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Sublethal Effects of Pesticides on Predator–Prey Interactions in Amphibians

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Increasing evidence suggests that contaminants in the environment can have important consequences on organismal interactions. While we have a good understanding of the lethal effects of contaminants on organisms, we have a weak understanding of how contaminants can affect organisms by altering the interactions that they have with other species in the community. Using tadpoles of two anuran species (Bullfrogs, *Lithobates* [*Rana*] *catesbeianus;* Green Frogs, *L. clamitans*), we investigated the effects of low nominal concentrations (1 and 10 ppb) of two pesticides (malathion and endosulfan) on tadpole activity and survival when exposed to four predator treatments (no predators; water bugs, *Belostoma flumineum*; newts, *Notophthalmus viridescens*; and dragonfly larvae, *Anax junius*). In both anuran species, adding predators reduced tadpole activity and survival, with increasing rates of mortality occurring with water bugs, newts, and dragonflies, respectively. Additionally, the highest concentration of endosulfan caused tadpole mortality after 48 hrs. Most significant, tadpole species also experienced interactive effects of predators and pesticides on survival after 48 hrs. In Bullfrog treatments, all predators reduced the amount of tadpole mortality when exposed to endosulfan. In Green Frogs, additive negative effects occurred, except that newts increased the tadpole mortality when exposed to endosulfan. Our findings illustrate that pesticide effects on predator–prey interactions are often complex and have the potential to alter aquatic community composition.

ORGANISMS are exposed to a variety of perturbations in natural communities. Specifically, environmental contaminants may affect organisms directly (e.g., reducing growth or developmental rates) or indirectly by altering their interactions with other organisms. While much research has been conducted on the former (Talmage and Walton, 1991; Furness and Camphuysen, 1997; Neff, 2004; Egea-Serrano et al., 2012), few studies have investigated the effects of contaminants on organism interactions, specifically between predators and prey (but see Clements, 2000; Weis et al., 2001; Relyea and Edwards, 2010).

In aquatic systems, pesticides are a common group of contaminants that enter aquatic systems through direct overspray, runoff, or spraydrift (Norris et al., 1983). Pesticides and often impact non-target organisms including aquatic plants, micro and macro invertebrates, and vertebrates such as fish and amphibians (LeNoir et al., 1999; Boone and James, 2003; Rohr and Crumrine, 2005; Relyea and Diecks, 2008; Sparling and Fellers, 2009; Smalling et al., 2012). In amphibians, pesticides have been implicated in reductions in survival, growth, and development (Sparling et al., 2010; Egea-Serrano et al., 2012). However, pesticides frequently occur in water bodies at concentrations that are sublethal to fish and amphibians (Smalling et al., 2012), but still can alter behavior, morphology, physiology, and oviposition site selection (Bridges, 1999; Hayes et al., 2006; Takahashi, 2007; Relyea, 2012).

While studies have examined the effects of pesticides on amphibian predator–prey interactions (Relyea and Mills, 2001; Relyea, 2005a; Boone et al., 2007; Jones et al., 2009; Rohr and Raffel, 2010; Kerby et al., 2011), the effects of pesticides on such interactions are not unique to amphibians. Numerous studies have shown that pesticides alter predator–prey interactions in rotifers, plankton, gastropods, insect larvae, and fish (Farr, 1977; Ali, 1990; Hanazato and Dodson, 1995; Schulz and Dabrowski, 2001). Many of these non-amphibian studies have focused on how pesticides alter the predator–prey interaction; however, most amphibian studies have investigated how pesticides interact with the chemical cues emitted by caged predators to affect prey behavior and survival (e.g., Bridges, 1999; Relyea and Mills, 2001; Sih et al., 2004). Only a few amphibian studies have examined whether pesticides can alter the predator–prey interaction (Bridges, 1999; Dodson et al., 1995; Schulz and Dabrowski, 2001; Broomhall, 2002, 2004; Relyea and Edwards, 2010; Kerby et al., 2012).

Pesticides may affect predator–prey interactions by altering the behaviors of either party. While many amphibian studies have examined the effects of pesticides on tadpole swimming performance and predator avoidance behavior (Bridges, 1997, 1999; Schulz and Dabrowski, 2001; Broomhall, 2002), few have actually examined how simultaneous pesticide exposure to both predators and prey affects prey behavior and subsequent survival (but see Bridges, 1999). Recently, Relyea and Edwards (2010) exposed three species of tadpoles to several concentrations of two insecticides (carbaryl and malathion) and predator cues from three predators. They found that reductions in tadpole activity were common when tadpoles were exposed to the insecticides. Predator cues in the study rarely affected tadpole activity, but the predators had been starved for 48 hrs, which reduces the amount of cue that is produced. Of the 12 predator–tadpole combinations, predator cues and pesticides only had interactive effects on behavior in one case; newts induced Green Frogs (*Lithobates clamitans*) to have very low activity, and the addition of the insecticides did not lower activity any farther (a trend that was not observed in other tadpole species). Taken together, such previous research suggests that pesticides have the potential to alter predator–prey interactions.

