The effect of a common pyrethroid insecticide on wetland communities

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Abstract

Within the past decade, the use of pyrethroid insecticides has drastically increased. While they are known to be directly toxic to aquatic organisms, based primarily on lab experiments, they are not expected to commonly contaminate aquatic ecosystems given their high binding affinities to organic material. However, increasing evidence suggest that pyrethroids do enter aquatic ecosystems via direct application, drift, and run-off. Despite the risks of these highly toxic chemicals, the full suite of direct and indirect effects of pyrethroid insecticides in wetland communities are not well understood. To address this gap, we examined the direct and indirect consequences of a common pyrethroid, permethrin, in complex aquatic mesocosms consisting of three trophic levels and 13 animal species. We found that permethrin was more lethal than laboratory toxicity assays suggest. Even the lowest concentrations of permethrin led to declines and extinctions in animal species across multiple trophic levels. The only animal species not negatively affected by permethrin were snails (Helisoma trivolvis and Physa acuta) and red-spotted newts (Notophthalmus viridescens). We also found that the direct effects of permethrin on anurans triggered indirect effects that facilitated periphyton abundance and increased the mass of those anurans that survived. As the use of pyrethroid insecticides continue to increase, understanding the direct and indirect effects of these insecticides on aquatic systems is critical to developing generalizations about their overall impact.

Introduction

With over 405 million kg of insecticides used annually, insecticides are a diverse global anthropogenic stressor to many ecological systems (Pimentel 2005, Grube et al 2011). In particular, aquatic systems are susceptible to insecticides due to direct application, drift, and run off (Gilliom 2007, Stone Gilliom and Ryberg 2014). Given the diversity of insecticides, assessing the effects for each of the 20 000 existing formulated products is a significant challenge (CDC 2011). Towards this goal, standardized laboratory toxicity assays (i.e., LC50 tests) provide a useful and highly replicable tools for identifying and comparing the direct effects of insecticides on aquatic organisms (Newman 2010). While these laboratory studies are useful for developing a foundational understanding of the direct effects on individual species, it is critical that we understand the degree to which laboratory tests predict outcomes in more complex realistic communities.

A common assumption in chemical risk assessment is that overall mortality should increase as the magnitude of disturbance increases (Calabrese 2004). However, due to the complexity of ecological interactions and the potential for indirect effects, this is not always the case. For example, low concentrations of insecticide (as low as 0.005 mg L⁻¹), which are not directly toxic to most amphibians, can initiate trophic cascades that can decrease amphibian survival (Downing et al 2008, Relyea and Diecks 2008). In more complex communities that include a predator trophic level, higher concentrations of insecticides (e.g., up to 0.5 mg L⁻¹) can indirectly facilitate the survival of amphibian species either by eliminating their insect predators or by eliminating more...
susceptible amphibian species and thereby decreasing resource competition (Relyea 2005, Relyea and Hoverman 2008, Jones Hua and Relyea 2015). Thus, the consequences of insecticides in communities are often more complicated than a general dose response. While several studies have considered how simplified aquatic communities respond to insecticides, fewer have considered the role of added trophic complexity in altering community responses to insecticides (Boone and Semlitsch 2005, Relyea 2005, Relyea and Hoverman 2008, Relyea and Edwards 2010, Hua and Relyea 2014).

In this study, we tracked the direct and indirect effects of a commonly applied, but relatively understudied pyrethroid insecticide (permethrin) on diverse wetland communities. Permethrin is an insecticide that is commonly used for agricultural, residential, and public pest-control and interferes with sodium (Na⁺) channel function (Cox , USEPA 2011b). Within the past decade, the use of pyrethroid insecticides has increased as organophosphate insecticides have declined (USEPA 2011a). A number of previous studies have demonstrated that pyrethroids are toxic to a diversity of invertebrates (Ephemeroptera Odonata Hemiptera; Conrad et al 1999, Samsøe-Petersen et al 2001, Rico and Van den Brink 2015, Antwi and Reddy 2015). Despite their toxicity to aquatic organisms, due to their high affinity to organic materials, pyrethroid insecticides bind to terrestrial substrates and are less likely to enter aquatic systems (USEPA 2011a, Palmquist et al 2012). As a consequence, the EPA reported that pyrethroids are considered good candidates for replacement of the more toxic organophosphate insecticides (USEPA 2009). Despite their recent surge in popularity, the impacts of pyrethroid insecticides on aquatic communities have not been well-studied (Fairchild et al 1992, Mian and Mullu 1992, Hanzas Jones and White 2011) compared to AChE-inhibiting insecticides (Fairchild et al 1992, Boone 2008, Relyea 2009). This is particularly relevant because permethrin not only enters aquatic bodies via direct application, drift, and runoff but with a half-life of 50 d can persist for extended periods (Bacey Starner and Spurlock 2004). In fact, recent research suggests that the incidences that regulatory threshold levels for surface water are exceeded are higher for pyrethroids even in the face of strict environmental regulations (Stehle and Schulz 2015). Notably, despite the likelihood for pyrethroids to accumulate in sediments of non-flowing aquatic systems (i.e. ponds, wetlands, drainage ditches), the majority of recent community studies have focused on stream systems (Li et al 2017, Wieczorek et al 2018). Indeed, concentrations up to 3.1 mg L⁻¹ have been detected in natural aquatic bodies though lower concentrations are more common in flowing water bodies (2.2–170 ng L⁻¹) (Bacey et al 2004, Schleier and Peterson 2013, Markle et al 2014, Werner and Young 2018). Thus, exploring the direct and indirect effects of pyrethroid insecticides on wetland communities has broad implications by providing important information about the potential consequences of a newer generation of insecticides on natural aquatic systems.

