Pesticide alters oviposition site selection in gray treefrogs

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Abstract Understanding the impacts of pesticides on non-target organisms is an important issue for conservation biology. Research into the environmental consequences of pesticides has largely focused on pesticide toxicity. We have less understanding of the nonlethal effects of pesticides, and the consequences of nonlethal effects for species and communities. For example, we know very little about whether pesticides alter habitat selection behavior. Understanding whether pesticides alter habitat selection is important because pesticide-induced shifts in habitat selection could either magnify or reduce the toxic effects of contaminants by funneling organisms into or directing them away from contaminated sites. Here we present four field experiments that examine the effect of the commercial pesticide Sevin® and its active ingredient, carbaryl, on oviposition site selection by the gray treefrog (*Hyla chrysoscelis*). Our results show that uncontaminated pools consistently received 2–3 times more eggs than contaminated pools; that treefrogs appeared to respond to Sevin® directly, not indirectly via its effects on the aquatic food web, and that this preference persisted across a range of temporal and spatial scales. Both Sevin® and carbaryl per se reduced oviposition, while other volatile chemicals (e.g., our solvent control, acetone) had no effect. These findings suggest that in order to understanding the consequences of contaminants in aquatic systems we will need to consider not only toxicity, but also how contaminant effects on habitat selection alter the way organisms distribute themselves in the environment.

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Introduction

The potential for pesticides to alter the distribution and abundance of aquatic organisms and the structure and function of aquatic communities is an important conservation concern (see recent reviews: Relyea and Hoverman 2006; Rohr et al. 2006a). Due to concerns about global amphibian decline (Houlahan et al. 2000; Stuart et al. 2004; Wake 1991) and because amphibians are often considered to be important indicators of environmental stress, a growing number of ecotoxicology studies have focused on the effects of agricultural pesticides on amphibians. Experimental studies have enabled researchers to isolate the direct toxic effects of specific pesticides (e.g., atrazine: Hayes et al. 2002; Rohr et al. 2003; carbaryl: Relyea and Mills 2001; malathion: Relyea 2004; Roundup: Relyea 2004) on the survival and growth of larval amphibians and have revealed some surprising interactions between chemical and biotic stressors (e.g., Relyea and Mills 2001; Kiesecker 2002; Relyea 2003).

While we are improving our knowledge of the toxic effects of pesticides and some types of nonlethal effects, a

recent review of contaminants in freshwater systems (Relyea and Hoverman 2006) highlights that we currently know very little about how contaminants affect habitat selection. Many organisms with complex life cycles colonize aquatic habitats via oviposition by terrestrial adult stages. Many of these species have been shown to exhibit nonrandom preferences when selecting aquatic breeding sites (e.g., frogs: Resetarits and Wilbur 1989; salamanders: Kats and Sih 1992; midges: Petranka and Fakhoury 1991; mosquitoes: Chesson 1984; Blaustein and Kotler 1993; beetles: Binckley and Resetarits 2005; Resetarits 2001). Oviposition site preference may be in response to variation in abiotic (e.g., habitat size: Spieler and Linsenmair 1997; Skelly 2001; Bosch and Martinez-Solano 2003; salinity: Viertel 1999; others: Fegraus and Marsh 2000) or biotic factors among potential sites (e.g., competition: Marsh and Borrell 2001; Resetarits and Wilbur 1989; predation risk: Blaustein and Kotler 1993; Spieler and Linsenmair 1997; Resetarits and Wilbur 1989).

Pesticide-induced shifts in habitat selection could arise in direct response to the chemical per se or indirectly via the effects of the pesticide on the aquatic food web. Several studies from the vector control literature show direct effects of chemicals on oviposition by mosquitoes. Xue et al. (2001, 2006) found that 18 of 21 commercial insect repellents tested in laboratory trials were effective oviposition deterrents for the mosquito *Aedes albopictus*, and Rajkumar and Jebanesan (2005) show *Solanum trilobatum* extract deters oviposition by *Anopheles stephensi*. In contrast, our own studies suggest that the insecticide Sevin® directly attracts oviposition in *Culex* mosquitoes (Vonesh, in preparation). These examples suggest that chemicals can directly alter habitat selection behavior; however, the mechanisms by which they affect this change remain unclear. We are unaware of any published examples of contaminants indirectly altering oviposition preference by directly affecting an intermediate species, but food-web-mediated scenarios are easy to hypothesize. For example, pesticides may reduce survival of predators, potentially making contaminated sites more attractive to species with risk-sensitive habitat selection. Alternatively, pesticides might reduce resources (e.g., a herbicide reduces algal biomass), making contaminated sites less attractive for species with resource-sensitive habitat selection.