To address the possibility that pesticides alter predator–prey interactions, we examined the effects of two insecticides (malathion and endosulfan)—each at two concentrations—in the presence of four predator treatments (no predators, adult water bugs [*Belostoma flumineum*], adult Red-spotted Newts [*Notophthalmus viridescens*], and dragonfly larvae [*Anax junius*]) on the activity and subsequent survival of two tadpole species (Bullfrogs [*L. catesbeianus*] and Green Frogs [*L. clamitans*]). We tested two hypotheses:

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1) predator cues and insecticides will both reduce tadpole activity levels; and 2) pesticides will reduce predation rates on tadpoles.

MATERIALS AND METHODS

Pesticide background.—Malathion is a broad-spectrum insecticide that acts through inhibition of acetylcholine esterase to disrupt nervous system function. As the most commonly applied insecticide in the United States, expected concentrations in natural water bodies range up to 1600 ppb and has been detected in amphibian habitats (McConnell et al., 1998; LeNoir et al., 1999; Sparling et al., 2001; Relyea, 2004). As an organophosphate, malathion is indirectly transformed via oxidative desulfuration into oxons or sulfon degradates, which can serve as potent inhibitors of acetylcholine esterase (Sparling and Fellers, 2007).

Endosulfan is an organochlorine insecticide that can alter amphibian neuromuscular activity and negatively affect tadpole eyes and gills by altering nitric oxide synthase production (Harris et al., 2000; Bernabó et al., 2008). With an average application rate of 19 kg/ha, endosulfan has been detected in water sources from 0.5–2.5 ppb, and the U.S. Environmental Protection Agency expects the environmental concentrations for surface drinking water to range from 0.5–23.9 ppb (Rossi, 2002). Endosulfan is highly lipophilic and known to accumulate in amphibian tissue and has been detected in amphibian habitats (Naqvi and Vaishnavi, 1993; Fagotti et al., 2005; Smalling et al., 2012). As such, the insecticide is highly toxic to amphibians, fish, and crustaceans (Berrill et al., 1998; Rossi, 2002; Wan et al., 2005; Jones et al., 2009; Relyea, 2009; Hammond et al., 2012).

Predator selection.—Dragonfly larvae and adult newts are generalist predators that consume a wide range of prey including zooplankton, amphipods, and tadpoles (Folsom and Collins, 1984; Bergelson, 1985). In contrast, aquatic water bugs are snail specialists that will opportunistically prey upon tadpoles (Kesler and Munns, 1989; Choe and Crespi, 1997). Compared to dragonfly larvae or adult newts, water bugs are not as efficient at preying on tadpoles (Relyea, 2003a). These predators allowed us to test predation abilities across a range of ecologically relevant amphibian predators.

Experimental design.—We conducted our laboratory experiments at the University of Pittsburgh's Pymatuning Laboratory of Ecology, Linesville PA. The experimental units were 14 L plastic tubs filled with 8 L of charcoal-filtered, UV-radiated water. Each container also contained three 15 cm stems of vegetation (*Potamogeton* sp.) for structure. The laboratory was held at 21°C with a 14:10 light:dark cycle. For each tadpole species, the 20 treatments were composed of five nominal pesticide treatments (a negative control [i.e., water] and 1 or 10 ppb of malathion or endosulfan) crossed with four predator treatments (a no-predator control, an adult water bug, an adult newt, or a larval dragonfly). Using Bullfrog and Green Frog tadpoles, we conducted separate experiments on each species by employing a randomized block design with four spatial blocks (i.e., experimental shelves). Each block contained a factorial arrangement of 20 experimental units. As a result, each experiment had a total of 80 experimental units.

The pesticide concentrations were chosen because they represent some of the lowest concentrations found in natural settings (Rossi, 2002). While such concentrations are sublethal, previous work has shown that the simple presence of predator cues is sufficient to increase the lethal effects of otherwise sublethal pesticide concentrations (Relyea and Mills, 2001; Relyea, 2003b, 2005b). The predator species (in their appropriate life stages [i.e., larvae vs. adult]) in our study were chosen because they coexist with both Bullfrogs and Green Frogs in natural wetlands. Predators were selected with a mean ($\pm$1 SE) mass of 273$\pm$11 mg for water bugs, 624$\pm$182 mg for dragonfly larvae, and 3.547$\pm$182 mg for newts.

