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IMPACT OF AN INSECTICIDE CHANGES WITH AMOUNT OF LEAF LITTER INPUT: IMPLICATIONS FOR AMPHIBIAN POPULATIONS

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Abstract—Changes in percentage of forest cover can influence nutrient levels in aquatic systems and change abiotic conditions that may influence species. The authors examined how increasing amounts of leaf litter influenced toxicity of the insecticide carbaryl using larval green frogs (*Rana clamitans*) in outdoor mesocosm ponds. Insecticides can have direct negative effects on individual physiology and behavior and indirect effects on the food web, which can result in trophic cascades. They predicted that direct effects of the insecticide would dominate when nutrients were low (resulting in negative impacts on amphibian development and survival), whereas indirect effects could offset direct effects when nutrients were more abundant through a trophic cascade that leads to more food for tadpoles (resulting in positive effects on amphibian development and survival). The authors found support for this hypothesis: first, total green frog survival was greatest with increased leaf litter input in the presence of carbaryl. Additionally, most green frogs that reached metamorphosis were from ponds with high leaf litter input and carbaryl. Second, the impact of carbaryl on developmental stage and tadpole mass varied depending on the amount of leaf litter present. With high amounts of leaf litter, carbaryl had a positive impact on development and growth; in contrast, with low amounts of leaf litter, carbaryl had negative or no effects on tadpole development and mass. The present study suggests that differences in nutrient levels between ponds exposed to pesticides could play a role in amphibian population dynamics. Environ. Toxicol. Chem. 2012;31:xxx–xxx. © 2012 SETAC

Keywords—Amphibian Carbaryl Green frog *Rana clamitans* Nutrient input

INTRODUCTION

Community composition shifts as critical habitat characteristics change across environmental gradients. Canopy cover variation, from open to closed canopy, represents a gradient that leads to changes in environmental temperature and moisture [1,2]; canopy cover can also impact aquatic systems through effects on pond pH, dissolved oxygen (DO) concentration, dissolved organic carbon (DOC), ultraviolet light (UV) availability, plankton communities, and nutrient input [1,3,4], which can influence the impact of anthropogenic factors such as pesticides. Because pesticides move with wind currents, runoff, and precipitation, they can be found in protected as well as disturbed habitats [5–7]. For this reason, pesticides are a relevant environmental factor for all natural systems. The impact of a pesticide on natural systems is not always predictable from laboratory tests, because environmental factors such as UV radiation or temperature can change a pesticide's effect. For instance, the amount of UV in an aquatic system can increase or decrease toxicity of some contaminants [8,9]; furthermore, UV penetration in aquatic environments is influenced by DOC [10], which is influenced by soil conditions and surrounding vegetation. Therefore, environmental factors can vary with habitat gradients, influencing the relative toxicity of a pesticide.

Amphibian communities are experiencing major changes in assemblages in some regions as a result of population declines and local extinctions. Amphibian population declines have been associated with disease pathogens (especially *Batrachochytrium dendrobatidis*) [11,12] as well as with upwind agriculture [13–15]. Many factors associated with declines are also present in areas where declines are not apparent, suggesting that key environmental factors may influence susceptibility. For example, amphibian declines are most prominent in the United States along the Pacific Coast and in mountainous regions in the west, but not in the East or the Midwest, although both regions have substantial pesticide use [16]. The midwestern corn belt has approximately 95.7 million acres in cropland, whereas Pacific states have dramatically less agriculture, with approximately 23.9 million acres in cropland [17]. Given that approximately 80% of the 389 million kg (857 million pounds) of active ingredients of pesticides used in the United States is applied to agricultural lands [18], the Midwest receives a greater input of contamination than the West Coast. However, few species appear to be declining in the Midwest [16]. This trend could also suggest that pesticides are not playing a significant role in declines farther from sources of contamination, as suggested by Bradford et al. [19]; however, given the ubiquity of environmental pesticides, it is critical to understand how toxicity may vary with conditions across environmental gradients.

Nutrient availability in aquatic systems is an environmental factor that varies among regions and with elevation, which could interact with factors such as pesticides associated with population declines. A greater portion of lakes in the Midwest and the eastern United States, where amphibian population declines are less frequent, are eutrophic or hypertrophic compared with lakes in the western United States [20]. Nearly 50% of the lakes in the Midwest are hypereutrophic, the highest U.S. rates [20]. Wetlands where declines have occurred in the western United States are often at high elevation [16], where aquatic systems are typically oligotrophic [21]. More than 50% of wetlands in the Pacific Coast states are oligotrophic, compared with <5% of those in the Midwest temperate plains [20].

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Although patterns of amphibian population declines have been associated with higher elevation [16], nutrient levels in ponds have not been implicated as a contributing factor.

Effects of pesticides and other environmental contaminants could be influenced by nutrient availability in aquatic systems. The effect of a pesticide in the natural environment will be a combination of effects from direct and indirect pathways. Direct effects include impacts on individual survival, physiology, or behavior; indirect effects include changes in the food web, which can alter predator communities and food resources. For instance, although a short-lived insecticide may reduce activity of tadpoles, it may also reduce the abundance of predators (especially arthropods) and zooplankton grazers; a reduction in zooplankton can result in algal blooms, with short-term increases in both phytoplankton and periphyton [22,23]. Therefore, the impact of pesticides may vary with species sensitivity to direct effects, as well as environmental conditions that influence the magnitude of indirect effects. Pesticides have been found to reduce anuran survival in mesocosm ponds [23–26]. However, some studies show that insecticides can have apparent positive effects on anuran growth and survival, which may result from changes in predator communities or food resources [27–30]. Positive effects on anuran survival with exposure to insecticides can also occur in more natural environments such as experimental farm ponds [31] as well as in areas that experience heavy chemical management such as golf courses [32]. Greater anuran survival with sublethal insecticide exposure relative to controls suggests that indirect effects of a pesticide could outweigh direct negative effects in some cases [29].

