What Doesn't Kill You Makes You Sluggish: How Sublethal Pesticides Alter Predator–Prey Interactions

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Pesticides commonly occur in ecological communities at relatively low concentrations, leading to growing interest in determining the sublethal effects of pesticides. Such effects should affect individuals and, in turn, alter interspecific interactions. We sought to determine how sublethal concentrations (0.1 and 1.0 mg/L) of two common pesticides (carbaryl and malathion) affected predator and prey behavior as well as subsequent predation rates. We conducted a series of experiments using three species of larval amphibians (Gray Treefrogs, *Hyla versicolor*; Green Frogs, *Rana clamitans*; and American Bullfrogs, *R. catesbeiana*) and three species of their predators (larval dragonflies, *Anax junius*; adult water bugs, *Belostoma flumineum*; and adult Red-spotted Newts, *Notophthalmus viridescens*). We found that the pesticides frequently reduced the activity of all three tadpole species. For the two invertebrate predators (*Anax* and *Belostoma*), the pesticides were lethal, precluding us from examining sublethal effects on predator–prey interactions. However, newt survival was high and the addition of the pesticides reduced the predation rates of newts in one of the three tadpole species. There were no effects of the pesticides on the striking frequency of the newts or on their prey capture efficiency. Thus, the mechanism underlying the pesticide-induced reduction in predation rates remains unclear. What is clear is that sublethal concentrations of pesticides have the potential to alter prey behavior and species interactions and thereby alter the composition of ecological communities.

THE application of pesticides to control undesirable organisms in terrestrial and aquatic habitats occurs throughout the world at an annual rate of 2.5 billion kg of active ingredients (Donaldson et al., 2002). While these pesticides are designed to kill a select group of target organisms, the impact of these chemicals on non-target organisms is typically unknown and can be difficult to predict. This situation occurs because very few non-target species are tested as part of the pesticide registration process (Cooney, 1995). When examining the impacts of pesticides on non-target organisms, one often tests the lethal impacts via single-species, LC50 tests (which estimate the concentration of a pesticide that is lethal to 50% of a test population) and subsequently derives exposure concentrations that minimize the risk of mortality. However, it is becoming increasingly clear that lower concentrations of pesticides can also have important sublethal effects on non-target organisms including changes in physiology, growth, and behavior (Little, 1990; Hayes et al., 2002; Relyea, 2004a; Relyea and Hoverman, 2006).

In natural communities, pesticide exposure may have lethal and sublethal effects on a focal species, as well as cascading effects on other species in the ecological community (reviewed in deNoyelles et al., 1994; Fleeger et al., 2003; Relyea and Hoverman, 2006; also see Special Features in Environmental Toxicology and Chemistry, 1996, Volume 15[4] and Ecological Applications, 1997, Volume 7[4]). When pesticide concentrations are lethal to members of a population, the density of the population declines. As a result, the population density of predators may also decrease, while the population density of competitors in the food web may increase due to an increase in resource availability. However, when pesticide concentrations are sublethal, they cause no immediate change in population density, but can cause important changes in behaviors, such as locomotion, foraging activity, and refuge use (Weis et al., 2001). If these changes affect per-capita interactions, then sublethal pesticide concentrations should also affect other members of the ecological community (Relyea and Hoverman, 2006).

Sublethal effects of pesticides on behavioral traits are receiving increased interest from ecotoxicologists because behavioral traits are important in determining both the foraging ability and predator resistance of many non-target species in nature (Little, 1990; Dodson et al., 1995; Hanazato, 2001). For example, many prey species depend on refuge use and reduced activity to avoid detection by predators and decrease their risk of predation. However, these anti-predator responses come at the cost of reduced foraging and growth (Lima, 1998). At the same time, predators depend on a variety of behaviors to increase per-capita predation rates (Werner and Anholt, 1993; Kats and Dill, 1998; Lima, 2002; Werner and Peacor, 2003). Because chemical contaminants can alter animal behavior (Zala and Penn, 2004), sublethal concentrations of pesticides may have profound effects on the survival of predators, prey, and other trophic levels (Dodson et al., 1995; Hanazato, 2001; Schulz and Dabrowski, 2001; Bengtsson et al., 2004; Singh et al., 2004). As a result, trophic cascades that are normally attributed to pesticides reducing predator densities (Relyea, 2005) may, in fact, also be caused by the effects of pesticides on the behavior of the surviving predators or prey (Boone and Semlitsch, 2001, 2003; Relyea et al., 2005; Relyea and Hoverman, 2008).

The effects of contaminants on animal behavior is receiving increased study (reviewed in Weis et al., 2001; Zala and Penn, 2004). For example, several insecticides cause reduced swimming activity (Bridges 1997, 1999a, 1999b; Relyea and Mills, 2001; Rohr et al., 2003; Broomhall, 2004; Punzo, 2005), reduced food consumption (Gurushankara et al., 2007), and impaired ability to retain an equilibrium posture (Fordham et al., 2001). However, we have few tests of how pesticides can affect the behavior of predators and prey together and even fewer that mechanistically link changes in behavior to changes in predation rates (Bridges, 1999a, 1999b; Dodson et al., 1995; Schulz and Dabrowski, 2001; Broomhall, 2002, 2004).