To understand the effect of permethrin on wetland communities, we used aquatic mesocosms consisting of three trophic levels with 13 species of animals and numerous species of periphytic and planktonic algae. We then exposed these communities to six concentrations of permethrin and tracked the abundance and survival of community members over time.

Methods

Experimental design
We conducted the aquatic mesocosm study at the University of Pittsburgh’s Pymatuning Laboratory of Ecology. We used a completely randomized design that contained six treatments: a no-pesticide control (i.e. water) and permethrin applied at five different nominal concentrations (0.05, 0.1, 0.5, 1, and 5 mg L⁻¹). While still environmentally-relevant, it is important to note that we chose these concentrations to represent worse-case scenarios for pyrethroid contamination of aquatic ecosystems (Bacey et al 2004, Schleier and Peterson 2013, Markle et al 2014, Werner and Young 2018). The six treatments were replicated three times for a total of 18 experimental units.

Experimental setup
The experimental units were plastic, 1200-L cattle watering tanks filled with ~1,044 L of well water on 9 May 2011. All tanks were covered using 60% shade cloth to prevent organisms from entering or leaving while still allowing high levels of primary productivity. We also added 25 g of rabbit chow and 300 g of dry leaves (primarily Quercus spp.) to provide nutrients and additional substrate for primary producers. We chose not to include soil in our mesocosms, which has been shown to remove insecticides from the water column (Palmquist et al 2012), but there was ample organic material (leaf litter and algae), which can allow for the comparable removal of insecticide from the water column (Maund 2009). To each mesocosm, we added algae, zooplankton, herbivores, and predators (figure 1).
Algae and zooplankton
We collected pond water from seven nearby ponds and visually screened the water for invertebrate predators. After removing the predators, we added equal aliquots of pond water to each mesocosm to provide a natural source of algae and bacteria. We vertically placed 2 unglazed ceramic tiles (15 × 15 cm) on the north side of each mesocosm to serve as periphyton samplers. On 19 May, we collected zooplankton from four local ponds using a 30-micron zooplankton tow. After removing predators from this sample we added equal aliquots to each mesocosm. After adding the algae and zooplankton, we let the mesocosms sit for 5 weeks to allow the algae and zooplankton populations to grow.

Herbivores
We collected five additional species of herbivores that are common in wetlands. For additional information regarding animal collection and husbandry prior to being placed into the experiment, see the Supplementary Information (table S1 is available online at stacks.iop.org/ERC/1/015003/mmedia). On June 22, we added two species of snails to each mesocosm: ramshorn snails (Helisoma trivolvis) and pond snails (Physa acuta; 50 individuals of each species). We also added 25 tadpoles of each of the following three species: gray treefrogs (Hyla versicolor; Gosner Stage 25; Mean mass ± SE = 458 ± 76 mg), leopard frogs (Lithobates pipiens; Gosner Stage 27; Mean mass ± SE = 115 ± 13 mg), green frogs (L. clamitans; Gosner Stage 25; Mean mass ± SE = 178 ± 26 mg species). Snails and tadpoles were size-selected to ensure that each mesocosm received similar sized organisms. To assess the handling effects, we set aside a subset of all herbivores and assessed survival at 24-hrs. We found 100% survival for all five species.

Predators
We also added four species of predators to each mesocosm. To prevent predation prior to applying our pesticide treatments, we delayed the addition of the predators until 2 d before the start of the experiment. On 25 June, we added two aeshnid dragonfly larvae (Anax junius) and five backswimmers (Notenecta undulata) to each mesocosm. On 26 June, we added two water bugs (Belostoma fluibuneum) and one red-spotted newt (Notophthalmus viridescens) to each mesocosm. All species added were size selected to ensure that all mesocosms received similar-sized predators. In the 24-hr survival test, there was 100% survival of all predators.

Insecticide applications
To simulate a single insecticide contamination event, we applied the permethrin treatments once on 26 June (referred to as day 1 of the experiment). We used a commercial grade formulation of permethrin (ORTHO® Basic). To achieve the nominal concentrations of 0.05, 0.1, 0.5, 1, and 5 mg L⁻¹ we added insecticides directly to each mesocosm and vigorously and uniformly mixed the water in each tank to homogenize insecticides (table 1). Four hours later, we collected duplicate, 150-mL samples of water from the middle of the water column from each mesocosm and pooled the samples within a given pesticide treatment. We sent these samples to Mississippi State Chemical Laboratory for high-pressure liquid chromatography analysis within 2 d of sampling (Mississippi State, Mississippi, USA; lower detection limit = 0.2 ppb). Actual concentrations for the following nominal

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**Figure 1.** Aquatic mesocosm community with three trophic levels and over 13 species represented. The values in parenthesis represent number of individuals added to each mesocosm.
concentrations 0, 0.05, 0.1, 0.5, 1 and 5 mg L\(^{-1}\) were 0, 0.08, 0.1, 0.8, 1.6 and 6.5 mg L\(^{-1}\). Because actual concentrations were relatively close to our nominal concentrations, we report the nominal concentrations in all text and figures.

**Biotic response variables**

To evaluate the effects of the permethrin treatments, we measured several biotic response variables. To prevent complete elimination of herbivores by predators, we terminated the experiment on day 26 (22 July). We drained all mesocosms and collected the surviving organisms. We recorded the survival and individual mass of the three tadpole species. We recorded the survival of the snails, backswimmers, aeshnid dragonfly larvae, water bugs, and newts. Though snails reproduced during the experiment, we were able to determine the survival of the original snails added to each mesocosm because there were large size differences between hatchlings and adults.

