. Thus, our measurement of periphyton includes a combination of periphyton, bacteria, fungi, and detritus. We filtered the periphyton–water mix through a Whatman GF/C filter. We dried all filters at 80°C for ≥24 h and weighed them before using them to collect periphyton. We dried periphyton collected on the filters for 24 h and then weighed it to measure periphyton biomass.

The anurans were the first amphibians to metamorphose. The first wood frog emerged on day 16 and the first Cascades frog emerged on day 22. Thus, beginning on day 16 until day 40, we checked the mesocosms daily for metamorphs. The last wood frog emerged on day 31 and all but 3 Cascade frogs emerged by day 40. We removed metamorphosing anurans when both forelimbs had emerged and tail length was <3 cm. We placed metamorphs in covered 1-L tubs with a layer of moist sphagnum moss until tail resorption was complete (Gosner stage 46; Gosner 1960). We euthanized completely metamorphosed individuals in 2% MS-222 and preserved them in 10% formalin.

We terminated the experiment on day 40. We drained all tanks, sorted through the leaf litter, and recovered the remaining amphibians. Any anurans that had forelimbs on this day were allowed to complete their metamorphosis. The 3 anurans that failed to achieve metamorphosis were treated as survivors in our analyses but were excluded from the mass analysis because metamorphosis causes substantial mass loss. For all anurans, our response variables were survival, mean time to metamorphosis, and mean mass at metamorphosis. Only 2 spotted salamanders metamorphosed by the end of the experiment, so we analyzed larval salamander mass on day 40. We excluded the metamorphosed salamanders from this analysis because metamorphosis causes substantial mass loss.

Statistical analyses

We used multivariate analysis of variance (MANOVA) to test for effects of malathion, amphibian assemblage, and salamander presence on the 4 abiotic response variables (pH, dissolved O_2, temperature, and light attenuation on day 22). Analyses of variance (ANOVAs) are usually regarded as robust to violations of assumptions, but failure to meet homogeneity of variance can lead to a misrepresentation of the data. We log(x)-transformed response variables that did not meet the homogeneity of variance assumption. A robust and common alternative to ANOVA when data cannot be log(x)-transformed successfully is to use rank transformation (Quinn and Keough 2002). We ran general linear

TABLE 1. Results (multivariate tests) of multivariate analysis of variance of the effects of insecticides, amphibian assemblage, and salamander presence on 4 abiotic factors (pH, dissolved O_2, temperature, and light attenuation) in experimental mesocosms.

Multivariate tests	df	*F*	*p*
Insecticide	8,66	3.1	0.005
Assemblage	4,33	2.6	0.056
Salamander presence	4,33	1.5	0.213
Insecticide × assemblage	8,66	0.9	0.515
Insecticide × salamander	8,66	1.1	0.364
Salamander × assemblage	4,33	0.9	0.498
Insecticide × assemblage × salamander	8,66	1.3	0.259

model (GLM) ANOVAs on rank-transformed data. We followed significant multivariate tests with individual ANOVAs and Student–Newman–Keuls (SNK) means comparison tests (all ranked data) or Tukey's tests (nonranked data).

We analyzed phytoplankton (chlorophyll *a*) abundance with a separate ANOVA because phytoplankton data from 2 mesocosms were accidentally lost. We used Tukey's test to compare means. We used MANOVA to test for effects of insecticide, amphibian assemblage, and salamander presence on 5 biotic response variables: periphyton abundance, cladoceran abundance, copepod abundance, anuran time to metamorphosis, and anuran mass at metamorphosis. Anuran survival was very high across all treatments (wood frogs = 97.1%, Cascades frogs = 99.4%), so we did not analyze treatment effects on anuran survival.

For the subset of treatments that contained salamanders, we used a separate ANOVA to assess salamander responses to the treatments. The survival of both salamander species was very high across all treatments (spotted salamanders = 97.5%, northwestern salamanders = 99.2%). Thus, we did not analyze salamander survival. We ran an ANOVA on the effects of insecticide and amphibian assemblage on salamander mass and compared means with Tukey's test.

Results

Abiotic variables

The MANOVA on water temperature, pH, dissolved O_2, and light attenuation revealed a significant effect of insecticide treatment but no effect of amphibian assemblage, salamander presence, or any interactions (Table 1). Subsequent univariate tests indicated that the multivariate effect of the insecticide treatment was driven by effects on pH, dissolved O_2, and light attenuation, but not temperature (Table 2).