Of the many taxonomic groups in which one could examine sublethal effects of pesticides, understanding the effects in amphibians is of particular interest because amphibians are experiencing population declines throughout the world (Alford and Richards, 1999; Houlihan et al., 2001; Collins and Storfer, 2003; Stuart et al., 2004). Although these declines have numerous causes, recent studies in the western United States suggest a connection with pesticides (Davidson et al., 2001, 2002), and the class of pesticides most closely correlated with this upwind agriculture are the insecticides that inhibit acetylcholine esterase (Davidson, 2004). Moreover, these pesticides are found in ponds and wetlands (McConnell et al., 1998; LeNoir et al., 1999), and examinations of frog tissues in ponds with declining populations have found reduced acetylcholine esterase activity (Sparling et al., 2001). Because the concentrations of pesticides that inhibit acetylcholine esterase (i.e., carbamates and organophosphates) are typically in the sublethal range (Sparling et al., 2001), we need to understand how sublethal concentrations affect amphibian behavior and how these behavioral changes affect interactions between amphibian prey and their predators. Despite this need, there have been few attempts to examine how sublethal pesticide concentrations affect predation on larval amphibians (Broomhall, 2002, 2004).

We examined the effects of two common insecticides (carbaryl and malathion; each at two concentrations) on the activity of three species of tadpoles and the predation rates of the tadpoles when each was reared in four different predator environments (a no-predator environment and three lethal predator environments). We also conducted a subsequent experiment examining the effects of the pesticides on the behavior of a predator by observing the frequency of newts striking at prey and the efficiency of newts in capturing their prey. We tested the following hypotheses: 1) concentrations of insecticides that are sublethal to tadpoles will alter the activity level of tadpoles; 2) concentrations of insecticides that are sublethal to predators will alter the ability of predators to capture prey; and 3) changes in predator and prey behavior due to sublethal concentrations of insecticides will alter predation rates on tadpoles.

MATERIALS AND METHODS

Information on pesticides.—Malathion and carbaryl are among the most commonly applied insecticides in the United States because they are broad-spectrum insecticides that are used against a wide variety of invertebrate pests (Kiely et al., 2004). Malathion is the most commonly applied insecticide in the United States; expected concentrations in natural water bodies are up to 1.6 mg/L and observed concentrations are 0 to 0.6 mg/L (California Department of Fish and Game, 1982; United States Department of Agriculture, 1997; LeNoir et al., 1999; McConnell et al., 1998; Relyea, 2004b). Maximum expected concentrations of carbaryl in wetlands is 4.8 mg/L (Norris et al., 1983; Peterson et al., 1994); however, typical concentrations are probably considerably lower but difficult to detect, due to carbaryl's short half-life (1 d at pH = 8; Aly and El-Dib, 1971). Both pesticides kill animals by inhibiting acetylcholine esterase either directly by binding to acetylcholine esterase molecules (enzymes which are essential for proper nerve transmission) or, in the case of organophosphates such as malathion, more indirectly by being transformed via

oxidative desulfuration into oxons or sulfon degradates, which can serve as even more potent inhibitors of acetylcholine esterase (Sparling and Fellers, 2007). In short, these pesticides impair the proper functioning of the nervous system.

Activity and survival of prey.—All experiments were conducted at the University of Pittsburgh's Pymatuning Laboratory of Ecology. Using three species of prey (Gray Treefrogs, *Hyla versicolor*; Green Frogs, *Rana clamitans*; and American Bullfrogs, *R. catesbeiana*), we conducted three separate experiments to determine how pesticide concentrations that are sublethal to amphibians affect predator–prey interactions with each species of frog. Egg masses for all three species (*H. versicolor*: $n = 9$; *R. clamitans*: $n = 8$; *R. catesbeiana*: $n = 5$) were collected from ponds within 20 km of the field station in early summer 2004. At these sites, there is no known history of pesticide application, and the frogs coexist with the three species of predators used in each experiment. Eggs were hatched in outdoor wading pools containing well water; the hatched tadpoles were fed rabbit chow *ad libitum* and kept predator-naïve until exposed to the experimental treatments.

For each tadpole species, we used a randomized block design with 20 treatments replicated four times (four spatial blocks) for a total of 80 experimental units. The 20 treatments were a factorial combination of four predator treatments (no predators, larval aeshnid dragonflies, *Anax junius*; adult Red-spotted Newts, *Notophthalmus viridescens*; and adult water bugs, *Belostoma flumineum*), crossed with five nominal pesticide concentrations (no pesticide, 0.1 mg/L or 1.0 mg/L malathion, and 0.1 mg/L or 1.0 mg/L carbaryl) that do not cause tadpole mortality in short-term (1-d) exposures (Relyea, 2003a, 2004b). The experimental units were 10-L plastic tubs containing 7.8-L charcoal-filtered, UV-irradiated well water. The pH in the tubs ranged from 8.0 to 8.1 and the temperature ranged from 19.9 to 21.3°C across the three experiments, which is within the range observed in ponds occupied by these three species (Werner and Glennemeier, 1999).