We measured periphyton abundance by removing a clay tile from each mesocosm at day 19. The periphyton on the tiles was scrubbed and rinsed with filtered well water. The periphyton-water mix was then filtered through a Whatman GF/C 7-cm filter that had been previously dried at 80 °C for 24 h. The filters containing periphyton were re-dried for 24 h and then re-weighed to determine periphyton biomass.

Phytoplankton abundance was measured at day 18 by sampling 500 ml of water from each mesocosm. Phytoplankton samples were filtered using GF/C 47 mm Whatman glass microfiber filters (Whatman, Incorporated, Florham Park, New Jersey, USA). To prevent sample degradation, each sample was vacuum filtered, wrapped in aluminum foil and then frozen to −29 °C. The frozen samples were later analyzed for chlorophyll-a concentrations following the protocols developed by (Arar and Collins 1997). We used a fluorometer (Model ED-700, Turner Designs, Sunnyvale, California, USA) to assay the chlorophyll-a concentrations.

Zooplankton assemblages were sampled at days 11 and 18. We identified all zooplankton to the level of species. Zooplankton assemblage was dominated by one species of cladoceran (92.1% Daphnia pulex) and one species of copepod (6% Microcyclops rubellus). The remaining species were relatively rare: Scaphelobes mucronata (1.7%), and Chydorus sphaericus (0.2%) so we pooled all zooplankton into either cladocerans or copepods.

**Water quality variables**

We measured several water-quality variables on day 18. Using a calibrated digital water meter (WTW, Woburn, Massachusetts, USA), we measured temperature, pH, and dissolved oxygen. Using an underwater light meter (LI-COR, Lincoln, Nebraska, USA), we quantified light attenuation by measuring the rate of sunlight decay in each mesocosm. We measured photosynthetically active radiation at depths of 10 cm and 30 cm on day 20. The decay rate of light with increased water depth (K) was determined using the following formula:

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

Where \(L_{10}\) is the intensity of sunlight at 10 cm from the surface, \(L_{30}\) is the intensity of sunlight at a depth of 30 cm, and \(d\) refers to the difference in depth between the two intensity measurements.

**Statistical analysis**

For response variables measured once (periphyton abundance, chlorophyll-a abundance, light attenuation, and survival of leopard frogs, gray tree frogs, green frogs, pond snails, ramshorn snails, aeshnid dragonflies, backswimmers, water bugs, and newts), we conducted non-parametric multivariate analyses because not all variables met the assumptions for ANOVA. To conduct non-parametric analyses, we first rank-transformed our

| Nominal conc. | Amount added | Actual conc. |
|---------------|--------------|--------------|
| 0 mg L\(^{-1}\) | —            | 0 mg L\(^{-1}\) |
| 0.05 mg L\(^{-1}\) | 2.08 ml | 0.08 mg L\(^{-1}\) |
| 0.1 mg L\(^{-1}\) | 4.18 ml | 0.1 mg L\(^{-1}\) |
| 0.5 mg L\(^{-1}\) | 20.9 ml | 0.8 mg L\(^{-1}\) |
| 1 mg L\(^{-1}\) | 41.8 ml | 1.6 mg L\(^{-1}\) |
| 5 | 208.8 ml | 6.5 mg L\(^{-1}\) |
response variables then we ran a multivariate analysis of variance (MANOVA; (Quinn and Keough 2002)). All significant multivariate effects were further explored using univariate analyses. For response variables that met ANOVA assumptions (chlorophyll-a, light attenuation, pond snail survival and ramshorn snail survival), we conducted a parametric ANOVA. For all other response variables, the normal distribution assumption was not met, so we conducted generalized linear models (GZLM) using a Tweedie distribution with a log-link function, which is more appropriate to model responses with mixtures of zeros and positive values (Tweedie 1984, Swallow et al 2016). For both ANOVA and GZLM, if all assumptions were met, we conducted analyses on untransformed data and if assumptions were not met, we conducted our analyses on ranked data.

To analyze the effect of permethrin treatments on anuran mass, we also conducted three separate univariate analysis on the average individual mass of anurans that survived until the end of the experiment. We conducted separate univariate analyses on anuran mass instead of multivariate analyses because sample sizes across the anuran species were not similar because permethrin had different effects on the survival of the different anuran species. Notably, gray tree frog and green frog tadpoles were completely eliminated by all but the lowest permethrin concentration (0.05 mg L$^{-1}$). Similarly, leopard frog tadpoles were completely eliminated by all but the two lowest permethrin concentrations (0.05 and 1 mg L$^{-1}$). Finally, to assess water quality measures (pH, dissolved oxygen, and temperature), we ranked-transformed the variables and then conducted a MANOVA.

For response variables measured more than once (cladoceran and copepod abundances), we conducted a repeated-measures, multivariate analysis of variance (rm-MANOVA). For significant time, insecticide, or insecticide-by-time interaction, we assessed the response of cladoceran and copepod abundances to permethrin treatments by conducting Bonferroni-corrected planned pairwise comparisons of each insecticide treatment compared to the control at each time point. Cladoceran and copepod abundances did not meet assumptions of analysis of variance so we conducted non-parametric analyses by first rank-transforming the data prior to running the rm-MANOVA (Quinn and Keough 2002).