We conducted mean comparisons to understand which insecticide concentrations affected these abiotic variables (averaged across assemblages and salamander presence) (Fig. 2A–D). Mean comparisons for pH revealed that 6 and 40 μg/L did not differ from the control (both $p > 0.1$; Fig. 2B). However, 6 μg/L caused an increase in pH compared to 40 μg/L ($p = 0.005$). Mean comparisons for dissolved O_2 revealed that 6 and 40 μg/L did not differ from the control (both $p \geq 0.09$; Fig. 2C). However, 6 μg/L caused an increase in dissolved O_2 compared to 40 μg/L ($p = 0.006$). Mean comparisons for light attenuation revealed that 6 μg/L did not differ from the control ($p = 0.42$) but 40 μg/L caused greater light attenuation than the control ($p = 0.003$; Fig. 2D). Light attenuation did not differ between 6 and 40 μg/L ($p = 0.07$).

Biotic variables

We found a significant multivariate effect of insecticide and amphibian assemblage, but no effect of salamander presence or any interactions on biotic response variables (cladoceran abundance, copepod abundance, periphyton abundance, anuran mass at metamorphosis, and anuran time to metamorphosis; Table 3). We then examined the effects of insecticide and amphibian assemblage on each taxonomic group (Table 4).

Zooplankton.—Copepod abundance was affected by insecticide, but not amphibian assemblage (Table 4, Fig. 3A). Copepod abundance was greater with 6 μg/L of malathion than in the control ($p < 0.001$), did not

TABLE 2. Results (univariate tests: *p*-values) of multivariate analysis of variance of the effects of insecticides, amphibian assemblage, and salamander presence on 4 abiotic factors (pH, dissolved O_2, temperature, and light attenuation) in experimental mesocosms.

Univariate tests	pH	Dissolved O_2	Temperature	Light attenuation
Insecticide	0.006	0.007	0.502	0.004