The objective of the present study was to examine whether changes in amount of leaf litter input influence toxicity of the insecticide carbaryl to green frog (*Rana clamitans*) tadpoles, because insecticide use has been correlated with population declines of other ranids [15,33]. Green frogs have larval periods often exceeding one year, but green frogs that reach a minimum size at metamorphosis can transform in a single season. The flexibility in this species' life history makes it ideal for examining how environmental factors might modulate relative toxicity. We predicted that the impact of the insecticide would differ with nutrient availability in the pond. We expected that tadpoles would be negatively affected by insecticide exposure when the amount of leaf litter was low, because direct negative effects of the insecticide would dominate, and low nutrient availability would reduce the potential for trophic cascades. We predicted that the effect of the insecticide exposure would positively influence growth and survival when nutrient availability was greater, because nutrient availability would increase the potential for a trophic cascade that resulted in increased food resources for anurans, allowing indirect positive effects of exposure to outweigh direct negative effects of exposure.

MATERIALS AND METHODS

Animal care and experimental design

We collected four clutches of green frogs (*R. clamitans*) from Miami University Natural Areas in Oxford, Ohio, USA, on June 20, 2007. Eggs were hatched in the laboratory at 23 to 25°C, and we kept them in the laboratory until tadpoles were freely swimming (Gosner stage 25 [34]). Clutches were mixed to homogenize genetic variation. We established aquatic communities in 32 polyethylene ponds (1.85 m in diameter; 1,480 L volume) at Miami University's Ecology Research Center in early July by adding 1,000 L water, leaf litter (amount described below), and plankton from natural ponds. Screen-mesh lids covered each pond to exclude incidental predators and anuran colonists.

We added 30 recently hatched green frog tadpoles to ponds on July 25, 2007 (experimental day 0). We experimentally manipulated two factors in a full-factorial design with four replicates: exposure to expected environmental concentrations of the insecticide carbaryl (0 or 1.75 mg/L) and amount of leaf litter (air-dried wt of 0.25, 0.50, 1.0, or 2.0 kg). We added the insecticide carbaryl as liquid Sevin (22.5% carbaryl; GardenTech) on experimental day 5 at a nominal concentration of 1.75 mg/L (7.8 g Sevin [22.5% carbaryl, GardenTech] added to 1,000 L water) after tadpoles had acclimated to mesocosm environments; in similar studies in our laboratory using identical approaches of insecticide application, carbaryl concentration has been confirmed to be near calculated concentrations [22,29], but carbaryl concentration was not confirmed in the present study because of cost constraints. To simulate an agricultural overspray event, we mixed carbaryl with 5 L of pond water taken from each pond and poured the mixture evenly across the pond surface with a watering can at 3:30 PM Eastern Standard Time to achieve a final concentration of 1.75 mg/L. The LC50s of carbaryl at 96 h for green frog tadpoles can range from 22.0 mg/L at 17°C to 11.3 mg/L at 27°C [35]. Therefore, the concentration used in our experiment was considerably lower than concentrations expected to cause direct mortality to tadpoles but is within the range that could alter swimming and feeding behaviors [36]; this concentration, however, can be directly lethal to zooplankton [22]. A concentration of 1.75 mg/L represents a worst-case scenario given that it is likely greater than concentrations observed in streams or ponds following a runoff event after forest spraying ($\leq$16.1 μg/L [37], $\leq$254 μg/L [38]) but is below expected environmental concentrations of 3.667 mg/L from direct exposure calculated by Peterson et al. [39]. At the time of insecticide addition, pond pH was 7.7 ± 0.02 (mean $\pm$ SE), temperature was 19.5 ± 0.05°C, and DO was 4.8 ± 0.2 mg/L. Leaf litter was collected from a mature, mixed deciduous forest (predominantly oak [*Quercus* spp.], beech [*Fagus grandifolia*], maple [*Acer* spp.], and sycamore [*Platanus occidentalis*]). We selected leaf litter levels to represent low to high amounts of litter input found in nature [40–43] and to span the range of additions researchers typically use in mesocosm studies (for review see Semlitsch and Boone [44]).

We checked ponds two or three times per week for metamorphs, which were removed from each pond and held in the laboratory until tail resorption (usually $\leq$3 d), at which time they were weighed to the nearest milligram. Green frogs did not reach metamorphosis in all treatments. We terminated the experiment on October 31 (experimental day 98), at which time it was likely that tadpoles would overwinter in mesocosms. At the end of the experiment, we drained the ponds and thoroughly searched for remaining tadpoles. Tadpoles remaining in the pond were weighed, staged (Gosner developmental stage [34]), and enumerated.