Results

For the response variables measured once (periphyton abundance; chlorophyll-a abundance; light attenuation the survival of aeshnid dragonflies, backswimmers, water bugs, newts, pond snails, and ramshorn snails; and the survival of leopard frogs, gray tree frogs, and green frogs), we found a significant multivariate effect ($F_{55,12} = 12.9; p < 0.001$). As a result, we investigated the effects of the treatments on the survival of each species. For tadpoles, we also examined the effect of insecticide treatment on individual mass.

Predators

Aeshnid dragonfly survival

We found a significant effect of permethrin treatment on the survival of larval aeshnid dragonflies (figure 2; Wald $X^2 = 32.1; df = 5; p < 0.001$). All permethrin concentrations except for 0.05 mg L$^{-1}$ resulted in a significant decrease in survival relative to the control ($p = 0.009$ for all comparisons). Additionally, aeshnid dragonflies exposed to 0.05 mg L$^{-1}$ had higher survival compared to all of the higher permethrin concentrations ($p = 0.018$ for all comparisons), but it was not different from the control ($p = 1.0$).

Water bug survival

We found a significant effect of permethrin treatment on the survival of water bugs (figure 2; Wald $X^2 = 4641.8; df = 5; p < 0.001$). All permethrin concentrations resulted in a significant decrease in survival compared to the control (all $p < 0.001$).

Backswimmer survival

We found a significant effect of permethrin treatment on the survival of backswimmers (figure 2; Wald $X^2 = 4507.2; df = 5; p < 0.001$). All permethrin concentrations resulted in a significant decrease in survival compared to the control (all $p < 0.001$).

Newt survival

We found no significant effect of permethrin treatment on the survival of newts (figure 2; Wald $X^2 = 2.4; df = 5; p = 0.78$).

Tadpoles

Gray tree frog survival and mass

We found a significant effect of permethrin treatment on the survival of gray tree frogs (figure 3; Wald $X^2 = 3205.7; df = 5; p < 0.001$). While 33% of gray tree frog tadpoles survived in the control, only about 9%
survived in 0.05 mg L\(^{-1}\) permethrin and none survived in any of the higher concentrations. When we examined the mass of the gray tree frog tadpoles in the two concentrations that had survivors, we found that tadpoles exposed to 0.05 mg L\(^{-1}\) were marginally heavier than those from the control (figure 3; Wald \(X^2 = 3.2;\) df = 1; \(p = 0.07\)).

**Leopard frog survival and mass**
We found a significant effect of permethrin treatment on the survival of leopard frogs (figure 3; Wald \(X^2 = 160.5;\) df = 5; \(p < 0.001\)). We found that 28% of leopard frog tadpoles survived in the control, 46% survived with 0.05 mg L\(^{-1}\) permethrin, and 1% survived with 1 mg L\(^{-1}\) permethrin. In contrast, no leopard frog tadpoles survived in any of the higher concentrations. When we examined the mass of the leopard frog tadpoles in the three concentrations that had survivors, we found that the mass of tadpoles did not differ between the 0, 0.05, and 1 mg L\(^{-1}\) permethrin treatments (figure 3; Wald \(X^2 = 0.41;\) df = 2; \(p = 0.82\)).

**Green frog survival and mass**
We found a significant effect of permethrin treatment on the survival of green frogs (figure 3; Wald \(X^2 = 3119.5;\) df = 5; \(p < 0.001\)). While 74% of green frog tadpoles survived in the control, only about 28% survived with 0.05 mg L\(^{-1}\) permethrin, and no green frog tadpoles survived in any of the higher concentrations. When we examined the mass of the green frog tadpoles in the two concentrations that had survivors, we found that tadpoles exposed to 0.05 mg L\(^{-1}\) were significantly heavier than those from the control (figure 3; Wald \(X^2 = 5.5;\) df = 1; \(p = 0.02\)).

**Snails**
In our analyses of snail survival, we found no significant effect of pesticide treatment on ramshorn snail survival (figure 4; \(F_{5,12} = 0.09;\) \(p = 0.99\)). We also found no significant effect of pesticide treatment on pond snail survival (figure 4; \(F_{5,12} = 1.3;\) \(p = 0.33\)).

**Algae**
We found a significant effect of permethrin treatment on periphyton abundance (figure 5; Wald \(X^2 = 35.3;\) df = 5; \(p < 0.001\)). Periphyton abundance in the 5 mg L\(^{-1}\) permethrin treatment was higher than in the 0.1 and
0.5 mg L\(^{-1}\) treatments (\(p = 0.04\) and \(p = 0.02\), respectively), marginally higher than in the 0.05, and 1 mg L\(^{-1}\) treatments (\(p = 0.058\) and \(p = 0.066\)) and not different than the control (\(p = 0.379\)). In our analysis of phytoplankton, we found no significant effect of permethrin treatment on chlorophyll-a concentration (figure 5; \(F_{5,12} = 1.3; p = 0.32\)).

**Water quality**

We found no significant multivariate effect of insecticide treatment on pH, dissolved oxygen, and temperature (\(F_{15,28} = 1.3; p = 0.26\); figure A1). We also found no significant effect of permethrin treatment on light attenuation (figure 5; \(F_{5,12} = 0.64; p = 0.68\)).