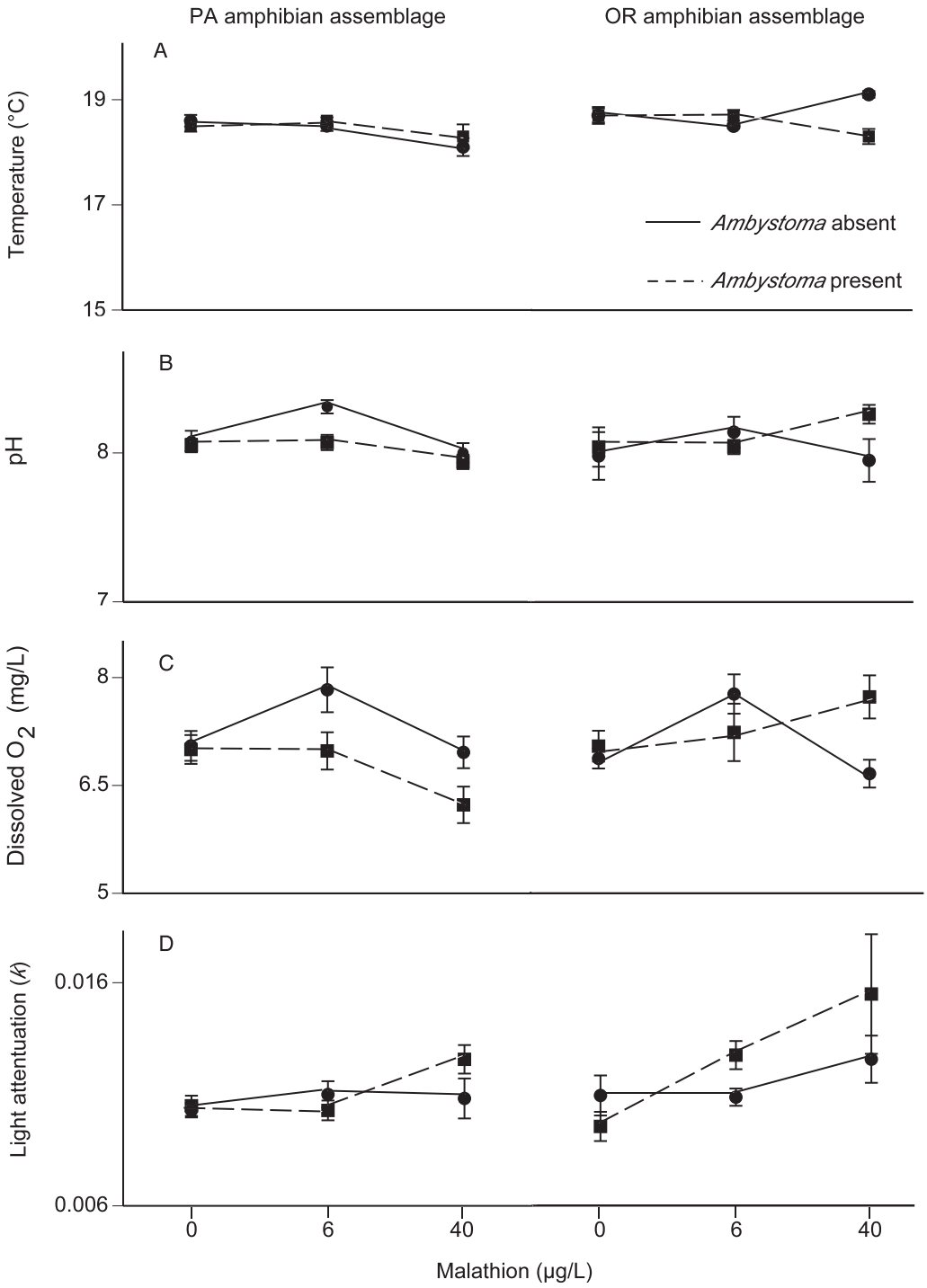

FIG. 2. Mean (±1 SE) temperature (A), pH (B), dissolved O_2 (C), and light attenuation (D) in experimental mesocosms treated with an insecticide (0, 6, or 40 µg/L of malathion), amphibian assemblage (Pennsylvania [PA] or Oregon [OR]), and salamanders (present or absent).

differ between 40 µg/L and the control ($p = 1.00$), and did not differ between 6 and 40 µg/L ($p = 0.06$). Cladoceran abundance also was affected by insecticide but not amphibian assemblage (Table 4, Fig. 3B). Cladoceran abundance was lower in 6 and 40 µg/L than in the control (both $p < 0.001$), but did not differ between 6 and 40 µg/L ($p = 0.375$).

Periphyton.—Periphyton biomass was significantly affected by insecticide but not by amphibian assemblage (Table 4, Fig. 3C). Periphyton biomass was

TABLE 3. Results (multivariate tests) of a multivariate analysis of variance of the effects of insecticide, amphibian assemblage, and salamander presence on the abundance of cladocerans and copepods, periphyton biomass, and the mass at and time to metamorphosis of the anurans.

Multivariate tests	df	F	p
Insecticide	10,64	14.8	<0.001
Assemblage	5,32	101.2	<0.001
Salamander presence	5,32	0.8	0.229
Insecticide × assemblage	10,64	0.9	0.550
Insecticide × salamander	10,64	1.1	0.370
Salamander × assemblage	5,32	1.3	0.307
Insecticide × assemblage × salamander	10,64	0.7	0.684

similar between 6 μg/L and the control ($p = 0.133$), but was lower in 40 μg/L than in the control ($p < 0.001$). Periphyton biomass was higher in 6 μg/L than in 40 μg/L ($p = 0.03$).

Anuran mass and time to metamorphosis.—Anuran mass at metamorphosis was affected by insecticide and amphibian assemblage (Table 4, Fig. 4A). Anuran metamorphs in both assemblages were smaller in the controls than in either 6 or 40 μg/L (both $p < 0.001$). Anuran mass did not differ between 6 and 40 μg/L malathion treatments ($p = 0.769$). Across all insecticide treatments, Cascades frog metamorphs were larger than wood frog metamorphs. Anuran time to metamorphosis was not affected by insecticide (Fig. 4B), but was affected by amphibian assemblage (Table 4, Fig. 4B). Cascades frogs metamorphosed later than wood frogs.