We measured periphyton abundance, a food resource of green frog tadpoles [45], on experimental days 7 and 35. Changes in the aquatic community following pesticide exposure can occur within a few days after exposure and may change the aquatic community for weeks [22]; therefore, these sampling times were sufficient to obtain a coarse assessment of changes in abundance of periphyton. We took periphyton samples by scraping a 3 cm × 1.3 cm patch directly below the waterline on the western side of each pond; by holding a razor blade at a

45° angle, periphyton can be successfully collected by this method without loss to provide a relative index of abundance of chlorophyll-a among experimental treatments [22]. Periphyton was then wiped onto a filter and placed in 15 ml neutralized 90% acetone in the dark at 5°C for 24 h and analyzed by fluorometry [46]. We measured water quality parameters including pH, DO, and temperature on experimental days 5 and 35. To determine whether DOC varied with leaf litter or carbaryl treatments, we took a composite sample from each pond on experimental day 64, and 100 ml was used for analysis. Water samples were filtered with 25-mm, 0.7-μm Whatman GF/F filters within 8 h of sample collection; samples were stored at 4°C until analysis with a Shimadzu Total Organic Carbon Analyzer (TOC-VCPH). Dissolved organic carbon was measured in standard sensitivity mode, subtracting Milli-Q deionized water blanks from standards and samples and calibrating to dilutions of a certified DOC standard.

Response variables and statistical analyses

We examined how carbaryl exposure, leaf litter input, and their interaction affected tadpole mass, Gosner developmental stage [34], percentage of green frogs reaching metamorphosis, and total survival (number of metamorphs plus tadpoles). Effects of treatments and their interaction on percentage of green frogs reaching metamorphosis were examined with analysis of variance (ANOVA). Because most green frogs that reached metamorphosis were from a single treatment, we could not test for treatment effects on mass or time to metamorphosis. Analysis for treatment effects and their interaction on the "tadpole response," consisting of total survival, Gosner developmental stage, and tadpole mass, was performed with multivariate analysis of variance. To normalize data and stabilize variances, we angularly transformed survival data and log transformed tadpole mass, Gosner developmental stage, mass at metamorphosis, and time to metamorphosis before analyses. Periphyton abundance was log transformed and analyzed with repeated-measures ANOVA to determine differences in exposure to treatments and their interaction over time. Pond pH, DO, and temperature were measured with repeated-measures ANOVA to determine effects of treatments over time. We analyzed DOC with ANOVA to determine differences between treatments and their interaction.

RESULTS

The percentage of green frogs that reached metamorphosis significantly increased with leaf litter input ($F_{3,23} = 11.62$, $p < 0.0001$; Fig. 1A) but was not affected by carbaryl exposure ($F_{1,23} = 2.79$, $p = 0.1083$). The addition of carbaryl and high amounts of leaf litter marginally affected the proportion of tadpoles that reached metamorphosis ($F_{3,23} = 2.87$, $p = 0.0584$; Fig. 1A).

The multivariate tadpole response (of total survival, mass, and developmental stage) was significantly affected by leaf litter input ($F_{9,51} = 10.41$, $p < 0.0001$), carbaryl exposure ($F_{3,21} = 9.66$, $p = 0.0003$), and the interaction of leaf litter and carbaryl ($F_{9,51} = 3.56$, $p = 0.0017$). Univariate analyses indicated that total survival (tadpoles plus metamorphs) significantly increased with the amount of leaf litter ($F_{3,23} = 13.02$, $p < 0.0001$; Fig. 1B) and with carbaryl exposure ($F_{1,23} = 20.09$, $p = 0.0002$; Fig. 1B); the interaction of these treatments ($F_{3,23} = 1.20$, $p = 0.3315$) did not appear to contribute to the multivariate response. Both stage ($F_{3,23} = 7.92$, $p = 0.0008$) and tadpole mass ($F_{3,23} = 7.00$, $p = 0.0016$) were

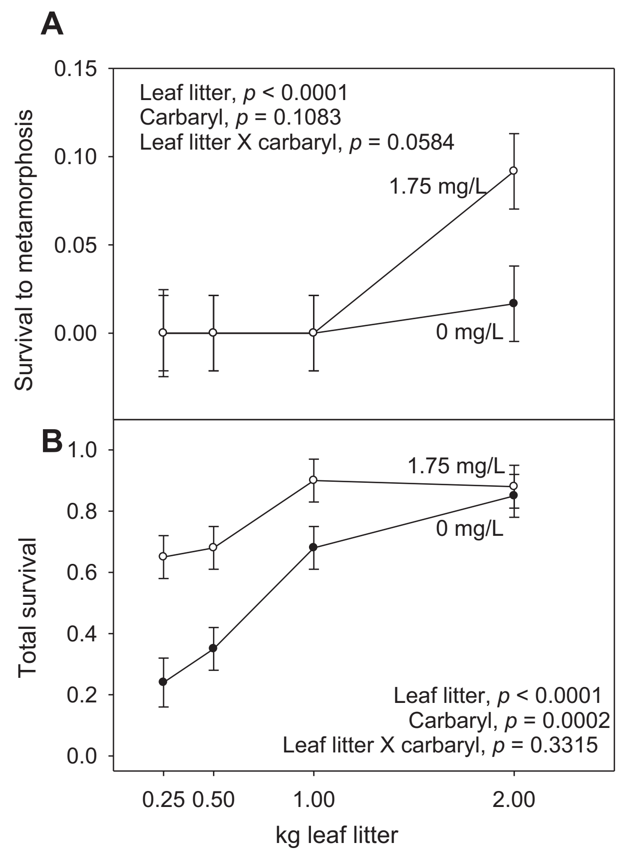

Fig. 1. Effects of leaf litter treatment and carbaryl exposure (0 or 1.75 mg/L carbaryl) on proportion reaching metamorphosis (**A**) and total survival (**B**; tadpole plus metamorph survival). Error bars represent ± 1 SE.

significantly affected by the interaction of leaf litter and carbaryl. Growth and mass were reduced with carbaryl exposure with 0.5 kg of leaf litter input, whereas carbaryl exposure increased growth and mass with 1 or 2 kg leaf litter (Fig. 2).