**Zooplankton**

In the rm-MANOVA on cladoceran and copepod abundance, we found a significant main effect of permethrin treatment (\(F_{10,22} = 5.1; p = 0.001\)) and a permethrin \(\times\) time interaction (\(F_{10,22} = 2.3; p = 0.048\)), but no effect of time (\(F_{1,11} = 0.0; p = 1.0\)). We then conducted univariate analyses on the abundance of cladocerans and copepods.
Cladocerans
We found an effect of permethrin ($F_{5,12} = 12.8; p < 0.001$) but no effect of time ($F_{1,12} = 0.0; p = 1.0$) and no permethrin*time interaction ($F_{5,12} = 1.3; p = 0.32$). To understand the significant effect of permethrin on cladoceran abundance, we conducted pairwise comparisons using Bonferroni-corrected planned contrasts. Relative to the control treatment, we found that all permethrin treatments ($0.05, 0.1, 0.5, 1, and 5$ mg $L^{-1}$) resulted in the near complete elimination of cladoceran abundances (figure 6). All other comparisons were not significant ($p > 0.05$).

Copepods
We found no main effect of permethrin ($F_{5,12} = 2.1; p = 0.14$) or time ($F_{1,12} = 0.0; p = 1.0$), but there was a permethrin*time interaction ($F_{5,12} = 3.7; p = 0.029$). To understand the significant permethrin*time interaction on copepod abundance, for each permethrin treatment, we compared copepod abundances across permethrin treatments on day 11 then on day 18. On day 11, we found no significant differences between the permethrin treatments ($p > 0.05$; figure 6). On day 18, copepods were marginally more abundant in the $0$ mg $L^{-1}$ treatment compared to all other permethrin treatments ($p = 0.07$ for all comparisons) and all other comparisons were non-significant ($p > 0.05$; figure 6).

Discussion

Direct effects of permethrin
Permethrin was significantly lethal for all animal taxa except for the snails and red-spotted newts. In particular, all but the lowest concentration of permethrin completely eliminated invertebrate predators. Toxicity estimates for many of the invertebrate predators used in our experiment (backswimmer, newt, water bug) were not previously available (table S2) but our findings suggest that these aquatic invertebrates are highly vulnerable to the worst-case scenario concentrations of permethrin used in this study. Use of pyrethroid insecticides has grown significantly within the past few years (USEPA 2011a, 2011b). Despite their known toxicity to aquatic organisms, these pyrethroids are believed to be safer due to their high affinity to organic materials, which allows them to bind to terrestrial substrates and thereby make them less likely to enter aquatic systems (Hill 1989,
USEPA 2011a, Palmquist et al 2012). However, recent surveys demonstrate that these insecticides are indeed reaching aquatic systems through drift and runoff; in some cases, they are even directly sprayed into aquatic bodies to eliminate mosquitoes (Schleier and Peterson 2013). Moreover, they are often detected well above the predicted no-effect concentration (Environmental Agency 2010, USEPA 2013). With the increasing popularity of pyrethroids, identifying their direct effects on species abundance has important implications on protecting the structure and function of aquatic systems.

The four highest concentrations of permethrin almost completely eliminated the three species of tadpoles. Interestingly, the direct effects permethrin on tadpoles found in this study were more severe than suggested by past studies (table S4). Berrill et al (1993) found no lethal effect of pyrethroid insecticides (permethrin and fenvelerate) ranging from 0.01–2 mg L$^{-1}$ on leopard and green frog tadpoles. In contrast, we found that 0.1, 0.5, 1, and 5 mg L$^{-1}$ of permethrin almost completely eliminated all tadpoles. According to past toxicity tests, except for 5 mg L$^{-1}$, our concentrations of permethrin should not have eliminated tadpoles. The duration of insecticide exposure is one explanation for these differences. Berrill et al (1993) exposed anurans to the insecticides for 22–96 h while our experiment lasted 26 d. Though we did not measure insecticide concentration in the mesocosms over time, the insecticide half-lives of permethrin suggest that the insecticides could have persisted in the mesocosms for up to 50 d. However, due to the high affinity of pyrethroid insecticides to organic matter, these insecticides likely did not persist long in the water column (Schleier and Peterson 2013). Instead, past studies indicate that these insecticides bind rapidly to benthic organic matter as well as periphytic and phytoplanktonic algae (Sundaram and Curry 1991, Schleier and Peterson 2013). Thus, it is possible that the
highly lethal effects of permethrin in our study occurred not through direct contact with the chemical but through the ingestion of contaminated food sources (Palmquist et al 2008, Rogers et al 2016). Therefore, in addition to assessing the toxicity of insecticides that occur via direct contact, future studies that consider other modes of exposure (i.e. ingestion) are critical to accurately assessing pyrethroid insecticide risk in aquatic systems.

Not only was permethrin more directly toxic than anticipated, we also found that permethrin had consequences on population recovery. For cladocerans, at day 11, all concentrations of permethrin caused a decline in abundance relative to the control. By day 18, we did not detect a single cladoceran in any of the mesocosms exposed to permethrin. These findings are consistent with past studies that also demonstrate that cladoceran population recovery can be inhibited following pyrethroid exposure (Kaushik et al 1985, Fairchild et al 1992, Giddings Solomon and Maund 2001, Reynaldi and Liess 2005). In this study, we assessed recovery across a relatively short time frame (Day 11 and Day 18). For some cladocerans, such as D. pulex, that have relatively short reproductive cycles of approximately one week, this time frame is sufficient for detecting recovery. However, assessing recovery across longer periods of time is likely to provide more insights. For instance, cladocerans produce durable dormant egg stages called ephippia that can hatch once more favorable conditions return. Thus, while we did not detect a single living cladoceran, the existence of an ephippia egg banks may potentially play a significant role in allowing cladoceran populations to recover in the future. Indeed, in our study, we detected D. pulex ephippia in the zooplankton samples of all treatments except for the highest permethrin treatment (5 mg L$^{-1}$; Appendix figure A2). Future studies that consider broader temporal scales are critical in further developing our understanding of permethrin in aquatic systems.