We collected Bullfrogs and Green Frogs as newly oviposited egg masses from nearby ponds. Bullfrogs were collected as 15 clutches of eggs from the Pymatuning State Fish Hatchery ponds on 6 June 2008. Green Frogs were collected as 15 clutches of eggs from Oberdick Pond on 27 to 28 May and 4 June 2008. At these sites, there is no known history of pesticide application. The egg masses were hatched in outdoor pools containing aged well water and covered with 60% shade cloth to prevent colonization of predators. Newly hatched tadpoles were fed rabbit chow ad libitum prior to being used in the experiment. Tadpoles were selected haphazardly from all clutches with a mean ($\pm$1 SE) mass of 253$\pm$32 mg for Bullfrogs and 318$\pm$47 mg for Green Frogs.

The three species of predators were collected from a local marsh. To ensure the predators were hungry, we starved them for 48 hrs prior to the experiment (sufficient time to starve predators but still ensure adequate predation rates [Relyea and Edwards, 2010]). At the start of each experiment, a single predator was added to the appropriate tubs. The predators were initially held in cages (plastic cups with a mesh screen over the top) for 1 hr and fed ~150 mg of the target prey to allow the secretion of predatory cues into the water before the pesticide was added. By keeping the predators caged, we allowed time for tadpoles to detect any chemical cues emitted by the predators and express any behavioral defenses prior to predator release.

After the caged predators were added, we applied the pesticide treatments. For both pesticides, we created stock solutions of 100 mg/mL EtOH using technical-grade pesticides (Chem Services Inc., West Chester, PA, 99% purity). For tubs assigned the 10 ppb concentration, we added 800 µL of the stock solution. For tubs assigned the 1 ppb concentration, we added 80 µL of the stock solution. For tubs assigned the no-pesticide treatment, we added 80 µL of water. We did not include an ethanol control because previous experiments have shown little or no effect of ethanol controls on amphibian health and behavior (Fraker et al., 2009; Jones et al., 2009; Relyea, 2009; Buck et al., 2012). All tubs were then mixed thoroughly. Pesticide concentrations were independently tested at the Mississippi State Chemical Laboratory using high-pressure liquid chromatography. Water samples from each replicate were collected and pooled into one sample per treatment for pesticide confirmation. The actual concentrations are reported in Table 1 and are representative of those found in natural settings. Because our actual concentrations differed from our nominal concentrations, we will refer to our 1 and 10 ppb treatments as ''low'' and ''high,'' respectively, hereafter.

Immediately after the pesticides were applied, we added the tadpoles (ten tadpoles per experimental unit). We then waited ~90 min for the tadpoles to acclimate to their new environment before quantifying their activity levels. Tadpole

Table 1. Nominal and Actual Pesticide Concentrations of (A) Malathion and (B) Endosulfan.

A. Malathion	Nominal	Actual (Bullfrogs)	Actual (Green Frogs)
Control	0 ppb	ND	0.3 ppb
Low	1 ppb	0.69 ppb	0.85 ppb
High	10 ppb	5.8 ppb	8.7 ppb

B. Endosulfan	Nominal	Actual (Bullfrogs)	Actual (Green Frogs)
Control	0 ppb	ND	0.15 ppb
Low	1 ppb	0.36 ppb	0.49 ppb
High	10 ppb	3.6 ppb	2.9 ppb

activity was quantified by having one researcher (consistent for all observations) slowly and quietly approach each tub and count the number of tadpoles that were moving at a given instant in time (i.e., scan sampling; Relyea and Mills, 2001; Relyea and Edwards, 2010). By dividing this number by the number of tadpoles in the tub, one can determine the percent of active (i.e., moving) tadpoles. Each of the 80 tubs was observed before the process was repeated for a total of ten times. We averaged the activity across the ten observations and used the mean activity in each tub as our behavioral response variable.

Once the behavioral observations were completed (which took ~75 min), we released the predators from their cages, removed the cages from the tubs, and allowed predation to commence. All tubs were covered with a perforated plastic lid to ensure that the predators would not escape. We quantified survival after 24 and 48 hrs to accurately assess the effects of pesticides on predator–prey interactions. At each time point, we counted the number of live tadpoles, the number of dead and unconsumed tadpoles, and, by subtraction, the number of consumed tadpoles.