Several weeks after hatching, tadpoles for the experiments were haphazardly selected from the pools of hatchlings. We purposely selected tadpoles less than 100 mg to ensure they were within the gape size of our predators. We placed 20 tadpoles into each 10-L tub and allowed the tadpoles to acclimate for 24 hours (initial mean mass ±1 SE: Gray Treefrogs = 57 ± 2 mg; Green Frogs = 64 ± 4 mg; Bullfrogs 59 ± 5 mg). The tadpoles were not fed while in the tubs because a lack of food can elevate tadpole activity and potentially increase predation risk (Relyea, 2002a); any effects would be standardized across all tubs. All tubs were placed on shelves in a 22°C animal room with a 14:10 day:night light cycle using standard fluorescent ceiling lights.

All predators were collected from local ponds (within 20 km of the field station) and, prior to the experiment, starved for 48 hours in individual cups under laboratory conditions. Water bugs, newts, and dragonflies were chosen because they represent a range of low, medium, and high predation risk (respectively), thereby allowing us to examine pesticide effects on predator–prey interactions across a gradient of predation-risk scenarios (Relyea, 2001a, 2003b). After a 24-hr acclimation period, we temporarily placed each predator in a small cage constructed of a 500-ml plastic cup

with a screen over the open end. Each tub assigned to the no-predator treatment received an empty cage. By keeping the predators caged for the first hour, we allowed the tadpoles time to detect any chemical cues emitted by the predators and express any behavioral defenses prior to predator release, although starved predators typically induce weak or absent antipredator behaviors in tadpoles (Schoeppner and Relyea, 2005). Thus, we faced a compromise between having greater induction of behavior via predator cues versus having hungry predators.

Immediately after adding the caged predators, we added the pesticides to the appropriate tubs using commercial forms of carbaryl (Sevin; 22.5% active ingredient) and malathion (Malathion Plus; 50% active ingredient). To attain nominal concentrations of 0.1 and 1.0 mg/L of carbaryl, we added 3.6 and 36 µl of Sevin, respectively. To attain nominal concentrations of 0.1 and 1.0 mg/L of malathion, we added 1.6 and 16 µl of Malathion Plus, respectively. Using commercial formulations is a relevant way to examine pesticide effects, but one must also recognize that the effects of the pesticides could be due to both the active ingredient as well as the additional ''inert'' ingredients. For all control tubs, we added 36 µl of filtered water as a sham control.

After adding the caged predators and the pesticides at approximately noon, we quantified tadpole activity (the proportion of time that tadpoles spent moving) to determine whether either factor affected tadpole activity (tadpoles typically respond to both factors within minutes). To quantify tadpole activity, we performed scan samples of each tub to count the number of tadpoles that were active (i.e., moving; Relyea 2001b). Over a one-hour period, we made ten observations of each tub and used the mean proportion of active tadpoles in each tub as our response variable. After observing tadpole activity, we released the predators and removed all cages.

After 22 hours, we quantified the final survival of the predators and prey and then terminated the experiment. Although we used pesticide concentrations that were expected to be sublethal to tadpoles, these concentrations were often lethal to the invertebrate predators (but never lethal to the newts). Because larval dragonflies and water bugs died in many of the tubs containing pesticides, we only analyzed survival of prey in the control and newt-predator treatment groups.

Behavior of predators.—We also conducted an experiment to quantify the predatory behavior of newts in response to the pesticide using tadpoles of Green Frog as prey (observing predator behavior was not feasible in the 80-tub experiments because prey observations required 30 min of observation per tub). Due to the lethal nature of the insecticides to dragonflies and water bugs, we focused the predator-behavior experiments on newts using the lower concentration (0.1 mg/L) of malathion and carbaryl. Thus, using the same experimental units as in the earlier experiments and a completely randomized design, we assigned three treatments (no pesticide, 0.1 mg/L of carbaryl, and 0.1 mg/L malathion) and replicated each treatment four times (for a total of 12 experimental units). As in the previous experiments, we attained the pesticide concentrations by adding either 0.36 µl of water (for the control), 0.36 µl of Sevin, or 0.16 µl of Malathion Plus. Twenty tadpoles of Green Frogs (initial mean mass ± 1 SE:

115 ± 5 mg) were added to each tub. After a 24-hour acclimation period, we added the pesticides and caged newts. After an additional hour, the newts were released in a pair of tubs. For the next 30 min, we quantified the number of strikes (defined as a quick movement in the direction of the tadpoles with mouth open), the number of strikes that resulted in captures, and from these two variables, the capture efficiency of the newt (number of captures/total number of strikes). Two tubs were visually observed at one time and the order of treatments being observed was random. Importantly, predatory behaviors are potentially a function of pesticide-induced changes in prey movement and pesticide-induced changes in a predator's capacity to strike at prey. All observations were conducted during the afternoon under fluorescent lighting.