While permethrin was lethal to cladocerans and prevented their recovery over time, we did not find a significant effect of permethrin on copepod abundance. This was surprising because copepods are predicted to be more susceptible to permethrin than cladocerans based on laboratory toxicity estimates (table S2). However, it is possible that the lack of a pesticide effect on copepods was due to the low number of copepods detected. At day 11, there were no copepods detected in any of the control treatments. However, by day 18, copepods were present in the control mesocosms while not a single copepod was detected in any of the permethrin-treated mesocosms. Though the differences in copepod abundances between the control and permethrin treatments at day 18 were not statistically significant, the susceptibility of copepods to the concentrations of permethrin used in our mesocosms is consistent with predicted laboratory toxicity estimates (table S4).
Snails and red-spotted newts were both relatively tolerant to all permethrin treatments. For both snail species, survival never declined relative to the control when exposed to the permethrin treatments. Past laboratory toxicity studies as well as community mesocosms studies find that snails are generally tolerant of most insecticides (Relyea 2005, Relyea and Hoverman 2008). For the red-spotted newts, survival was not clearly affected by any concentration of permethrin. Past studies found that red-spotted newts are tolerant to other insecticides including malathion and carbaryl at 0.32 and 0.51 mg L$^{-1}$, respectively (Relyea 2005) but we are the first to show that this amphibian species is also relatively tolerant to similar concentrations of a pyrethroid insecticide. Thus, while permethrin is highly toxic to most aquatic organisms, we demonstrate that at least a few species are tolerant. Though future work should consider how the persistence of these tolerant species may lead to shifts in community structure over time.

### Indirect effects of permethrin

The indirect effects of insecticides have been well-documented in the literature (Downing et al. 2008). For instance, studies have demonstrated that when sensitive zooplankton species are eliminated by insecticides, phytoplankton abundance can be indirectly facilitated via reduced grazing pressure (Relyea and Diecks 2008). Interestingly, in our study, though permethrin caused a significant decrease in zooplankton abundance, we did not find any evidence of a phytoplankton bloom. Instead, we found that communities exposed to the highest concentrations of permethrin exhibited a bloom in filamentous periphyton. Past studies examining pyrethroid insecticides in ponds also found an increase in filamentous algal abundance and attributed this increase to the mortality of herbivores that normally graze on the algae (Crossland 1982, Davies and Cook 1993). Indeed, the highest permethrin treatments completely eliminated the anurans from our mesocosms. The inverse relationship between periphyton and phytoplankton biomass due competitive interactions for nutrient- or light- resources are well-documented (Havens et al. 1996). Thus, a reduction in anuran survival and the subsequent facilitation of periphyton may have resulted in indirect suppression of phytoplankton preventing the typical phytoplankton bloom seen after insecticide contamination in past studies (Boone and Semlitsch 2002, Mills and Semlitsch 2004, Relyea and Diecks 2008). To our knowledge, this is the first study to demonstrate that a pyrethroid insecticide can indirectly facilitate periphyton by eliminating anuran grazers. This work also underscores the need for future studies that continue to investigate indirect effects of insecticides that arise via different pathways.

Permethrin also had indirect effects on the mass of surviving anurans. Surviving gray tree frogs and green frogs from the 0.05 mg L$^{-1}$ treatment were 13.4% and 44.9% heavier than their counterparts in the control treatment, respectively. Some studies suggest that insecticides can indirectly facilitate anurans by eliminating their insect predators (Relyea 2005, Relyea and Hoverman 2008). In this study, the survival of both vertebrate and invertebrate anuran predators (newt and aeshnid dragonflies) were not significantly affected by 0.05 mg L$^{-1}$ treatment relative to the control suggesting that another mechanism is likely driving the increase in anuran mass. Indeed, gray tree frogs and green frogs exposed to the 0.05 mg L$^{-1}$ permethrin treatment experienced a 72% and 62.5% reduction in survival, respectively. These results are consistent with previous works which suggest that a reduction in overall density can indirectly facilitate surviving anurans by reducing overall competition for resources (Relyea and Diecks 2008, Relyea 2009, Hua and Relyea 2014). While permethrin contamination was more directly toxic than anticipated, the persistence and facilitation of some anurans has some potentially optimistic long-term evolutionary implications. For example, increasing evidence suggests that populations of anurans living close to agriculture that commonly encounter pesticides can evolved increased tolerance to pesticides (Hua et al. 2013, 2015, Cothran Brown and Relyea 2013). Given that some individuals appear to be tolerant to permethrin, assessing the possibility for populations to evolve tolerance to permethrin is an area for future research.

To sum, we found that permethrin was more lethal than laboratory toxicity assays predict. Past studies suggest that the adverse effects of pyrethroids are short-term and recovery likely rapid at the population and community levels (Palmoquist et al. 2012). However, in our study, we find that even the lowest concentrations of permethrin led to dramatic reductions and even extinctions in a number of animal species. The only animal species not negatively affected by permethrin were snails and newts. The lethal effects of permethrin on larval anurans also indirectly facilitated periphyton abundance and led to an indirect increase in the mass of surviving anurans. Despite the risks of contamination by these highly toxic chemicals, few studies have considered the consequences of pyrethroid insecticides in wetland communities (Mian and Mulla 1992, Hanzas et al. 2011). As the use of pyrethroid insecticides continue to increase, understanding their impacts will become increasingly important to developing generalizations and predictions about their overall impact on aquatic ecosystems.
Author contribution