Statistical analysis.—We analyzed the activity and survival data in each of the experiments using general linear model analysis of variance (GLM ANOVA) using the PROC MIXED function in SAS. Tadpole activity data were normally distributed and had homogenous errors for both species. When significant treatments effects were observed, we conducted mean comparisons using Tukey's HSD test. Tadpole survival data were not normally distributed and did not contain homogeneous errors. As a result, we rank-transformed the survival data (Quinn and Keough, 2002). Repeated-measures GLM ANOVAs on rank-transformed data were used to analyze tadpole survival at 24 and 48 hrs. Because we never detected significant block effects, we pooled the block error degrees of freedom with the error term for both activity and survival measures. When significant treatments effects were observed, we conducted mean comparisons using Student-Newman-Kuels test. The PROC MIXED function allowed us to obtain specific P-values associated with our mean comparisons tests.

RESULTS

Prey activity.—When we analyzed Bullfrog activity, we found a significant effect of predators, but no effect of pesticides or the interaction (Table 2, Fig. 1A). Compared to the no-predator control, all predators induced lower activity ($P < 0.020$). Water bugs decreased activity the least while dragonflies decreased activity the most.

Table 2. Results of the ANOVAs for Tadpole Activity When Exposed to Pesticides and Predators. Activity was defined as the proportion of tadpoles moving in a tub, averaged over ten observations in a one-hour period. Boldface fonts indicate significance ($P < 0.05$).

Factor	Bullfrogs	Green Frogs
Pesticides (df = 4,60)	$F = 1.0$ $P = 0.412$	$F = 0.7$ $P = 0.592$
Predators (df = 3,60)	$F = 11.1$ $\mathbf{P < 0.001}$	$F = 7.0$ $\mathbf{P < 0.001}$
Pesticides*Predators (df = 12,60)	$F = 1.7$ $P = 0.093$	$F = 0.2$ $P = 0.999$

When we analyzed Green Frog activity, we found a significant effect of predators, but no effect of pesticides or the interaction (Table 2, Fig. 1B). Compared to the no-predator control, caged water bugs had no effect ($P = 0.111$)

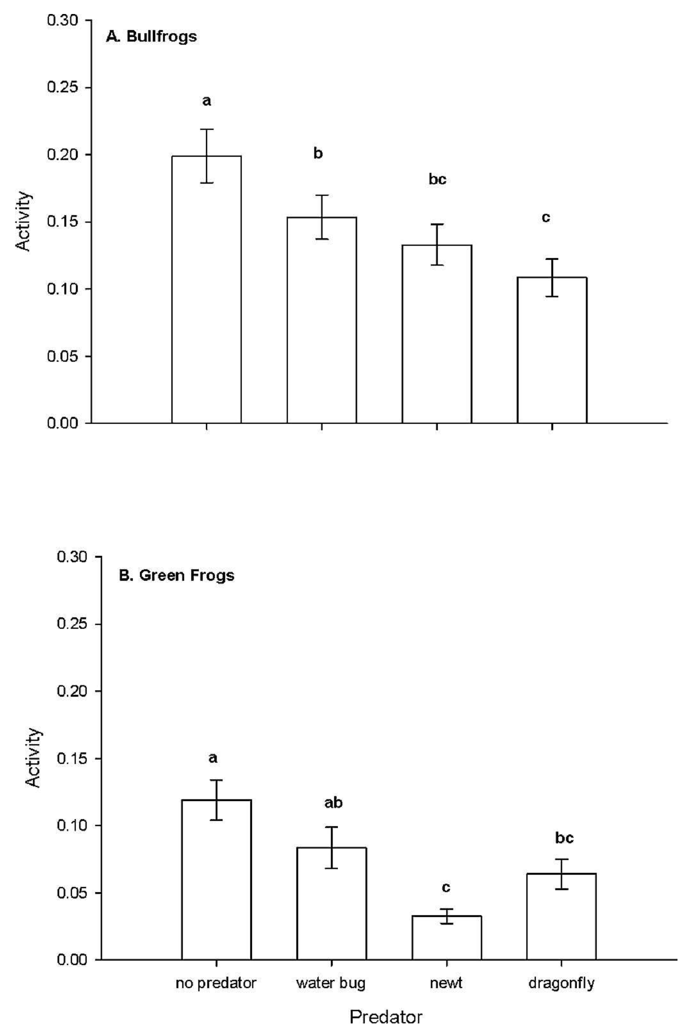

Fig. 1. Activity of (A) Bullfrog and (B) Green Frog tadpoles when exposed to four predator treatments. Activity was defined as the proportion of tadpoles moving in a tub, averaged over ten observations in a one-hour period. Different letters above histograms indicate significant differences between predator treatments ($P < 0.05$). Values plotted are least-squares means ±1 SE.