Statistical analysis.—We analyzed the data using analyses of variance (ANOVA). First, we analyzed the activity of the three tadpole species in response to the pesticides and caged predators (i.e., prior to predator release). The activity data (the mean proportion of moving tadpoles in a tub, averaged over ten observations in one hour) were normally distributed for all three tadpole species and had homogeneous errors in two of the three tadpole species (Gray Treefrogs and Green Frogs). Second, we analyzed the survival of each tadpole species after 22 hours in the three predation experiments for the no-predator and newt-predator treatments. Because the survival data did not contain homogeneous errors (some treatments had 100% survival), we ranked the data before analysis. For the predator-behavior experiments, we analyzed the number of strikes and the capture efficiency of the predators using separate ANOVAs because an experimental unit with no strikes necessarily contained no data for capture efficiency (behaviors are not independent). In all analyses containing experimental blocks, the block effects were not significant, so we pooled the block error degrees of freedom with the error term. In all analyses that produced significant treatment effects, we then conducted mean comparisons using Fisher's LSD test.

RESULTS

Activity of prey.—The first analyses examined tadpole activity in response to pesticide treatments and predators. For Gray Treefrogs, activity was affected by pesticides, but not predators or the pesticide-by-predator interaction (Table 1; Fig. 1A). Compared to the no-pesticide control (averaged across all predator treatments), Gray Treefrog activity increased by 3% with 0.1 mg/L of carbaryl ($P = 0.024$) but decreased by 5 to 8% with the other three pesticide treatments (all $P < 0.001$).

For Green Frogs, activity was affected by pesticides, predators, and the pesticide-by-predator interaction (Table 1; Fig. 1B). Based on mean comparisons when no pesticide was present, Green Frog activity was not affected by caged dragonflies or water bugs (all $P > 0.2$), but was reduced by 7% with caged newts ($P = 0.003$) compared to the control. With dragonflies, water bugs, or no predators, Green Frog activity was not affected by 0.1 mg/L of malathion (all $P > 0.06$), but was reduced by 6 to 10% with the other three pesticide treatments (all $P \leq 0.012$). With newts, Green Frog activity was already quite low without pesticides present (3% activity) and was not further reduced by any of the four pesticide treatments (all $P > 0.6$).

Table 1. 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.

Factor	*Hyla versicolor*	*Rana clamitans*	*Rana catesbeiana*
Pesticides (df = 4,60)	$F = 27.7$ $P < 0.001$	$F = 24.7$ $P < 0.001$	$F = 65.8$ $P < 0.001$
Predators (df = 3,60)	$F = 0.922$ $P = 0.436$	$F = 5.57$ $P = 0.002$	$F = 0.687$ $P = 0.563$
Pesticides * Predators (df = 12,60)	$F = 1.10$ $P = 0.375$	$F = 2.51$ $P = 0.010$	$F = 0.373$ $P = 0.968$

For Bullfrogs, activity was affected by pesticide treatments, but not by predators or the pesticide-by-predator interaction (Table 1; Fig. 1C). Compared to the no-pesticide control (averaged across all predator treatments), Bullfrogs had 19 to 27% lower activity with all four pesticide treatments (all $P < 0.001$), and the higher concentrations of each pesticide induced lower tadpole activity than the lower concentrations (all $P < 0.001$).

In summary, while each tadpole species had a different baseline level of activity, all three species commonly exhibited lower activity in the presence of the two insecticides, particularly under the higher of the two concentrations. The exception was when Gray Treefrogs were exposed to 0.1 mg/L of carbaryl, which caused a small increase in activity.

Prey survival.—The second analysis examined tadpole survival after being exposed to pesticides and either no predators or newt predators for 22 hours. For Gray Treefrogs, there was a significant effect of predators, pesticides, and their interaction (Table 2; Fig. 2A). With no predators, Gray Treefrogs experienced 100% survival regardless of pesticide presence. With newts, survival declined to 39% in the absence of pesticides ($P < 0.001$). Survival improved to 78% with 0.1 mg/L of malathion and improved to 61% and 81% with 0.1 to 1.0 mg/L of carbaryl (all $P \leq 0.05$), but was unaffected by 1.0 mg/L of malathion ($P = 0.359$).

For Green Frogs, there also was a significant effect of the predators, but no effect of pesticides and no interaction effect (Table 2; Fig. 2B). In the absence of predators, survival was 100%. In the presence of newts, survival averaged 75%.

For Bullfrogs, there was a main effect of predators, no main effect of pesticides, and a predator-by-pesticide interaction (Table 2; Fig. 2C). In the absence of pesticides, we observed 100% survival in the no-predator treatment but only 71% survival in the newt treatment ($P < 0.001$). With no predators, Bullfrogs generally experienced high survival, although there was a 10% decrease in survival with 1.0 mg/L of malathion compared to the no-pesticide control ($P < 0.001$). With newts, survival was not affected by any of the pesticide treatments relative to the no-pesticide control (all $P > 0.1$).