JH and RR conceived the ideas and designed methodology; JH collected the data and analyzed the data; JH and RR led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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References

Arar E J and Collins G B 1997 In Vitro Determination of Chlorophyll a and Phaeophytin a in Marine and Freshwater Algae by Fluorescence (Methods No. 445.0). (Cincinnati, OH: USEPA)
Antwi F B and Reddy G V P 2015 Toxicological effects of pyrethroids on non-target aquatic insects Environmental Toxicology and Pharmacology 40 915–23
Bacej J, Storner K and Spurlock F 2004 The Occurrence and Concentration of Esfenvalerate and Permethrin in Water and Sediment in the Sacramento and San Joaquin watersheds (No. EH04-01). (Sacramento, CA: California Environmental Protection Agency)
Berrill M, Bertram S, Wilson A, Louis S, Brigham D and Stromberg C 1993 Lethal and sublethal impacts of pyrethroid insecticides on amphibian embryos and tadpoles Environ. Toxicol. Chem. 12 525–39
Boone M D 2008 Examining the single and interactive effects of three insecticides on amphibian metamorphosis Environ. Toxicol. Chem. SETAC 27 1561–8
Boone M D and Semlitsch R D 2002 Interactions of an insecticide with competition and pond drying in amphibian communities Ecol. Appl. 12 307–16
Boone M D and Semlitsch R D 2003 Interactions of bullfrog tadpole predators and an insecticide: predation release and facilitation Oecologia 137 610–6
Calabrese E J 2004 Hormesis: a revolution in toxicology, risk assessment and medicine EMBO Rep. 5 S37–40
CDC 2011 Pesticide Illness & Injury Surveillance – NIOSH Workplace Safety and Health Topic. National institute for Occupational Safety and Health (GA: Atlanta)
Cothran R D, Brown J M and Relyea R 2013 Proximity to agriculture is correlated with pesticide tolerance: evidence for the evolution of amphibian resistance to modern pesticides Evol. Appl. 6 832–41
Conrad A U, Fleming R J and Craze M 1999 Laboratory and field response of Chironomus riparius to a pyrethroid insecticide Water Res. 33 1603–10
Crossland N O 1982 Aquatic toxicology of cypermethrin. II. Fate and biological effects in pond experiments Aquat. Toxicol. 2 205–22
Davies P E and Cook I S J 1993 Catastrophic macroinvertebrate drift and sublethal effects on brown trout, Salmo trutta, caused by cypermethrin spraying on a Tasmanian stream Aquat. Toxicol. 27 201–24
Downing A L, DeVanna K M, Rubeck-Schurtz C N, Tuhela L and Grunkemeyer H 2008 Community and ecosystem responses to a pulsed pesticide disturbance in freshwater ecosystems Ecotoxicology 17 539–48
Environmental Agency 2010 An evaluation of the impact of cypermethrin use in forestry on Welsh streams Fish. Aquat. Sci. 34 2011–12
Fairchild J F, La Point T W, Zajicek J L, Nelson M K, Dwyer F J and Lovely P A 1992 Population-, community- and ecosystem-level responses to the common mode of action of three insecticides in the environment J. Plankton Res. 14 832–41
Fiorello J, Jones D K, Relyea R and Hoverman J T 2015 The contribution of phenotypic plasticity to the evolution of amphibian resistance to modern pesticides Evol. Appl. 8 386–96
Giddings J M, Solomon K R and Maund S J 2001 Probabilistic risk assessment of cotton pyrethroids: II. Aquatic mesocosm and field studies Environ. Toxicol. Chem. 20 660–8
Gilliom R J 2007 Pesticides in US streams and groundwater Environ. Sci. Technol. 41 3408–14
Grube A, Donaldson D, Kielty T and Wu L 2011 Pesticides Industry Sales and Usage: 2006 and 2007 Market Estimates. (Washington, DC: US EPA)
Hanzas J P, Jones R L and White J W 2011 Runoff transport of pyrethroids from a residential lawn in central California Environ. Toxicol. Chem. 30 610–8
Havens K E, East T L, Meeker R H, Davis W P and Steinman A D 1996 Phytoplankton and periphyton responses to in situ experimental nutrient enrichment in a shallow subtropical lake J. Plankton Res. 18 551–66
Hill I R 1989 Aquatic organisms and pyrethroids Pestic. Sci. 27 429–57
Hua J, Cothran R, Stoler A and Relyea R 2013 Cross-tolerance in amphibians: wood frog mortality when exposed to three insecticides with a common mode of action Environ. Toxicol. Chem. 32 932–6
Hua J, Jones D K, Matzes B M, Cothran R D, Relyea R and Hoverman J T 2015 The contribution of phenotypic plasticity to the evolution of insecticide tolerance in amphibian populations Evol. Appl. 8 386–96
Hua J and Relyea R 2014 Chemical cocktails in aquatic systems: pesticide effects on the response and recovery of >20 animal taxa Environ. Pollut. 189 18–26
Jones D K, Hua J and Relyea R 2015 Effects of endosulfan in southern pond communities Freshw. Sci. 35 152–63
Kaushik N K, Stephenson G L, Solomon K R and Day K E 1985 Impact of permethrin on zooplankton communities in limnocorals Can. J. Fish. Aquat. Sci. 42 77–85
Li H, Cheng F, Wei Y, Lydy M J and You J 2017 Global occurrence of pyrethroid insecticides in sediment and the associated toxicological effects on benthic invertebrates: An overview J. Hazard. Mater. 324 236–71