Table 3. (A) Results of the rm-ANOVAs for Tadpole Survival When Exposed to Pesticides and Predators. Boldface fonts indicate significance ($P < 0.05$).

Factor	Bullfrogs	Green Frogs
Pesticides (df = 4,60)	$F = 4.13$ **$P = 0.005$**	$F = 116.10$ **$P < 0.001$**
Predators (df = 3,60)	$F = 22.77$ **$P < 0.001$**	$F = 9.7$ **$P < 0.001$**
Pesticides*Predators (df = 12,60)	$F = 1.99$ **$P = 0.041$**	$F = 1.33$ $P = 0.225$
Time (df = 1,60)	$F = 0.00$ $P = 0.990$	$F = 0.00$ $P = 1.000$
Time*Pesticides (df = 4,60)	$F = 0.21$ $P = 0.933$	$F = 0.46$ $P = 0.767$
Time*Predators (df = 3,60)	$F = 2.41$ $P = 0.076$	$F = 0.88$ $P = 0.456$
Time*Pesticides*Predators (df = 12,60)	$F = 4.07$ **$P < 0.001$**	$F = 1.09$ $P = 0.383$

(B) Results of the ANOVAs for Tadpole Survival after 24 and 48 hrs of Exposure to Pesticides and Predators. Boldface fonts indicate significance ($P < 0.05$).

Bullfrog survival	24 hrs	48 hrs
Pesticides (df = 4,60)	$F = 2.82$ **$P = 0.033$**	$F = 4.78$ **$P = 0.002$**
Predators (df = 3,60)	$F = 21.37$ **$P < 0.001$**	$F = 19.68$ **$P < 0.001$**
Pesticides*Predators (df = 12,60)	$F = 0.73$ $P = 0.720$	$F = 3.9$ **$P < 0.001$**

Green Frog survival	24 hrs	48 hrs
Pesticides (df = 4,60)	$F = 7.26$ **$P < 0.001$**	$F = 10.84$ **$P < 0.001$**
Predators (df = 3,60)	$F = 94.73$ **$P < 0.001$**	$F = 102.53$ **$P < 0.001$**
Pesticides*Predators (df = 12,60)	$F = 0.97$ $P = 0.480$	$F = 20.2$ **$P = 0.038$**

whereas caged newts and dragonflies induced lower activity ($P < 0.006$).

Prey survival.—We did not observe any dead, unconsumed tadpoles through the experiment. Accordingly, we assumed that all missing tadpoles were consumed by the predators.

In the repeated-measures analysis of Bullfrog survival after 24 and 48 hrs, we found significant effects of pesticides, predators, their interaction, and a time-by-treatment interaction (Table 3A). As a result, we examined the treatment effects within each time period.

At 24 hrs, predators and pesticides both affected Bullfrog survival but there was no interaction between the two factors (Table 3B, Fig. 2A). Predators significantly reduced Bullfrog survival; compared to when predators were absent, survival declined with water bugs, newts, and dragonflies ($P < 0.001$). Among the three predators, dragonfly larvae and newts reduced survival more than water bugs ($P < 0.050$). Compared to the no-pesticide control, the high concentration of endosulfan caused a reduction in survival ($P < 0.009$) but the other pesticide treatments did not ($P > 0.850$).

At 48 hrs, predators and pesticides again affected Bullfrog survival, but now there was an interaction (Table 3B, Fig. 2B). The interaction was driven by the fact that the high concentration of endosulfan reduced tadpole survival to 0% in no-predator treatment but it had no effect when any of the three predators were present. When we re-ran the analysis on Bullfrog survival without the high endosulfan treatment, we found that there was an effect of predators on survival ($P < 0.001$) but there was no pesticide effect ($P = 0.991$) and no interaction ($P = 0.749$); survival was significantly reduced by all predator treatments compared to the control ($P < 0.001$).

In the repeated-measures analysis of Green Frog survival after 24 and 48 hrs, we found significant effects of pesticides and predators (Table 3A). As a result, we examined the treatment effects within each time period.

At 24 hrs, there was an effect of pesticides and predators on survival, but there was no interaction (Table 3B, Fig. 3A). Among the pesticide treatments, only the high concentration of endosulfan reduced the survival of Green Frogs compared to the no-pesticide treatment ($P < 0.001$). The three predators all caused lower tadpole survival than the no-predator treatment ($P < 0.001$).