In this study we examine the effect of an insecticide on oviposition site selection in the gray treefrog. We focus on the gray treefrog because adaptive oviposition behavior is well documented in this species (e.g., Resetarits and Wilbur 1989) and occurs over multiple spatial scales (Resetarits 2005). Here we examine whether the commercially available insecticide Sevin® and its active ingredient, carbaryl (1-naphthyl *N*-methylcarbamate) alter patterns of oviposition. We focus on this insecticide because carbaryl is applied widely and can contaminant aquatic habitats via a variety of pathways (e.g., Relyea and Mills 2001). Furthermore, recent laboratory and mesocosm experiments have shown that Sevin® (or carbaryl, specifically) can reduce larval survival in gray treefrogs, and have revealed dramatic synergistic interactions between carbaryl and predation risk (e.g., Boone and Semlitsch 2001; Semlitsch et al. 2000; Relyea and Mills 2001; Relyea 2003). We present the results from four experiments. First we test for main and interactive effects of predation risk and Sevin® on oviposition site selection by gray treefrogs. We then explore whether Sevin® alters oviposition patterns directly or whether these effects are mediated through changes in oviposition by other organisms. Third, we examine whether the patterns of oviposition observed in the first two experiments hold at larger spatial scales. Finally, we examine in greater detail the nature of the chemical giving rise to the observed shifts in habitat selection.

Methods

This research was conducted during spring and summer 2005 and 2006 at Washington University in Saint Louis' Tyson Research Center (http://biology4.wustl.edu/tyson/; Fig. S1). Tyson encompasses approximately 2,000 acres of predominately oak–hickory woodlands situated near the Meramec River, approximately 40 km west of St Louis, MO. Gray treefrogs (*Hyla chrysoscelis*) are common at Tyson and readily breed in a number of man-made ponds established at the station. We conducted four experiments in which we manipulated insecticide (or solvent control) presence/absence in plastic wading pools (1.38 m diameter × 0.29 m deep) spaced 0.5 m apart (except "Experiment 3") filled with 300 l well water and arrayed in fields adjacent to the field laboratory (Fig. S1B,C). We then monitored subsequent natural oviposition by gray treefrogs in these pools daily (Fig. S1D). New eggs were removed, digitally photographed and counted using ImageJ image analysis software (http://rsb.info.nih.gov/ij/).

Insecticide concentrations

We established initial concentrations of carbaryl at 7 mg/l. In several recent studies that examine the effects of carbaryl on gray treefrog larvae, this concentration was used to represent the high end of ecologically realistic concentrations (Boone and Semilitsch 2001; Boone and Semlitsch 2002; Boone and James 2003; Relyea 2003). The breakdown of carbaryl in aquatic environments is often fairly rapid but depends upon a variety of factors. For example, hydrolytic

breakdown of carbaryl in natural ponds depends upon pH (at pH 8, half-life ≈ 1 d; at pH 7, half-life ≈ 10 d; Aly and El-Dib 1971; Sharom et al. 1980; Wauchope and Haque 1973), photolytic breakdown depends upon light exposure (half-life = 4–7 d in full sun; Wolfe et al. 1978), and biological breakdown is negligible (Wolfe et al. 1978). Our approach follows that of other studies (e.g., Boone and Semlitsch 2001) in using an initial pulse of carbaryl at the beginning of the experiment, which breaks down over time. We did not attempt to maintain or quantify carbaryl concentration throughout the experiment. Initially, we focus on the widely used commercial form, Sevin (Sevin Concentrate Bug Killer from GardenTech™, Lexington, KY, USA; 22% carbaryl, 78% inert ingredients). In "Experiment 4" we compare the effects of this commercial form with technical-grade 1-naphthyl *N*-methylcarbamate.

Experiment 1: *Effect of Sevin® and predation risk on oviposition*

Our first experiment was designed to test the independent and combined effects of the insecticide and predation risk on oviposition site selection. We employed a 2×3 factorial experimental design in which presence or absence of Sevin® was crossed with presence or absence of caged (i.e., nonlethal) predators, either a spothanded crayfish (*Orconectes punctimanus*) or a spotted salamander larva (*Ambystoma maculatum*). These six treatments were replicated ten times in five spatial blocks of 12 pools each (Fig. S1B). Predator cages consisted of 4-l plastic flowerpots with large holes cut in their sides that were covered with screening. Empty predator cages were placed in nonpredator treatments (Fig. S1C). On May 20, we added 100 ml of concentrated zooplankton collected from nearby ponds, 200 g leaf litter, and 100 ml of cat food to each pool to establish a simple aquatic food web. On May 24, we added 9.33 g Sevin® to treatment pools in to establish initial insecticide concentrations at 7 mg/l. The experiment was terminated on June 8, 2005.