Phytoplankton.—Phytoplankton abundance (measured as chlorophyll *a* concentration) was affected by insecticide ($F_{2,31} = 12.04$, $p < 0.001$), but not amphibian assemblage ($F_{1,31} = 1.28$, $p = 0.27$), salamander presence ($F_{1,31} = 3.36$, $p = 0.076$), or interactions ($p > 0.22$). Compared to the control, phytoplankton abundance was greater in 6 and 40 μg/L of malathion (both $p \leq 0.047$). Phytoplankton abundance did not differ between 6 and 40 μg/L ($p = 0.08$; Fig. 3D).

Salamander mass.—We used a separate ANOVA to analyze the effect of insecticide and amphibian assemblage in mesocosms that contained salamanders (Fig. 4C). Salamander mass was affected by insecticide ($F_{2,18} = 79.9$, $p < 0.001$) and amphibian assemblage ($F_{1,18} = 6.41$, $p = 0.02$), but not the interaction ($F_{1,31} = 3.36$, $p = 0.076$). Salamanders in 6 and 40 μg/L were smaller than in the control ($p < 0.001$), and salamanders in 40 μg/L were smaller than those in 6 μg/L ($p < 0.001$). The amphibian assemblage effect occurred because northwestern salamanders were smaller than the spotted salamanders.

Discussion

Our results indicate that low concentrations of a globally common insecticide can have diverse effects on aquatic communities. Six and 40 μg/L of malathion initiated a chain of events across multiple trophic levels from primary producers to secondary consumers. Moreover, the effects of malathion could be generalized across communities with different amphibian assemblages. Both anuran species grew larger in insecticide treatments. Last, growth of zooplankton predators (larval salamanders) decreased with increases in malathion, but salamanders did not interact with malathion to affect zooplankton abundance.

Malathion had a direct lethal effect on zooplankton, and the magnitude of this effect differed between cladocerans and copepods. These lethal consequences at low concentrations of insecticide (<1 mg/L) are well established in aquatic mesocosm studies (Relyea and Diecks 2008, Relyea 2009), are consistent with laboratory-derived $LC50_{2d}$ values (1 to 2 μg/L; Naqvi and Hawkins 1989, Wong et al. 1995), and are well

TABLE 4. Results (univariate tests: p-values) of a multivariate analysis of variance of the effects of insecticide, amphibian assemblage, and salamander presence on the abundance of cladocerans and copepods, periphyton biomass, and the mass at and time to metamorphosis of the anurans.

Univariate tests	Copepod abundance	Cladoceran abundance	Periphyton biomass	Anuran mass at metamorphosis	Anuran time to metamorphosis
Insecticide	<0.001	<0.001	<0.001	<0.001	0.08
Assemblage	0.525	0.316	0.145	<0.001	<0.001