At 48 hrs, pesticides and predators both affected green frog survival, and there was an interaction (Table 3B, Fig. 3B). When no predators were present, the high concentration of endosulfan reduced survival by 60% compared to the no-pesticide treatment ($P = 0.001$) while the other pesticide treatments had no effect ($P = 0.517$). When water bugs were present, the high concentration of endosulfan also caused a reduction in survival ($P = 0.013$) whereas the other three pesticide treatments had no effect ($P > 0.444$). With newts, the low and high concentrations of endosulfan both reduced survival compared to the no-pesticide treatment ($P = 0.003$), but the low and high concentrations of malathion had no effect ($P = 0.214$). Because the dragonflies killed nearly every Green Frog tadpole across all pesticide treatments, there was no scope of response available to assess any additional mortality effects of endosulfan or malathion. To assess the singular effects of predation on tadpole survival, we examined the effects of predators on survival in the no-pesticide treatment. The three predators all caused lower tadpole survival than the no-predator treatment ($P < 0.005$). Among the three predators, dragonfly larvae and newts reduced survival to lower levels ($P < 0.001$) than water bugs ($P < 0.005$).

DISCUSSION

The results of this study suggest that predator cues may have greater effects on tadpole activity than low concentrations of insecticides. Predator-induced reduction in prey activity is well documented in both amphibian and non-amphibian taxa (Petranka, 1989; Dodson et al., 1995; Bridges, 1999; Relyea and Mills, 2001; Schulz and Dabrowski, 2001; Fraker et al., 2009; Kerby et al., 2011). In our study, newts and dragonflies significantly reduced tadpole activity compared to the water bug and no-predator treatments in both tadpole species. This is consistent with studies showing that newts and dragonflies pose a high risk of predation to tadpoles (Werner, 1991), whereas water bugs tend to be snail specialists and therefore less effective predators on tadpoles (Relyea, 2001, 2003a).