Relative abundance of periphyton significantly changed over time ($F_{1,23} = 19.22$, $p = 0.0002$) but did not vary with treatments over time ($p > 0.0942$). However, between-subjects analysis indicated that the interaction of leaf litter and carbaryl concentration ($F_{3,23} = 3.47$, $p = 0.0326$) significantly affected periphyton abundance overall; generally, there were greater amounts of periphyton in ponds with carbaryl and more leaf litter (Fig. 3), although this pattern is not apparent by day 35. Pond pH decreased significantly with leaf litter input ($F_{3,23} = 8.37$, $p = 0.0006$) but was not significantly affected by other treatment combinations, and treatments did not vary with time (Fig. 4A). Dissolved oxygen significantly decreased with leaf litter input ($F_{3,23} = 67.92$, $p < 0.0001$; Fig. 4B). Carbaryl exposure also reduced DO by approximately 6.6% ($F_{1,23} = 5.42$, $p = 0.0291$), but there was no interaction between carbaryl and leaf litter. Dissolved oxygen varied significantly over time ($F_{1,23} = 76.44$, $p < 0.0001$), and there was an interaction between time and leaf litter input ($F_{3,23} = 6.42$, $p = 0.0026$; Fig. 4B). Leaf litter input had a small but statistically significant effect on pond temperature ($F_{3,23} = 5.89$, $p = 0.0039$; Fig. 4C); however, temperature was not affected by carbaryl exposure or the interaction. Temperature also varied over time ($F_{1,23} = 14173.2$, $p < 0.0001$) and there was an interaction between time and leaf litter input ($F_{3,23} = 17.02$, $p < 0.0001$; Fig. 4C). On experimental day 5, ponds with

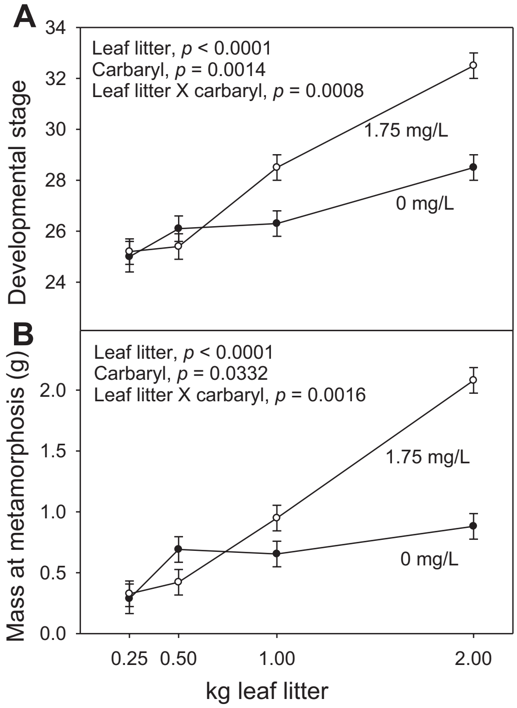

Fig. 2. Effects of leaf litter treatment and carbaryl exposure (0 or 1.75 mg/L carbaryl) on Gosner developmental stage [34] (**A**) and tadpole mass (**B**). Error bars represent ± 1 SE.

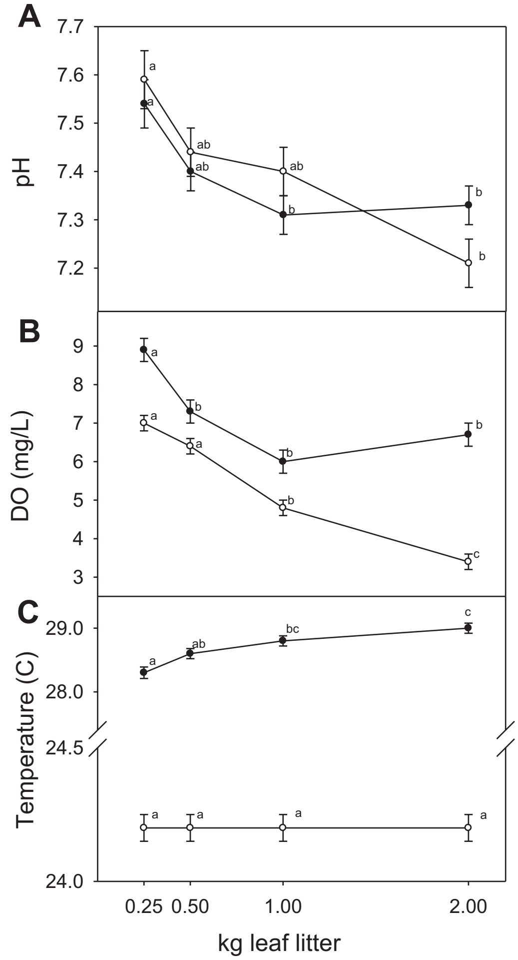

Fig. 4. Effects of leaf litter treatment on pond pH (**A**), dissolved oxygen (DO) (**B**), and temperature (**C**). Solid circles are samples taken on experimental day 5; open circles were taken on experimental day 35. Differing letters indicate significant differences among treatments at a given time measurement according to Scheffe's multiple comparison tests. Error bars represent ± 1 SE.