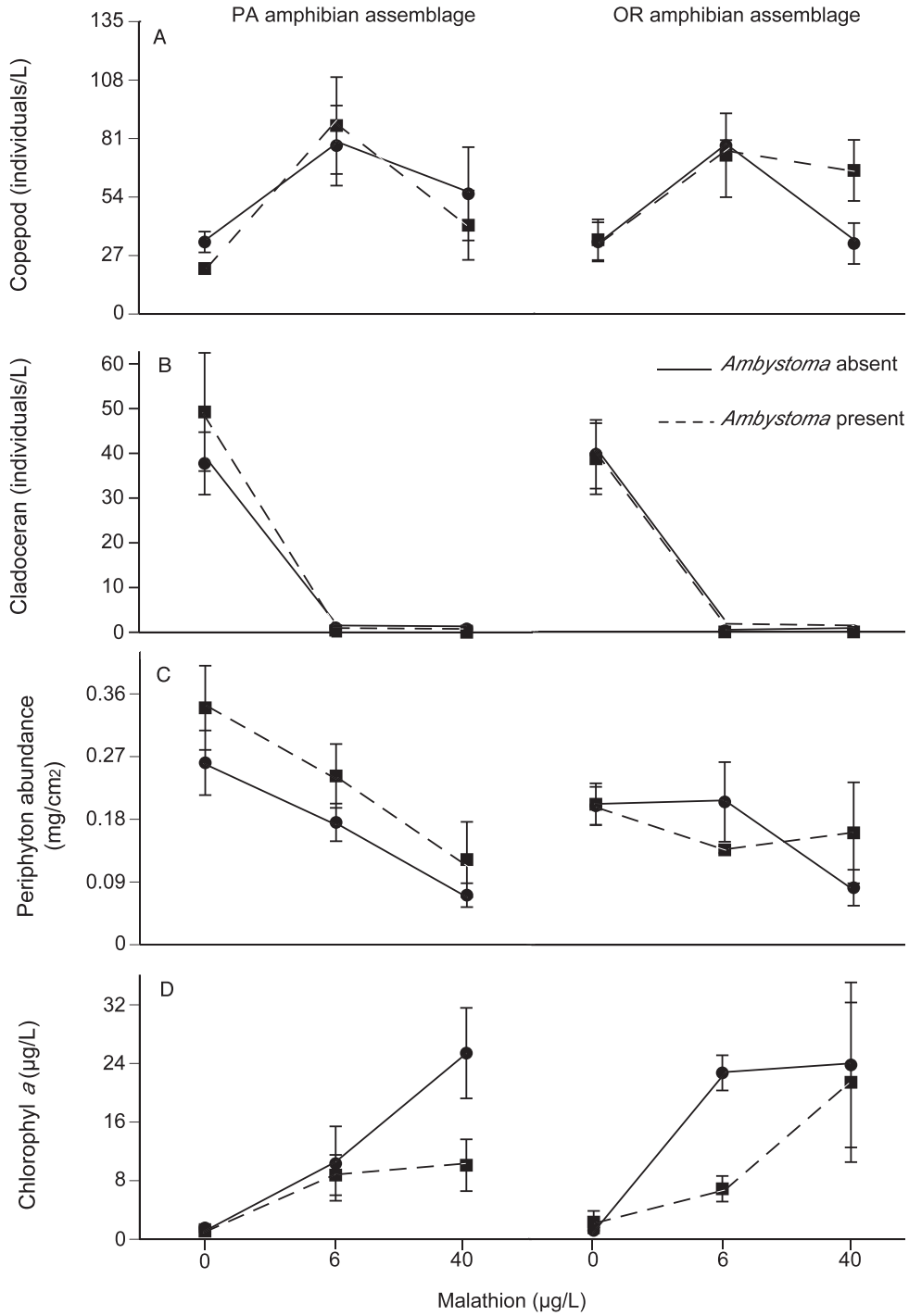

FIG. 3. Mean (±1 SE) copepod abundance (A), cladoceran abundance (B), periphyton biomass (C), and (A), chlorophyll *a* (D) in experimental mesocosms treated with an insecticide (0, 6, or 40 µg/L of malathion), amphibian assemblage (Pennsylvania [PA] or Oregon [OR]), and salamanders (present or absent).

within the range found in nature (Finlayson et al. 1982). Moreover, zooplankton exhibit a similar lethal response to low concentrations of a variety of insecticides including carbaryl, diazinon, endosulfan, and chlorpyrifos (Hanazato and Yasuno 1987, Bridges and Boone 2003, Relyea 2005, Rohr and Crumrine 2005), suggesting that our results may be generalizable across a variety of commonly used insecticides.

In our experiment, the initially abundant cladocerans suffered nearly complete elimination when