We monitored subsequent oviposition by treefrogs daily, and on three dates we measured the pH, temperature, and dissolved oxygen concentration in each pool using YSI digital pH and oxygen probes. We tested for treatment and block effects on (1) mean number of treefrog eggs per pool, (2) predator survival, (3) mean pH, (4) mean temperature, and (5) mean dissolved oxygen of pools using a mixed-model ANOVA in which block was a random factor and predator and carbaryl were fixed factors. In the analyses of all experiments, data were square-root-transformed to better fit ANOVA assumptions of normality and homoscedacity, as assessed using Shapiro–Wilks and Bartlett tests, and nonsignificant block terms were dropped from subsequent analyses.

Experiment 2: *Oviposition in the absence of food web*

Our second experiment was conducted to further investigate the pattern of oviposition we observed in "Experiment 1." Specifically, we tested whether we would observe a similar response to Sevin® in the absence of the aquatic trophic structure that develops in response to colonization. To accomplish this, we manipulated Sevin® presence/absence as above; however, in this experiment we did not add plankton, leaf litter, or nutrients, and organisms colonizing pools were removed daily with fine dip-nets. These two treatments were replicated ten times in 20 pools arrayed in two blocks of ten pools each (Fig. S1B). We used a mixed-model ANOVA to test for block effects and for whether Sevin® pools received fewer eggs (i.e., one-tailed test). The experiment ran from 1 to 19 July 2005.

Experiment 3: *Increasing the spatial scale*

Natural amphibian breeding sites are typically more widely dispersed in the landscape than reflected by the spatial arrangement of the pools in our first two experiments. Because the degree to which breeding sites are isolated may alter the tradeoffs associated with habitat choice decisions (e.g., marginal value theorem; Charnov 1976), our third experiment examined whether the response to Sevin® observed in the previous experiments was also observed when the experiment was conducted over a larger spatial scale. We accomplished this by manipulating Sevin® presence/absence in pools that were isolated from any other pond habitat by at least 100 m (Fig. S1A). Recent studies of movement patterns in gray treefrogs suggest that adults are unlikely to move this distance over short timescales (Johnson 2005). These two treatments were replicated ten times in 20 wading pools arrayed throughout the field station. Aquatic trophic structure (20 June) and initial Sevin® concentrations (23 June) were established as above. We used ANOVA to test whether Sevin® pools received fewer eggs (i.e., one-tailed test). The experiment was terminated on August 2.

Experiment 4: *Isolating the chemical mechanism*

Experiments 1–3 include only Sevin® and water treatments, and thus do not enable us to directly ascertain whether carbaryl per se or other inert ingredients in the commercial form Sevin® cause the observed shift in habitat selection. Furthermore, it is possible that any volatile chemical could elicit a similar response. In our final experiment, we specifically examine the nature of the chemical driving patterns observed in earlier experiments. We accomplished this using four treatments: (1) water control, (2) solvent control (acetone), (3) carbaryl + solvent, and (4) Sevin®. Each

treatment was replicated nine times in 36 wading pools filled with 140 l of well water and arrayed in three blocks of 12 pools each (Fig. S1B). In both the carbaryl + solvent and Sevin® treatments, initial carbaryl concentrations were established at 7 mg/l. For the carbaryl + solvent treatment this involved adding 16.3 ml of a stock solution of 60 mg/ml technical-grade carbaryl (Sigma-Aldrich, St. Louis, MO, USA) dissolved in acetone. For the Sevin® treatment, we added 4.35 g of Sevin concentrate to pools. We added 16.3 mL of water or acetone to the water and solvent control pools. No aquatic trophic structure was established and colonists were removed daily. We used a mixed-model ANOVA to test for treatment and block effects. The experiment ran from 1 to 20 July 2006.

Results

Experiment 1

Over the 15 days that we conducted experiment 1, a total of 70,516 *Hyla chrysoscelis* eggs were oviposited in pools over four dates (days 5, 7, 8, and 9). Sixty-two percent of tanks received eggs (37/60). Pool pH [range: 7.75 ± 0.015 to 7.84 ± 0.014 (mean $\pm$ SE), $F_{(4,2.8)} = 25.4$, $P = 0.015$], temperature (range: 16.87 ± 0.07 to 20.5 ± 0.19 °C, $F_{(4,1.98)} = 138.4$, $P = 0.007$), and dissolved oxygen (range:

1.31 ± 0.22 to 3.5 ± 0.42 mg/l, $F_{(4,0.9)} = 2810.1$, $P < 0.001$) varied among the five spatial blocks, but not among predators (pH: $F_{(2,8)} = 3.24$, $P = 0.93$; °C: $F_{(2,8)} = 0.782$, $P = 0.490$; $[O_2]$: $F_{(2,8)} = 3.56$, $P = 0.077$) or carbaryl treatments (pH: $F_{(1,4)} = 1.60$, $P = 0.275$; °C: $F_{(1,4)} = 0.165$, $P = 0.705$; $[O_2]$: $F_{(1,4)} = 3.54$, $P = 0.133$). There were no block effects on the number of eggs oviposited ($F_{(4,1.6)} = 1.301$, $P = 0.509$). Predators had no effect on oviposition site selection ($F_{(2,54)} = 0.126$, $P = 0.882$; Fig. 2A). Sevin® significantly reduced oviposition ($F_{(1,54)} = 7.27$, $P = 0.009$; Fig. 1A). Pools treated with Sevin® received 71% fewer eggs on average (533.1 ± 154.5 eggs) compared to untreated pools (1817.4 ± 436.0 eggs). There was no interaction between the effects of predators and Sevin® on oviposition ($F_{(2,54)} = 0.219$, $P = 0.804$; Fig. 1A). Sevin® did not significantly reduce *A. maculatum* survival ($P = 0.193$), but caused 100% mortality in crayfish.

Experiment 2

Over the 19 days that we conducted our second experiment, a total of 100,656 eggs were laid in the experimental pools over seven nights when oviposition occurred (Fig. 1B). The earliest oviposition occurred on day five, but more than 87% of eggs were laid >10 days after the start of the experiment. The number of eggs laid per breeding night ranged from 1,686 to 47,100 eggs (mean $\pm$ SD: $14,379 \pm 16,274$).
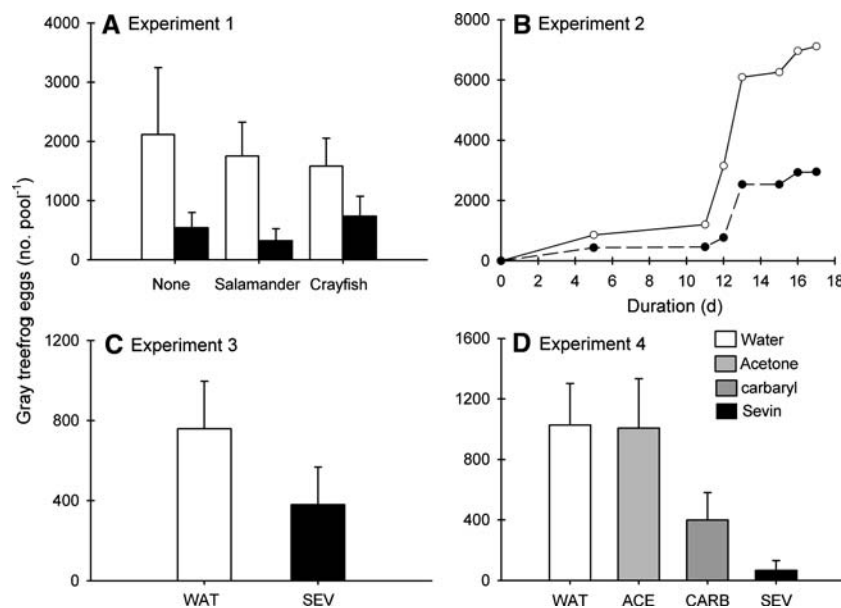
Fig. 1A–D Gray tree frog (*Hyla chrysoscelis*) egg oviposition in four experiments conducted in experimental pools at Tyson Research Center, St. Louis, MO, USA, in 2005–2006. **A** Experiment 1: number of gray treefrog eggs oviposited in response to Sevin® and predator treatments (none, salamander, crayfish). **B** Experiment 2: cumulative number of treefrog eggs oviposited in Sevin® and Sevin®-free (water) pools over the seven breeding nights that occurred during the duration of the

experiment. Each datapoint represents a breeding night. **C** Experiment 3: number of treefrog eggs oviposited in Sevin® and sevin®-free (water) pools when pools were isolated by 100 m. **D** Experiment 4: number of treefrog eggs oviposited in water, solvent control (acetone, ACE), carbaryl (CARB) and Sevin® (SEV)-treated pools. *Bars* show mean total number of eggs for each treatment (+1 SE). Legend in (**D**) is applicable to all panels

All 20 pools received eggs on at least one night. There was no block effect on oviposition ($F_{(1,1)} = 0.013$, $P = 0.928$). Even in the absence of aquatic trophic structure, Sevin® significantly reduced oviposition ($F_{(1,18)} = 7.75$, $P = 0.012$; Fig. 1B). Pools treated with Sevin® received 59% fewer eggs on average (mean ± SE: 2,949.9 ± 434.6 eggs) compared to untreated pools (7115.7 ± 1431.9 eggs).