2 kg leaf litter had temperatures that were 0.7°C greater than those with 0.25 kg leaf litter; by day 35, there are no differences in temperature among treatments.

Leaf litter input significantly increased DOC ($F_{3,27} = 3.78$, $p < 0.0001$), but carbaryl exposure alone did not ($F_{1,27} = 1.83$, $p = 0.1869$). The interaction of leaf litter and carbaryl significantly affected DOC ($F_{3,27} = 3.57$, $p = 0.0269$; Fig. 5). Lower DOC levels were found in ponds with low leaf litter input and carbaryl, and higher levels were found in ponds with high leaf litter and carbaryl compared to ponds without carbaryl.

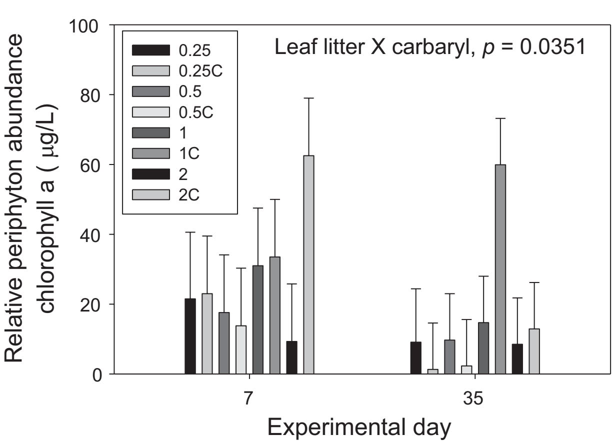

Fig. 3. Effects of leaf litter treatment and carbaryl exposure (0 or 1.75 mg/L carbaryl) on relative abundance of periphtyon (measured as chlorophyll a). Numbers indicate the amount of leaf litter added to ponds (kg), and C indicates exposure to 1.75 mg/L carbaryl. Carbaryl exposure occurred on experimental day 5. The *p* value represents the interaction of leaf litter and carbaryl exposure from between-subjects repeated-measures analysis of variance. Error bars represent ± 1 SE.

DISCUSSION

Amphibian population declines have been correlated with upwind agriculture in California [13,14,33]. This relationship is surprising given that the amount of pesticides that would move through the atmosphere would likely be below lethal concentrations and given that other areas of high pesticide use do not

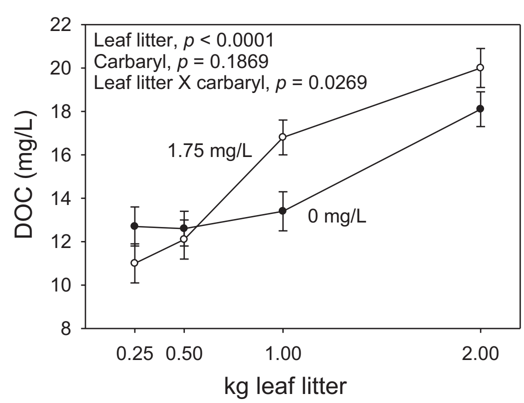

Fig. 5. Effects of leaf litter treatment and carbaryl exposure (0 or 1.75 mg/L carbaryl) on dissolved organic carbon (DOC). Error bars represent ± 1 SE.

have widespread population declines. However, Bradford et al. [19] suggests that declines evaluated by Davidson et al. [13,14] and Davidson and Knapp [47] were more likely attributable to the amphibian chytrid fungus. Disparity in amphibian decline frequency among regions could be a consequence of differences in pesticides used among regions or sensitivities of species, differences in other factors that may alter the toxicity, or differences in the presence of another factor (such as disease) altogether. The present study suggests that the differences in impacts of pesticides on amphibian survival and development can result from varying abiotic conditions related to nutrient availability, which could potentially explain or contribute to different regional patterns observed in the United States.

Our central prediction was that carbaryl and leaf litter would interact, resulting in negative effects of carbaryl at low nutrient levels (because of a lower likelihood of increases in algal food resources via trophic cascades) and positive effects at high nutrients (because of greater likelihood for increases in algal food resources via trophic cascades). These predictions were partially verified. The interaction of carbaryl and leaf litter input significantly impacted the multivariate response (of total survival, tadpole mass, and tadpole developmental stage) and marginally influenced percentage of green frogs reaching metamorphosis. The insecticide carbaryl increased the percentage of green frogs reaching metamorphosis, total survival, tadpole mass, and tadpole development with leaf litter inputs of 1 kg or greater. Almost all of the individuals that reached metamorphosis in a single season came from ponds exposed to carbaryl and 2 kg leaf litter. This suggests that any sublethal direct effects [36] might have been offset by short-term trophic cascades that resulted in increased food resources, allowing animals to reach metamorphosis. Individuals that reach metamorphosis before winter may be more vulnerable than they would be if they overwintered in a pond as tadpoles. However, studies with recently metamorphosed green frogs found a 48% probability of surviving the winter, similar to other anuran species that typically metamorphose in a single season [48], suggesting that when green frogs reach or exceed a minimum size at metamorphosis, the probability of overwinter survival as a metamorph is greater than or equal to the probability of overwinter survival as a tadpole.