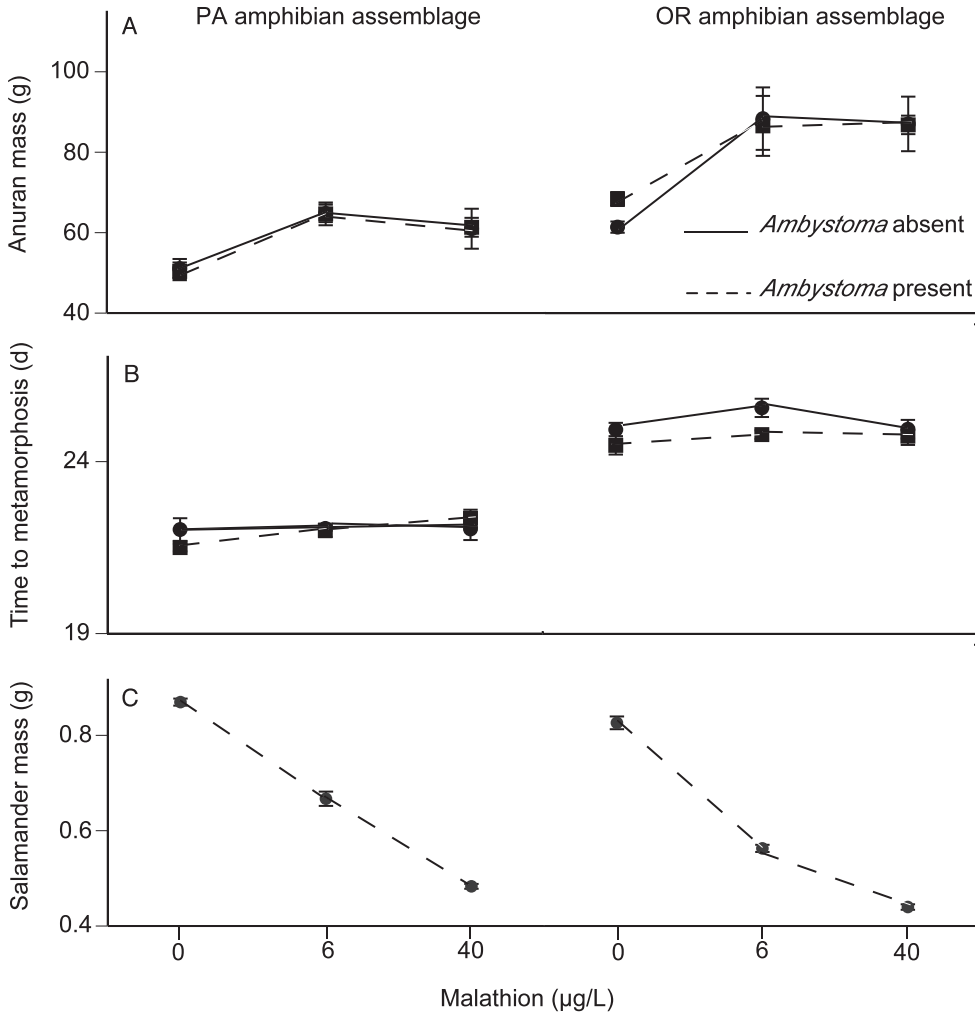

FIG. 4. Mean (±1 SE) anuran mass at metamorphosis (A), anuran time to metamorphosis (B), and salamander mass as larvae on day 40 of the experiment (C) in experimental mesocosms treated with an insecticide (0, 6, or 40 µg/L of malathion), amphibian assemblage (Pennsylvania [PA] or Oregon [OR]), and salamanders (present or absent).

exposed to 6 and 40 µg/L malathion. In contrast, copepod abundance increased relative to in controls in 6 µg/L but not in 40 µg/L. Insecticides, such as malathion, are more lethal to cladocerans than copepods (Hanazato and Yasuno 1987, Havens 1995, Mills and Semlitsch 2004, Relyea 2005, Relyea and Diecks 2008). The sharp decline in cladocerans resulting from insecticide exposure releases copepods from interspecific competition, resulting in an increase in copepod densities. However, at 40 µg/L, this trend was not observed because copepods are susceptible to malathion at high concentrations.

Cladocerans and copepods compete for the same resources (phytoplankton), but copepods process phytoplankton less efficiently than cladocerans (Haney 1973). Thus, a shift in zooplankton composition can indirectly affect the abundance of other members of the community, such as phytoplankton (Brooks and Dodson 1965). In our study, malathion caused a significant bloom in the phytoplankton by removing the major consumers of phytoplankton (zooplankton). Further evidence of a phytoplankton bloom was seen in the increases in pH and dissolved O_2, both indicators of increased photosynthesis. Higher light attenuation in tanks exposed to insecticides also suggests the presence of a phytoplankton bloom. Similar phytoplankton blooms have been seen in past studies of effects of malathion (Relyea and Diecks 2008, Relyea and Hoverman 2008) and other insecticides (Hanazato and Yasuno 1987, Boone et al. 2004, Downing et al. 1990, Relyea and Diecks 2008).

We expected a bloom in phytoplankton to inhibit periphyton growth because periphyton and phytoplankton compete for light and nutrients (Sand-Jensen

and Borum 1991, Axler and Reuter 1996). Periphyton biomass was lower in malathion than in control treatments. Others have reported lower periphyton biomass in response to pesticide-initiated phytoplankton blooms (Sand-Jensen and Borum 1991, Fleeger et al. 2003, Relyea and Diecks 2008).