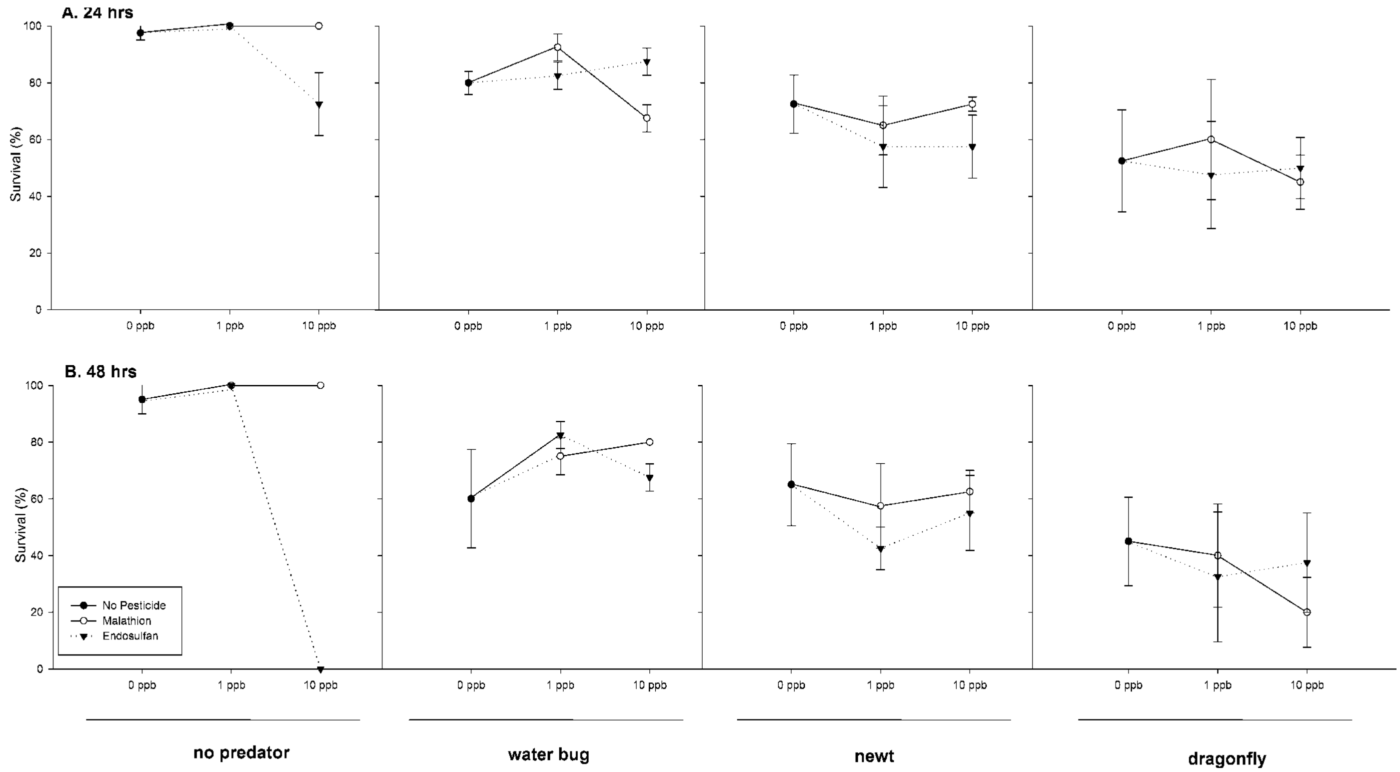

Fig. 2. Survival of Bullfrogs after (A) 24 hrs and (B) 48 hrs of being exposed to four predator treatments and two concentrations of insecticides (endosulfan and malathion). Values plotted are least-squares means ±1 SE.

We found no effect of the pesticide treatments on tadpole activity. Although other experiments (i.e., Relyea and Edwards, 2010) found a dose-dependent effect of pesticides on tadpole activity, the two insecticides used in that study (malathion and carbaryl) were applied at 100 and 1000 ppb, which is 100 times higher than the concentrations used in the current study. Thus, the lack of behavioral effects in our study likely reflects the very low concentrations that we used.

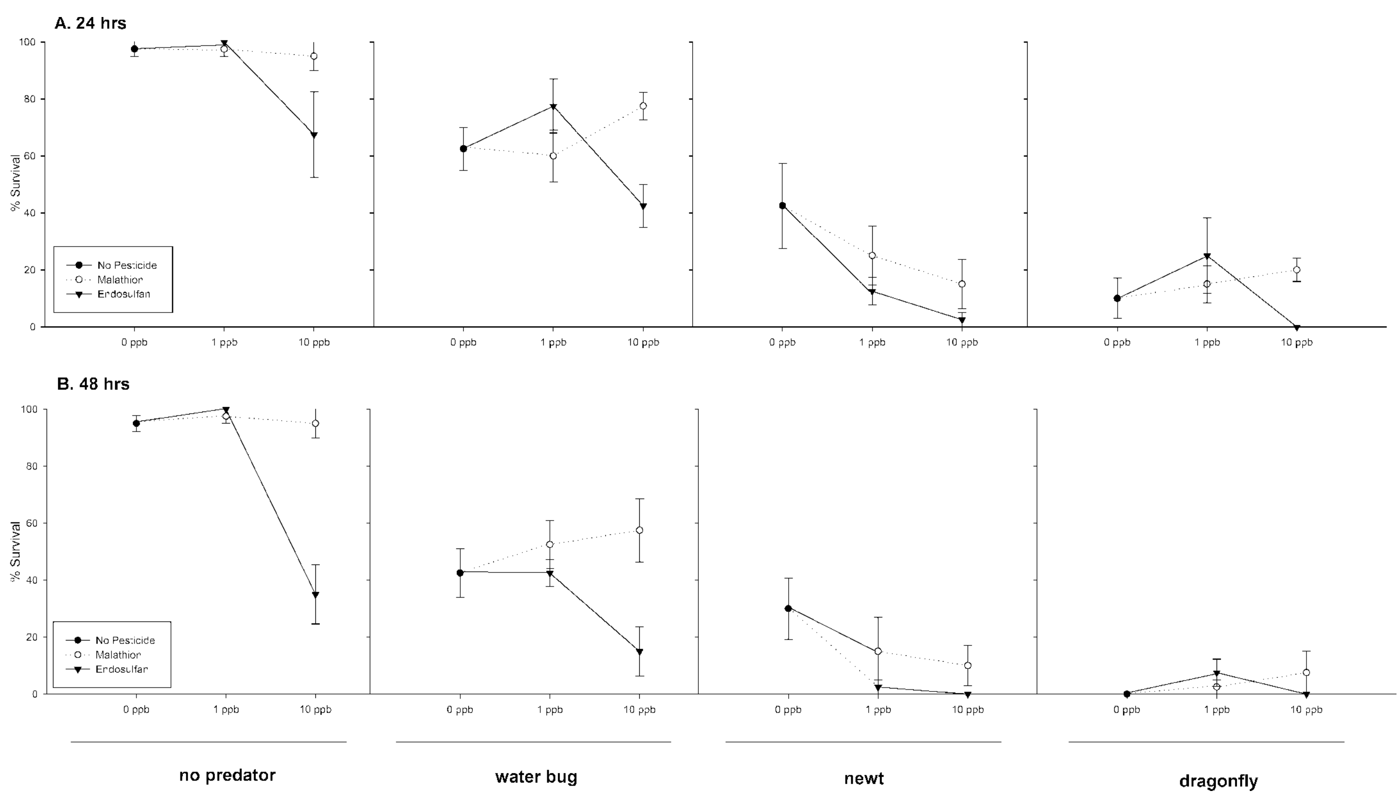

Fig. 3. Survival of Green Frogs after (A) 24 hrs and (B) 48 hrs of being exposed to four predator treatments and two concentrations of insecticides (endosulfan and malathion). Values plotted are least-squares means ±1 SE.