Behavior of predators.—In the predator-behavior experiment, we tested whether the addition of 0.1 mg/L of carbaryl or malathion affected the predatory behavior of newts. There was no effect of the pesticides on either the number of strikes ($F_{2,9} = 0.2$, $P = 0.854$; Fig. 3) or capture efficiency ($F_{2,9} = 1.4$, $P = 0.308$). Thus, newt behavior was not affected by 0.1 mg/L of the two pesticides.

DISCUSSION

The results of this experiment demonstrate that short-term exposure to pesticide concentrations that are sublethal to tadpoles and whose mode of action is to inhibit acetylcholine esterase can frequently reduce prey activity in some species. Activity was reduced by predators in one species, but reduced by the pesticides in all three species. The weak behavioral responses to the predators were not surprising given that the behavioral observations were taken after the predators had been starved for 48 hours. As reviewed by Schoeppner and Relyea (2005), starved predators typically induce weak behavioral responses in amphibian prey probably because they are emitting few chemical cues. Although visual and tactile cues can be important, chemical cues often appear to be the most important cue for inducing anti-predator behaviors in tadpoles (Relyea, 2002b).

Although numerous studies have examined the effects of contaminants on animal behavior (reviewed in Weis et al., 2001; Zala and Penn, 2004), few have examined the effects of pesticides on amphibian behavior. Studies using carbaryl have found that both lethal and sublethal concentrations can reduce activity in tadpoles (Bridges 1997, 1999a, 1999b; Relyea and Mills, 2001; Punzo, 2005). In studies using other insecticides (endosulfan and octylphenol), reduced activity also appears to be common (Rohr et al., 2003; Broomhall, 2004). Despite the fact that malathion is the most widely applied insecticide in the United States (Kiely et al., 2004), there are only a few studies examining the impacts of malathion on tadpole behavior including equilibrium posture (Fordham et al., 2001), food consumption (Gurushankara et al., 2007), swimming behavior (Webb and Crain, 2006), and activity (Mackey and Boone, 2009). In other aquatic organisms, pesticide-induced reductions in activity and other behaviors are common observations (Kruzynski and Birtwell, 1994; Kruzynski et al., 1994; Brewer et al., 2001; Hanazato, 2001). For carbamate and organophosphate insecticides, the mechanism underlying reduced activity is the inhibition of acetylcholine esterase. Such inhibition would typically reduce normal neuron firing which would inhibit tadpole movement (Sparling and Fellers, 2007) and therefore likely affect a variety of other tadpole behaviors that could also alter predation rates (e.g., refuge use). Hence, it was surprising that Gray Treefrogs exhibited activity reductions with three of the pesticide treatments, but exhibited an increase in activity under the highest concentration of carbaryl. Possible explanations include species differences in physiological responses to the pesticides and the fact that the commercial formulations can contain inert ingredients that might also affect behavior in a species-specific fashion.
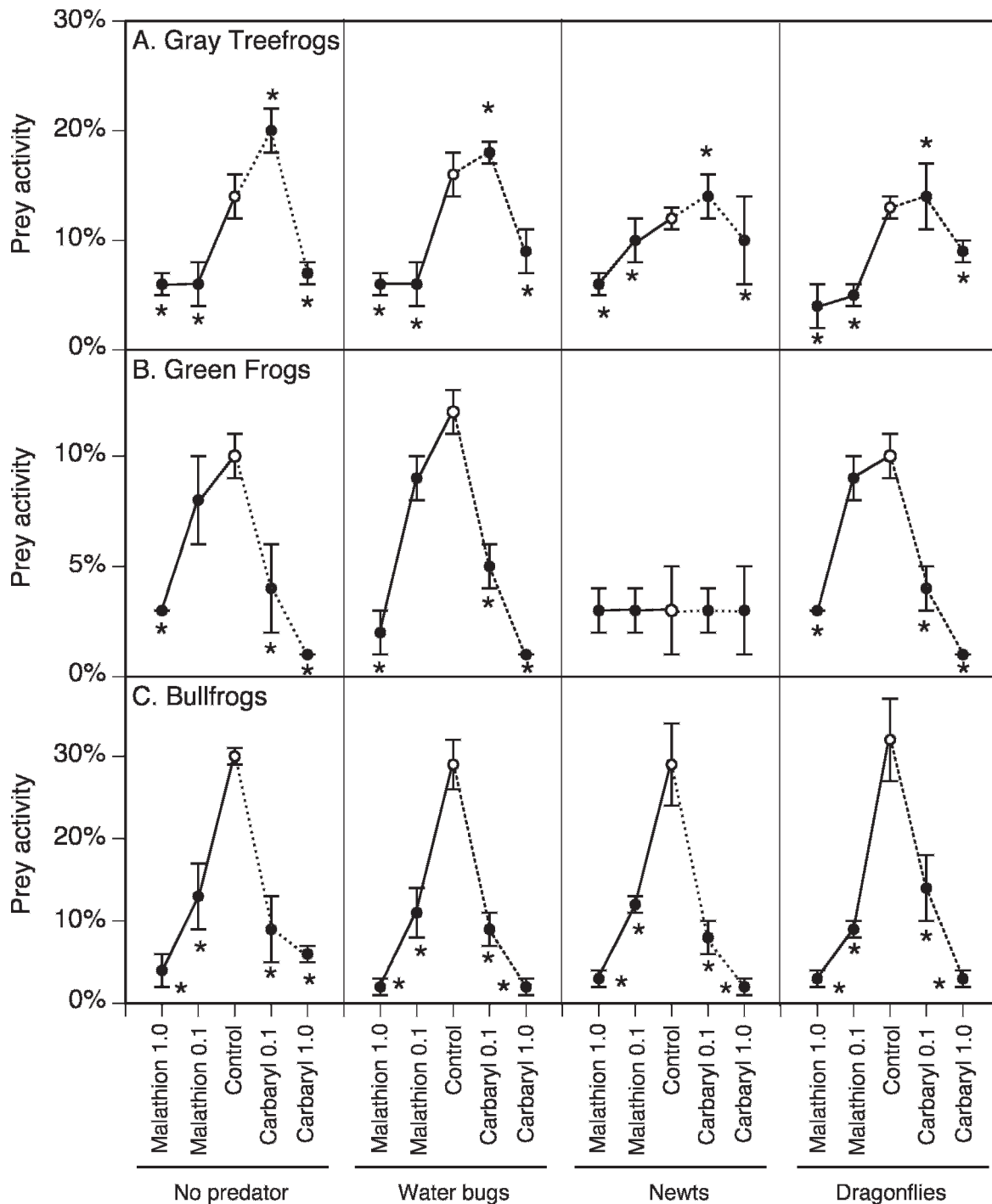
Fig. 1. The activity of tadpoles (A. Gray Treefrogs, *H. versicolor*; B. Green Frogs, *R. clamitans*; and C. Bullfrogs, *R. catesbeiana*) when exposed to four predator environments and two concentrations of insecticides (malathion and carbaryl). Activity was defined as the proportion of tadpoles moving in a tub, averaged over ten observations in a one-hour period. Note that each panel has a different y-axis scale to reveal the detail of each species. Data are means ± 1 SE. Asterisks indicate those pesticide treatments that differ from the control treatment.