The decline in zooplankton caused by malathion was associated with a decline in the mass of larval salamanders, which depend heavily on zooplankton prey (Freda 1983). However, the decrease in zooplankton abundance did not cause a decrease in salamander survival. Previous studies have shown indirect, negative effects of the insecticide carbaryl at 3.5 and 7.0 mg/L on survival (62% reduction) and mass (39% reduction) of spotted salamanders (Boone and James 2003, Metts et al. 2005). We used insecticide concentrations 3 orders of magnitude lower than the aforementioned studies (6–40 μg/L) and found no effects of salamander survival but still found substantial declines in salamander mass. Reduction in mass of salamanders at metamorphosis has further reaching indirect consequences by negatively affecting adult fitness (Semlitsch et al. 1988). Our results suggest that very low concentrations of insecticides commonly detected in nature can have significant negative effects on amphibians.

Both insecticides and larval salamanders target zooplankton (via toxicity and predation, respectively), so we predicted that both factors would be important reducers of zooplankton abundance. We found large insecticide effects, but no salamander effect even though we observed both salamander species consuming zooplankton. This result suggests that predation on zooplankton by salamanders was offset by zooplankton reproduction at all insecticide concentrations. Alternatively, malathion could have induced antipredator defenses in the zooplankton, as has been documented for cladocerans exposed to low concentrations of organophosphate and carbamate insecticides (Hanazato and Yasuno 1990, Oda et al. 2011).

Malathion had a negative effect on salamander mass, but it had a positive effect on anuran mass. Pesticide-initiated trophic cascades decrease periphyton biomass and lead to decreased growth and development of periphyton-consuming tadpoles (Mills and Semlitsch 2004, Relyea and Diecks 2008). However, wood frogs exposed to malathion were significantly heavier at metamorphosis than those not exposed to malathion (Smith et al. 2010). In contrast, American toads exposed to increasing levels of malathion decreased in mass (Smith et al. 2010). Smith et al. (2010) pointed to a combination of interacting mechanisms including food consumption rates, activity levels, and lipid metabolisms to explain the insecticide-induced variation in mass within and across amphibian species (Gurushankara et al. 2007). Anuran life history has also been cited as a mechanism for increased biomass caused by insecticides. Insecticides can have a positive effect on mass at metamorphosis for anurans with short larval periods (<10 wk), possibly because of a measurable, but brief, initial increase in periphyton abundance after insecticide application (Boone and Semlitsch 2002, Boone et al. 2005, Boone and Bridges-Britton 2006, Relyea and Diecks 2008, Mackey and Boone 2009). Wood frogs and Cascades frogs spend their relatively brief larval period in ephemeral ponds. Because of their short larval period, wood frogs and Cascades frogs are able to complete metamorphosis before the insecticide-initiated decline in periphyton food source. Future studies that measure the effect of insecticides across amphibian species that vary in life-history traits, such as oviposition phenology, are necessary to tease apart the mechanism underlying this insecticide-induced variation in amphibian growth.

Conclusions

We demonstrated that low concentrations of a globally common insecticide had both direct lethal effects on aquatic communities and numerous indirect effects. The responses of aquatic communities exposed to malathion were generalizable across 2 different amphibian assemblages, suggesting a great deal of generality in how aquatic communities possessing similar community structure are affected by a given contaminant. Last, despite the fact that both the insecticide and zooplankton predator killed zooplankton, they did not have interactive effects on the zooplankton. A trophic cascade was initiated for both assemblages, and malathion had direct, lethal effects on zooplankton that led to negative effects on salamander mass but positive effects on anuran mass. To better understand the effects of insecticides on different assemblages, future investigators should explore how different contaminants affect different assemblages and communities to achieve some sense of the generality of insecticide perturbations. In addition, future studies should examine whether different types of predators, especially invertebrate predators, might interact with insecticides differently than salamanders. The decline of major taxonomic groups that live in aquatic habitats (including amphibians; Stuart et al. 2004) makes it imperative to understand the conservation implications of how environmentally realistic levels of exposure to insecticides and variation in trophic structure affect these organisms directly and indirectly.

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