Markle J C, van Buuren B H, Moran K and Barefoot A C 2014 Pyrethroid pesticides in municipal wastewater: a baseline survey of publicly owned treatment works facilities in California in 2013 Describing the Behavior and Effects of Pesticides in Urban and Agricultural Settings (ACS Symposium Series: American Chemical Society) pp 177–94
Maund S 2009 The aquatic ecotoxicology of the synthetic pyrethroids: from laboratory to landscape Thesis The Netherlands Wageningen University
Mills N E and Semlitsch R D 2004 Competition and predation mediate the indirect effects of an insecticide on Southern Leopard Frogs Ecol. Appl. 14 1041–54
Mian I S and Mulla M S 1992 Effects of pyrethroid insecticides on nontarget invertebrates in aquatic ecosystems J. Agric. Entomol. 9 73–98
Newman M C 2010 Fundamentals of Ecotoxicology. (Boca Raton: CRC Press)
Palmquist K R, Jenkins J J and Jesup P C 2008 Effects of dietary esfenvalerate exposures on three aquatic insect species representing different functional feeding groups Environ. Toxicol. Chem. 27 1721–7
Palmquist K, Salatas J and Fairbrother A 2012 Pyrethroid Insecticides: Use, Environmental Fate, and Ecotoxicology ed F Perveen (London: Intech Open)
Pimentel D 2005 Environmental and economic costs of the application of pesticides primarily in the United States Environ. Dev. Sustain. 7 229–52
Quinn G P and Keough M J 2002 Experimental Design and Data Analysis for Biologists. (Cambridge: Cambridge University Press)
Relyea R A 2009 A cocktail of contaminants: how mixtures of pesticides at low concentrations affect aquatic communities Oecologia 159 363–76
Relyea R A and Diecks N 2008 An unforeseen chain of events: lethal effects of pesticides on frogs at sublethal concentrations Ecol. Appl. 18 1728–42
Relyea R A 2005 The impact of insecticides and herbicides on the biodiversity and productivity of aquatic communities Ecol. Appl. 15 618–27
Relyea R A and Edwards K 2010 What doesn’t kill you makes you sluggish: how sublethal pesticides alter predator–prey interactions Oecologia 4 558–67
Relyea R A and Hoverman J T 2008 Interactive effects of predators and a pesticide on aquatic communities Oikos 117 1647–58
Reynaldi S and Liess M 2005 Influence of duration of exposure to the pyrethroid fenvalerate on sublethal responses and recovery of Daphnia magna straus Environ. Toxicol. Chem. 24 1160–4
Rico A and Van den Brink P J 2015 Evaluating aquatic invertebrate vulnerability to insecticides based on intrinsic sensitivity, biological traits, and toxic mode of action Environ. Toxicol. Chem. 34 1907–17
Rogers H A, Schmidt T S, Dabney B L, Hladik M L, Mahler B J and Van Metre P C 2016 Bifenthrin causes trophic cascade and altered insect emergence in mesocosms: implications for small streams Environ. Sci. Technol. 50 11974–83
Samse-Petersen L, Gustavson K, Madsen T, Mogensen B B, Lassen P, Skjernov K, Christoffersen K and Jørgensen E 2001 Fate and effects of esfenvalerate in agricultural ponds Environ. Toxicol. Chem. 20 1570–8
Schleier I 3rd and Peterson R K D 2013 A refined aquatic ecological risk assessment for a pyrethroid insecticide used for adult mosquito management Environ. Toxicol. Chem. SETAC 32 948–53
Steihle S and Schulz R 2015 Agricultural insecticides threaten surface waters at the global scale Proc. Natl. Acad. Sci. 112 5750–5
Stone W W, Gilliom R J and Ryberg K R 2014 Pesticides in US streams and rivers: occurrence and trends during 1992–2011 Environ. Sci. Technol. 48 11025–30
Sundaram K M S and Curry J 1991 Partitioning and uptake of permethrin by stream invertebrates and periphyton J. Environ. Sci. Health Part B 26 219–39
Swallow B, Buckland S T, King R and Toms M P 2016 Bayesian hierarchical modelling of continuous non-negative longitudinal data with a spike at zero: an application to a study of birds visiting gardens in winter Biom. J. 58 357–71
Tweedie M 1984 An index which distinguishes between some important exponential families Stat. Appl. New Dir. Proc. Indian Stat. Inst. Gold. Jubil. Int. Conf. Indian Stat. Inst. Calcutta pp 579–604
USEPA 2013 Common Mechanism Grouping for the Pyrethrins and Synthetic Pyrethroids (No. EPA-HQ-OPP-2011-0746-0046) U.S. Environmental Protection Agency, Washington, DC
USEPA 2011a Cumulative Risk Assessments: Pyrethrins/Pyrethroid (No. EPA-HQ-OPP-2011-0746-0001) U.S. Environmental Protection Agency, Washington, DC
USEPA 2011b Pyrethrin/Pyrethroid Cumulative Risk Assessment (No. EPA-HQ-OPP-2011-0746-0019) U.S. Environmental Protection Agency, Washington, DC
Werner I and Young T M 2018 Pyrethroid Insecticides—Exposure and Impacts in the Aquatic Environment Encyclopedia Anther ed D DellaSala and M Goldenstein
Wieczorek M V, Bakanov N, Bilancia D, Szöcs E, Steihle S, Bundschuh M and Schulz R 2018 Structural and functional effects of a short-term pyrethroid pulse exposure on invertebrates in outdoor stream mesocosms Sci. Total Environ. 610–11 810–19