The relative magnitude of the impact of the insecticides on tadpole behavior was related to the concentration of the insecticides. In general, higher pesticide concentrations caused greater reductions in tadpole activity. Interestingly, equivalent concentrations of malathion and carbaryl often produced similar reductions in prey activity. Previous LC50 tests for the three species demonstrate quite similar sensitivities to carbaryl (Gray Treefrogs = 2.5 mg/L, Green Frogs = 2.6 mg/L, Bullfrogs = 2.3 mg/L; Relyea, 2003a) and malathion (Gray Treefrogs = 4.1 mg/L, Green Frogs =

3.7 mg/L, Bullfrogs = 1.5 mg/L; Relyea, 2004b). This suggests that these species have similar lethal sensitivities to two classes of insecticides with the same mode of action and also have similar sublethal sensitivities on their behavior, at least at the higher sublethal concentration. This allows us to generalize not only the qualitative effect of these two contaminants (i.e., a reduction in activity) but also, to some degree, the quantitative effect of the two contaminants (i.e., the magnitude of the activity reduction). Whether this pattern exists for a wider range of carbamates and organo-

Table 2.   Results of the ANOVAs for Tadpole Survival over a 22-Hour Period when Exposed to Pesticides and Newt Predators.

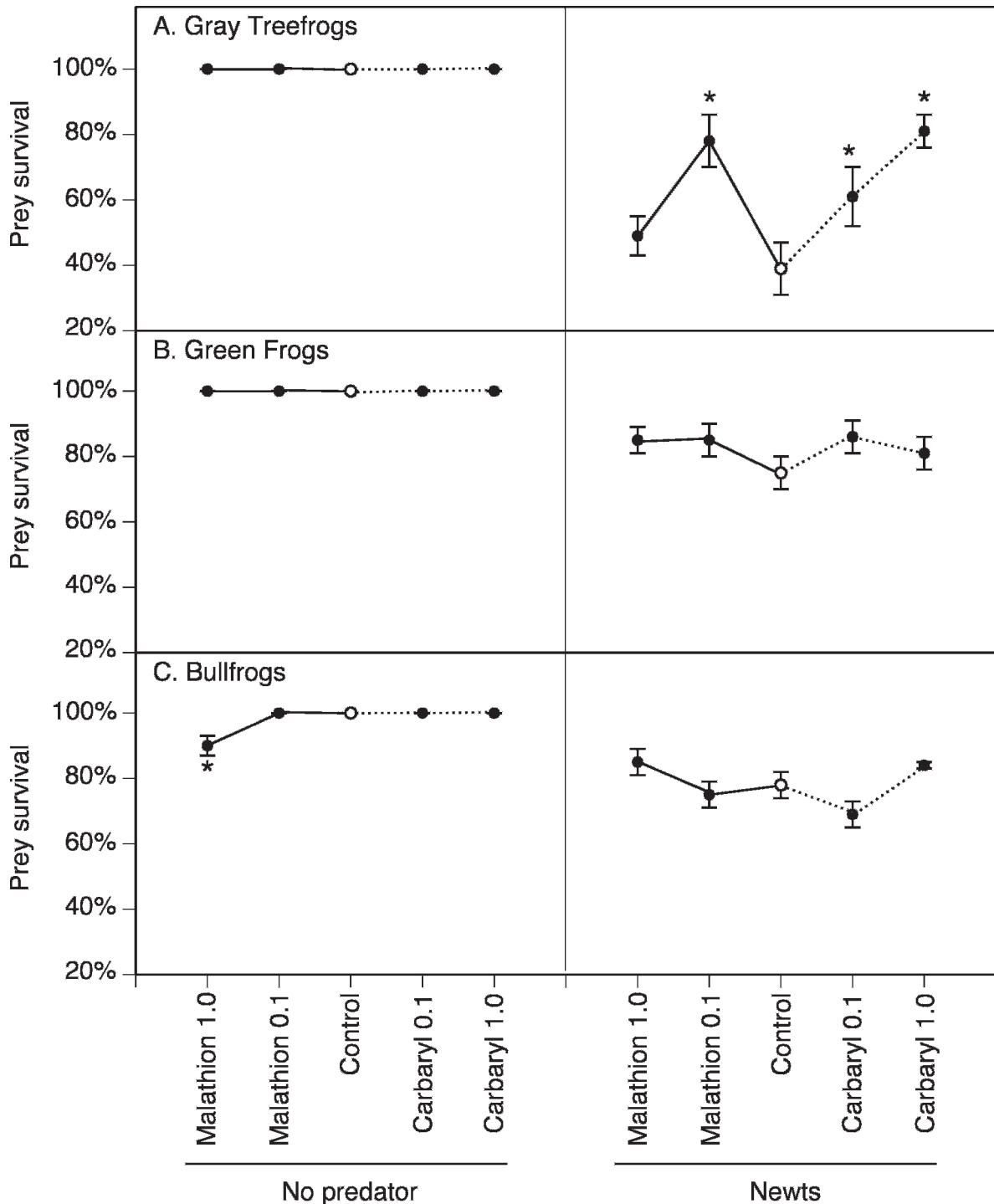| Factor	Gray Treefrogs	Green Frogs	Bullfrogs
Pesticides (df = 4,30)	$F = 5.60$ $P = 0.002$	$F = 0.867$ $P = 0.495$	$F = 2.53$ $P = 0.061$
Predators (df = 1,30)	$F = 455$ $P < 0.001$	$F = 143$ $P < 0.001$	$F = 239$ $P < 0.001$
Pesticides * Predators (df = 4,30)	$F = 5.60$ $P = 0.002$	$F = 0.867$ $P = 0.495$	$F = 12.5$ $P < 0.001$

Fig. 2.   The survival of tadpoles (A. Gray Treefrogs, *H. versicolor*; B. Green Frogs, *R. clamitans*; and C. Bullfrogs, *R. catesbeiana*) when exposed to two predator environments and two concentrations of insecticides (malathion and carbaryl) over a 22-hour period. Data are means ± 1 SE. Asterisks indicate those pesticide treatments that differ from the control treatment.
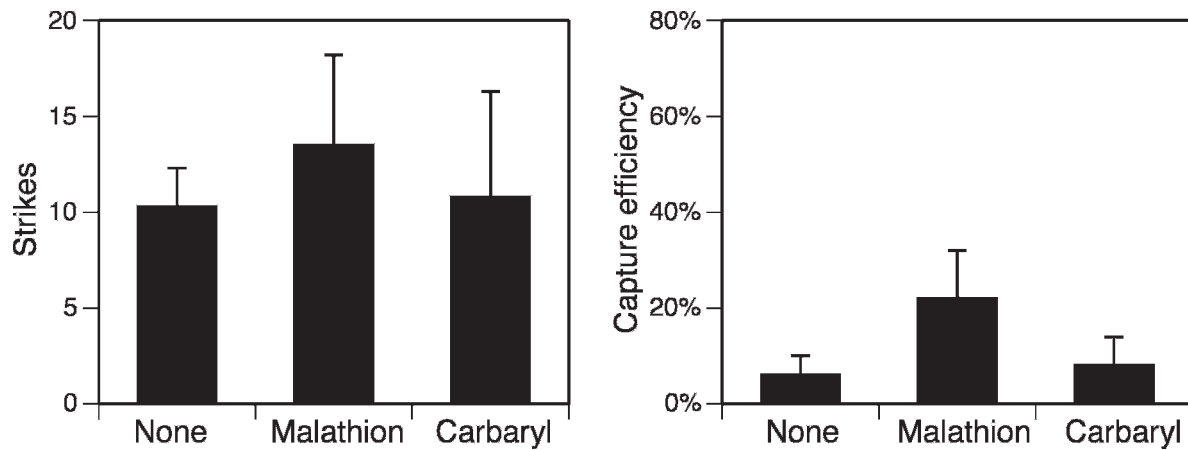
Fig. 3. The predatory behavior (number of strikes and capture efficiency, number of captures/total number of strikes) of Red-spotted Newts (*Notophthalmus viridescens*) when exposed to 0.1 mg/L of insecticides (malathion and carbaryl). Data are means ± 1 SE.

phosphates remains to be tested, but the results suggest that we may be able to arrive at some useful generalities across a diversity of insecticides.