Total survival of metamorphs and tadpoles combined (analyzed in the multivariate response) was significantly and positively affected by both insecticide exposure and increased leaf litter input, but there was no interaction between treatments. Increased survival with carbaryl exposure at low leaf litter concentrations suggests that tadpoles still benefited from exposure at lower nutrient levels. There were no large observable effects on periphyton food resources with low amounts of leaf litter additions with or without carbaryl. Because green frogs can also feed on phytoplankton and other food resources [45], it is possible that they fed on resources not measured in the present study or that trophic cascades do not explain the increased growth and development in ponds with high leaf litter content [49].

The amount of leaf litter influenced the impact of carbaryl on tadpole mass and developmental stage. Mass increased and tadpoles were more developed with increases in leaf litter, with carbaryl exposure augmenting this effect. However, with low leaf litter input of 0.25 or 0.5 kg, mass and tadpole development either were not affected or were negatively affected relative to controls. The experimental conditions influenced whether carbaryl had a positive or negative effect on growth or development. If the experiment was conducted under low leaf litter conditions only, we would have concluded that pesticides have negative or no effects; in contrast, if the present study were conducted with high levels of leaf litter input only, we would conclude that positive effects can result from exposure. Although treatment effects on survival have the most straightforward impacts on populations, time to metamorphosis, and size at metamorphosis are traits correlated with fitness, and they have important consequences for terrestrial survival and reproductive success [50–53]. Furthermore, population models suggest that reductions in larval survival, whether from pesticides or from other factors, might not be as significant as factors that influence juvenile survival [54–57], which can be impacted by time and mass at metamorphosis. Because earlier time at metamorphosis and larger size at metamorphosis (which are correlated with tadpole development and tadpole mass) are positively associated with overwinter survival, earlier time to first reproduction, and greater reproductive success, time and size at metamorphosis could have a large impact on population dynamics. Furthermore, smaller mass and slower developmental rate could also increase the likelihood of either disease exposure or susceptibility to disease [58]. Given that many of the enigmatic amphibian population declines have been associated with chytridiomycosis resulting from lethal infections of *B. dendrobatidis*, impacts on metamorph quality that could be influenced by environmental factors such as pesticide exposure and nutrient levels in the pond could have significant long-term impacts.

Additionally, low nutrient levels alone could make species more vulnerable to additional stressors. Survival, developmental stage, and mass at metamorphosis are at their lowest levels in ponds with small amounts of leaf litter. Other studies have shown that survival and developmental rate increased with increasing amounts of leaf litter [59] and that litter type can vary in quality, impacting metamorphosis [60,61]. Increasing the amount of leaf litter input can increase microbial production, thus increasing nutrient availability for primary production. Lower levels of DO were associated with increasing leaf litter in the present study and suggest that microbial respiration was greater in ponds with more leaf litter [59,62]. We also saw greater temperatures and lower pH between ponds with high versus low leaf litter input. The amount of leaf litter results in many shifts in the abiotic environment that could influence the response of an amphibian to a pesticide.

The difference in the impact of carbaryl exposure with low versus high leaf litter input explains some of the differences observed in the effects of pesticide among laboratories that use mesocosms (for review see Semlitsch and Boone [44]). However, the results in the present study also might help to explain how pesticide-influenced population declines could occur in some regions but not in others based on important differences in nutrient availability. One of the major risk factors for amphibians is living at higher elevations in the United States, Central America, South America, and Australia [16]. Higher elevations have characteristics that differ from lower elevation in terms of temperature, variation in temperature, ultraviolet radiation, and the amount of nutrients available in ponds. The present study suggests that nutrient input can influence amphibians in its own right and that it can modulate the impact of the pesticide.