In amphibian systems, studies have found that predator cues alone can cause sublethal pesticide concentrations to become more lethal. This may occur through direct pesticide-induced mortality (Relyea and Mills, 2001; Relyea, 2003b, 2005b) or indirectly through reduced predator recognition (Mandrillon and Saglio, 2007), predator avoidance (Bridges, 1999; Broomhall, 2004), or growth (Kerby et al., 2011). While previous studies have highlighted the positive effects of pesticides in the presence of predators (Boone and Semlitsch, 2001, 2003), such observations were likely the result of complex community interactions including trophic cascades. However, our study is the first to show a pesticide becoming less lethal to Bullfrogs in the presence of predators.

There are two possible mechanisms for endosulfan becoming less lethal to Bullfrogs in the presence of predators. First, pesticide residue might accumulate in the tissues of the predators (similar to amphibians [Naqvi and Vaishnavi, 1993; Fagotti et al., 2005]). This would not only reduce the amount of pesticide remaining in the water to adversely affect tadpole survival, but likely also reduce the predators' abilities to capture prey. However, this mechanism would not explain why predators did not make endosulfan less lethal to Green Frog tadpoles. Alternatively, the predators might induce a stress response in the tadpole (perhaps mediated through corticosterone secretion), which would improve survival via a heightened physiological response, similar to observations in other taxa (Apfelbach et al., 2005; Monclús et al., 2005). Clearly much more research is needed to determine the mechanism responsible for predators causing endosulfan to become less lethal in their presence.

In Green Frogs, predators and pesticides both significantly reduced survival and they had interactive effects. At 48 hrs, the high concentration of endosulfan was significantly more lethal than no-pesticide controls in no-predator, water bug, and newt treatments. However, the effect was lost in dragonfly treatments due to the large direct effects of dragonflies on tadpole survival. Our hypothesis that pesticides would reduce predation rates was refuted in newt treatments; we observed a significantly higher rate of mortality when Green Frogs were exposed to endosulfan and newts together compared to endosulfan alone. Also, while survival in high-endosulfan dragonfly treatments was not different than no-pesticide controls, survival in low-endosulfan treatments was greater than no-pesticide control. We conclude that while this observation is statistically significant, it is likely not biologically significant due to the low overall tadpole survival due to the direct effects of dragonflies.

While we observed significant effects of pesticides on predation rates, such effects were driven by endosulfan. Malathion did not alter predation rates compared to no-pesticide treatments and there were significant differences between the effects of malathion and endosulfan. Previously, malathion has been shown to significantly alter newt predation rates on tadpoles (Relyea and Edwards, 2010); however, we did not observe such effects because the concentrations used in our current study were 100–1000X lower than the previous study. Furthermore, previous research has shown that endosulfan is more lethal to both Bullfrog and Green Frog tadpoles than malathion (Relyea, 2004; Hammond et al., 2012).

Our results suggest that while predators and pesticides impact tadpole survival, both do so to varying degrees. Among the three species of predators, newts and dragonflies caused the greatest amounts of amphibian death. In the absence of newts and dragonflies, endosulfan at the high concentration was the most influential factor to affect tadpole survival. We hypothesized that pesticides would alter predation rates of tadpoles, but we found this to rarely be the case; only in the presence of newts did the one of the pesticides (i.e., endosulfan) cause increased Green Frog death when newts were present. While studies examining the separate effects of pesticides and predators on amphibian survival are important, examinations into the effects of pesticides on the direct predator–prey interaction provide information in a more ecologically relevant context. Furthermore, while our study showed that endosulfan facilitated increased Green Frog death in the presence of newts, the pesticide became less lethal to Bullfrogs in the presence of predators. Such findings illustrate the complex nature of multiple stressors, especially potential interactions between biotic (predators) and abiotic (pesticides) pressures. Our study highlights the importance of examining the effects of pesticides on amphibians under environmentally relevant situations (i.e., in the presence of predators). Because of the wide variety of pesticides currently used throughout the world, studies must continue to examine the varying effects of pesticides on predator–prey relationships to better understand how abiotic stressors alter biotic interactions.

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