At pesticide concentrations that were sublethal to the newts, Gray Treefrogs experienced improved survival (except with 1.0 mg/L of malathion), whereas Green Frogs and Bullfrogs did not. Several studies have documented pesticide-induced changes in amphibian behavior and extrapolated these behavioral changes to potential impacts on predator–prey interactions (Bridges 1997, 1999a, 1999b; Relyea and Mills, 2001; Rohr et al., 2003) or observed interactive effects between predator and pesticide manipulations and suggested a potential role of pesticide-induced behaviors (Boone and Semlitsch, 2001, 2003). Surprisingly, however, the actual impact of pesticides on predator–prey interactions has rarely been quantified. Broomhall (2002, 2004) documented reduced per-capita predation rates at pesticide concentrations in tadpoles (using 0.03 and 1.3 μg/L of the insecticide endosulfan), although the mechanism underlying this altered predation rate was not identified. Relyea and Hoverman (2008) exposed aquatic communities to malathion and found reduced predation rates on two species of tadpoles as malathion concentration was increased across a range of sublethal concentrations.

In the current experiment, we expected that a pesticide-induced reduction in prey activity would play an important role in reducing predation rates. When prey reduce their activity, encounter rates with predators decline, thereby reducing the risk of predation (Skelly, 1994; Relyea, 2001a). Interestingly, whereas all three tadpole species in the current study exhibited reductions in activity under most pesticide concentrations, activity reductions were not consistently related to changes in predation rates by Red-spotted Newts. Pesticide-altered predation rates have been documented in non-amphibian taxa, but there is a similar paucity of studies in these taxa as well (Kruzynski and Birtwell, 1994; Dodson et al., 1995; Schulz and Dabrowski, 2001). There have also been a few studies demonstrating how other contaminants (e.g., pH or heavy metals) can alter predator–prey interactions (Kiesecker, 1996; Lefcort et al., 1999). These studies underscore the importance of testing sublethal concentrations because they can play a major role in prey survival when put into a more natural context of environments containing predators.

The predatory newts exhibited no change in their number of strikes or capture efficiency at 0.1 mg/L of malathion or carbaryl. Thus these two behaviors of the predator also offered no explanatory mechanism for Gray Treefrogs experiencing higher survival from predation with several of the insecticide treatments. However, Bridges (1999b) found that Red-spotted Newt movement was reduced with exposure to carbaryl, although the cabaryl concentrations used (2.5 mg/L) were an order of magnitude higher than that used in our predator behavior experiment. It will likely require a complete assessment of predator and prey behaviors, including studies of predation with refuges present and studies for longer durations, to mechanistically understand the causes of altered predation rates.

Whatever the mechanism that allowed improved survival in Gray Treefrogs, it is clear that the improvement in prey survival did not require a reduction in predator number. This is an important point in the study of community ecotoxicology in which the default explanation for improved prey survival is typically via predator death (deNoyelles et al., 1994; Fleeger et al., 2003; Boone and Semlitsch, 2003; Relyea and Hoverman, 2006; Relyea et al., 2005). For example, in a study of food web changes with the application of pesticides, Relyea (2005) observed that carbaryl and malathion caused a 34–49% reduction in predator biomass and a 49–86% increase in tadpole biomass. This positive effect on the tadpoles was attributed to the pesticides killing off many of the predators and thereby allowing more prey to survive. The current study makes it clear that pesticides can also cause an increase in tadpole biomass, at least in some species, by reducing per-capita predation rates and allowing more prey to survive. These results may extend beyond the species of predators and prey used in this study and extend to other pesticides that share the same mode of action of carbaryl and malathion (e.g., diazinon, chlorpyrifos).

Pesticides can play an important role in species interactions either by killing individuals or by altering their behavior. With pesticides, we may be able to predict the relative importance of each process by simply understanding how different pesticide concentrations impact the survival and behavior of the various taxa in a community (reviewed in Relyea and Hoverman, 2006). At lower (i.e., sublethal) concentrations, only behavioral effects will occur.

However, as concentrations increase, we should expect simultaneous mortality and behavioral effects, although mortality effects will likely dominate at highly lethal concentrations. Moreover, whereas this study focused on predator–prey interactions, pesticide-induced changes in individual behavior (i.e., locomotion, foraging, mating) likely change the magnitude and direction of many interspecific interactions including herbivory, competition, and disease dynamics (Relyea and Hoverman, 2006). Therefore, to understand how pesticides affect communities, we need to give greater attention to sublethal concentrations of pesticides affect not only individuals, but also to how they affect interspecific interactions and the larger ecological community. By tackling these challenges, we will make considerable progress in understanding how contaminants impact ecological communities.

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