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REFERENCES

1. Skelly DK, Werner EE, Cortwright SA. 1999. Long-term distributional dynamics of a Michigan amphibian assemblage. *Ecology* 80:2326–2337.
2. Werner EE, Skelly DK, Relyea RA, Yurewicz KL. 2007. Amphibian species richness across environmental gradients. *Oikos* 116:1697–1712.
3. Skelly DK, Halverson MA, Freidenburg LK, Urban MC. 2005. Canopy closure and amphibian diversity in forested wetlands. *Wetl Ecol Manag* 13:261–268.
4. Bastazini CV, Munduruca JFV, Rocha PLB, Napoli MF. 2007. Which environmental variables better explain changes in anuran community composition? A case study in Restinga of Mata de Sao Joao, Bahia, Brazil. *Herpetologica* 63:459–471.
5. Bidleman TF. 1999. Atmospheric transport and air–surface exchange of pesticides. *Water Air Soil Pollut* 115:115–166.
6. LeNoir JS, McConnell LL, Fellers GM, Cahill TM, Seiber JN. 1999. Summertime transport of current-use pesticides from California's Central Valley to the Sierra Nevada mountain range. *Environ Toxicol Chem* 18:2716–2722.
7. van Dijk HFG, Guicherit R. 1999. Atmospheric dispersion of current-use pesticides: A review of the evidence from monitoring studies. *Water Air Soil Pollut* 115:21–70.
8. Oris JT, Giesy JP. 1985. The photoenhanced toxicity of anthracene to juvenile sunfish (*Lepomis* spp.). *Aquat Toxicol* 6:133–146.
9. Zaga A, Little EE, Rabeni CF, Ellersieck MR. 1998. Photoenhanced toxicity of a carbamate insecticide to early life stage anuran amphibians. *Environ Toxicol Chem* 17:2543–2553.
10. Williamson CE, Rose KC. 2010. When UV meets water. *Science* 329:637–639.
11. Skerrat LF, Berger L, Speare R, Cashins S, McDonald KR, Phillott AD, Hines HB, Kenyon N. 2007. Spread of chytridiomycosis has caused the rapid global decline and extinction of frogs. *EcoHealth* 4:125–134.
12. Lips KR, Diffendorfer J, Mendelson JR III, Sears MW. 2008. Riding the wave: Reconciling the roles of disease and climate change in amphibian declines. *PLoS Biol* 6:441–454.
13. Davidson C, Shaffer HB, Jennings MR. 2001. Declines of the California red-legged frog: Climate, UV-B, habitat, and pesticides hypotheses. *Ecol Appl* 14:464–479.
14. Davidson C, Shaffer HB, Jennings MR. 2002. Spatial tests of the pesticide drift, habitat destruction, UV-B, and climate-change hypotheses for California amphibian declines. *Conserv Biol* 16:1588–1601.
15. Sparling DW, Fellers GM, McConnell LL. 2001. Pesticides and amphibian population declines in California, USA. *Environ Toxicol Chem* 20:1591–1595.
16. Collins JP, Crump ML. 2009. *Extinction in Our Times: Global Amphibian Decline*. Oxford University Press, New York, NY, USA.
17. Lubowski RN, Vesterby M, Bucholtz S, Baez A, Roberts MJ. 2005. Major uses of land in the United States, 2002. USDA, Economic Informational Bulletin 14.
18. Grube A, Donaldson D, Kiely T, Wu L. 2011. Pesticides industry sales and usage: 2006 and 2007 market estimates. EPA 733/R-11/001. U.S. Environmental Protection Agency, Washington, DC.
19. Bradford DF, Knapp RA, Sparling DW, Nash MS, Stanley KA, Tallent-Halsell NG, McConnell LL, Simonich SM. 2011. Pesticide distributions and population declines of California, USA, alpine frogs, *Rana muscosa* and *Rana sierra*. *Environ Toxicol Chem* 30:682–691.
20. U.S. Environmental Protection Agency. 2009. National lakes assessment: A collaborative survey of the nation's lakes. EPA 841/R-04/001. Washington, DC.
21. Knapp RA, Matthews KR, Sarnelle O. 2001. Resistance and resilience of alpine lake fauna to fish introductions. *Ecol Monogr* 71:401–421.
22. Distel CA, Boone MD. 2009. Effects of aquatic exposure to the insecticide carbaryl and density on aquatic and terrestrial growth and survival in American toads. *Environ Toxicol Chem* 28:1963–1969.
23. Distel CA, Boone MD. 2010. Effects of aquatic exposure to the insecticide carbaryl are species-specific across life stages and mediated by heterospecific competitors in anurans. *Funct Ecol* 24:1342–1352.
24. Boone MD, Semlitsch RD. 2001. Interactions of insecticide with larval density and predation in experimental amphibian communities. *Conserv Biol* 15:228–238.
25. James SM, Little EE, Semlitsch RD. 2005. Metamorphosis of two amphibians after chronic cadmium exposure in outdoor aquatic mesocosms. *Environ Toxicol Chem* 24:1994–2001.
26. Relyea RA. 2009. A cocktail of contaminants: How mixtures of pesticides at low concentrations affect aquatic communities. *Oecologia* 159:363–376.
27. Boone MD, Semlitsch RD. 2003. Interactions of bullfrog tadpole predators and an insecticide: predation release and facilitation. *Oecologia* 442:610–616.
28. Relyea RA. 2005. The impact of insecticides and herbicides on the biodiversity and productivity of aquatic communities. *Ecol Appl* 15: 618–627.
29. Boone MD. 2008. Examining the single and interactive effects of three insecticides on amphibian metamorphosis. *Environ Toxicol Chem* 27: 1561–1568.
30. Mackey MJ, Boone MD. 2009. Single and interactive effects of malathion, overwintered green frog tadpoles, and cyanobacteria on gray treefrog tadpoles. *Environ Toxicol Chem* 28:637–643.
31. Boone MD, Semlitsch RD, Fairchild JF, Rothermel BB. 2004. Effects of an insecticide on amphibians in large-scale experimental ponds. *Ecol Appl* 14:685–691.
32. Boone MD, Semlitsch RD, Mosby C. 2008. Suitability of golf course ponds for amphibian metamorphosis when bullfrogs are removed. *Conserv Biol* 22:172–179.
33. Davidson C. 2004. Declining downwind: Amphibian population declines in California and historical pesticide use. *Ecol Appl* 14: 1892–1902.
34. Gosner N. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* 16:183–190.
35. Boone MD, Bridges CM. 1999. The effect of temperature on the potency of carbaryl for survival of tadpoles of the green frog, *Rana clamitans*. *Environ Toxicol Chem* 18:1482–1484.
36. Bridges CM. 1997. Tadpole swimming performance and activity affected by acute exposure to sublethal levels of carbaryl. *Environ Toxicol Chem* 16:1935–1939.
37. Stanley JG, Trial JG. 1980. Disappearance constants of carbaryl from streams contaminated by forest spraying. *Bull Environ Contam Toxicol* 25:771–776.
38. Gibbs KE, Mingo TM, Courtemanch DL. 1984. Persistence of carbaryl (Sevin-4-oil) in woodland ponds and its effects on pond macroinvertebrates following forest spraying. *Can Entomol* 116:203–213.
39. Peterson HG, Boutin C, Martin PA, Freemark KE, Ruecker NJ, Moody MJ. 1994. Aquatic phyto-toxicity of 23 pesticides applied at expected environmental concentrations. *Aquat Toxicol* 28:275–292.
40. Wallace JB, Eggert SL, Meyer JL, Webster JR. 1999. Effects of resource limitation on detrital-based ecosystem. *Ecol Monogr* 69:409–442.
41. Finzi AC, Allen AS, DeLucia EH, Ellsworth DS, Schlesinger WH. 2001. Forest litter production, chemistry, and decomposition following two years of free-air CO_2 enrichment. *Ecology* 82:470–484.
42. Mitchell MJ, Driscoll CT, Owen JS, Schaefer D, Michener R, Raynal DJ. 2001. Nitrogen biogeochemistry of three hardwood ecosystems in the Adirondack region of New York. *Biogeochemistry* 56:93–133.
43. Colon-Gaud C, Peterson S, Whiles MR, Kilham SS, Lips KR, Pringle CM. 2008. Allochthonous litter inputs, organic matter standing stocks, and organic seston dynamics in upland Panamanian streams: Potential effects of larval amphibians on organic matter dynamics. *Hydrobiologia* 603:301–312.
44. Semlitsch RD, Boone MD. 2009. Using aquatic mesocosms in amphibian ecology and conservation. In Dodd K, ed, *Amphibian Ecology and Conservation: A Handbook of Techniques*. Oxford University Press, New York, NY, USA, pp 87–104.
45. Jenssen TA. 1967. Food habits of the green frog, *Rana clamitans*, before and during metamorphosis. *Copeia* 1967:214–218.
46. Greenberg AE, Clesceri LS, Eaton AD. 1992. *Standard Methods for the Examination of Water and Wastewater*, 18th ed. American Public Health Association, Baltimore, MD, USA.
47. Davidson C, Knapp RA. 2007. Multiple stressors and amphibian declines: Dual impacts of pesticides and fish on yellow-legged frogs. *Ecol Appl* 17:587–597.
48. Boone MD. 2005. Juvenile frogs compensate for small metamorph size with terrestrial growth: Overcoming the effects of larval density and insecticide exposure. *J Herpetol* 39:416–423.
49. Bulen BJ, Distel CA. 2011. Carbaryl concentration gradients in realistic environments and their influence on our understanding of the tapdole food web. *Arch Environ Contam Toxicol* 60:343–350.

50. Semlitsch RD, Scott DE, Pechmann JHK. 1988. Time and size at metamorphosis related to adult fitness in *Ambystoma talpoideum*. *Ecology* 69:184–192.
51. Berven KA. 1990. Factors affecting population fluctuations in larval and adult stages of the wood frog (*Rana sylvatica*). *Ecology* 71:1599–1608.
52. Scott DE. 1994. The effect of larval density on adult demographic traits in *Ambystoma opacum*. *Ecology* 75:1383–1396.
53. Altwegg R. 2003. Multistage density dependence in an amphibian. *Oecologia* 136:46–50.
54. Biek R, Funk WC, Maxell BA, Mills LS. 2002. What is missing in amphibian decline research: Insights from ecological sensitivity analysis. *Conserv Biol* 16:728–734.
55. Vonesh JR, De la Cruz O. 2002. Complex life cycles and density dependence: Assessing the contribution of egg mortality to amphibian declines. *Oecologia* 133:325–333.
56. Govindarajulu P, Altwegg R, Anholt BR. 2005. Matrix model investigation of invasive species control: Bullfrogs on Vancouver Island. *Ecol Appl* 15:2161–2170.
57. Distel CA. 2010. Effects of an insecticide on competition in anurans: Could pesticide-induced competitive exclusion be a mechanism for amphibian declines? PhD thesis. Miami University, Oxford, OH, USA.
58. Carey C, Cohen N, Rollins-Smith L. 1999. Amphibian declines: An immunological perspective. *Dev Comp Immunol* 23:459–472.
59. Rubbo MJ, Belden LK, Kiesecker JM. 2008. Differential responses of aquatic consumers to variations in leaf-litter inputs. *Hydrobiologia* 605:37–44.
60. Rubbo MJ, Kiesecker JM. 2004. Leaf litter composition and community structure: Translating regional species changes into local dynamics. *Ecology* 85:2519–2525.
61. Williams BK, Rittenhouse TAG, Semlitsch RD. 2008. Leaf litter inputs mediates tadpole performance across forest canopy treatments. *Oecologia* 155:377–384.
62. Rubbo MJ, Cole JJ, Kiesecker JM. 2006. Terrestrial subsidies of organic carbon support net ecosystem production in temporary forest ponds: Evidence from an ecosystem experiment. *Ecosystems* 9:1170–1176.