In Experiments 1 and 2, the preference for Sevin®-free pools was consistent across breeding nights. Sevin® pools received fewer eggs than control pools on all nights when more than one pool received eggs. However, the strength of the preference varied across breeding nights. Uncontaminated pools received 1.7–13 times more eggs than Sevin® pools, depending upon the breeding night. We examined whether (1) time since start of the experiment or (2) the number of eggs oviposited on a given night explain this variation. To examine how the strength of the oviposition site preference changed as a function of time since the start of the experiment, we calculated the log response ratio (L), $L = $ ln(mean eggs in control) $-$ ln(mean eggs in carbaryl pools), for each date when oviposition occurred in more than a single pond ($n = 9$) and then regressed L against days since the start of the experiment. The results show no change in the strength of the preference over time (intercept: $1.1 \pm $ SE 0.715, slope: $0.027 \pm $ SE 0.063, $R^2 = 0.025$, $F_{(1,7)} = 0.180$, $P = 0.684$; Fig. 2A). We employed a similar approach to test the hypothesis that the strength of the preference decreased with the number of eggs oviposited on a given night. Preference for Sevin-free pools tended to decrease with increasing oviposition (intercept: 1.77 ± 0.309, slope: -0.0003 ± 0.000016, $R^2 = 0.310$, $F_{(1,7)} = 3.15$, $P = 0.059$; Fig. 2B).

Experiment 3

Over the 40 days that we conducted our third experiment, a total of 11,390 eggs were laid in experimental pools over eight dates when oviposition occurred. Oviposition occurred as early as day five and as late as day 39. On nights when breeding occurred, between one and six pools

received eggs (mean ± SD: 1.7 ± 1.5). At the end of the experiment, 60% of ponds had received eggs (12/20). The absence of eggs in a pool over the duration of this experiment may reflect habitat selection (i.e., frogs found it, but decided not to lay eggs); however, because of the isolated spatial arrangement of pools in this experiment, the absence of eggs may simply reflect the failure of frogs to locate that pool. This motivated us to analyze the data first with all pools included and secondarily with only pools that received eggs (i.e., the subset of pools we are certain frogs found). When all pools were considered, pools treated with Sevin® received 50% fewer eggs on average (mean ± SD: 380.4 ± 626.8, $n = 10$) compared to untreated pools (758.6 ± 790.2, $n = 10$, $F_{(1,18)} = 1.1$, $P = 0.15$). When we included only pools that received eggs, Sevin® pools received 51% fewer eggs on average (mean ± SD: 634.0 ± 717.0, $n = 6$) compared to untreated pools (1264.3 ± 597.3, $n = 6$, $F_{(1>,18)} = 3.8$, $P = 0.04$; Fig. 1C).

Experiment 4

Over the 23 days that we conducted experiment 4, a total of 22,513 *Hyla chrysoscelis* eggs were oviposited in pools over four dates (days 2, 5, 9 and 12). Fifty-six percent of tanks received eggs (20/36). There was no block effect ($F_{(2, 6)} = 2.77$, $P = 0.14$) or block by treatment interaction ($F_{(6, 24)} = 1.99$, $P = 0.11$) on the number of eggs oviposited. Oviposition by treefrogs differed significantly among treatments ($F_{(3,32)} = 5.83$, $P = 0.003$; Fig. 1D). Pools treated with Sevin® received 93% fewer eggs on average (mean ± SD: 66 ± 128.5 eggs) than both the water ($1,028 \pm 537.4$ eggs; Fisher's LSD $P = 0.001$) and acetone treatments ($1,008 \pm 640. 6$ eggs, Fisher's LSD $P = 0.002$). Pools treated with carbaryl received 61% fewer eggs on average (mean ± SD: 399 ± 356.7 eggs) than water (Fisher's LSD $P = 0.034$) and acetone treatments on average (Fisher's LSD $P = 0.07$). Sevin® and carbaryl (Fisher's LSD $P = 0.16$) and water and acetone (Fisher's LSD $P = 0.74$) treatments received similar egg numbers.

Fig. 2A–B Relationships among the strength of the preference for Sevin® or Sevin®-free pools as measured by the log response ratio, L, of egg oviposition and **A** experiment duration (days since the start of the experiment) and the **B** the total number of eggs oviposited on a given night when reproduction occurred (e.g., log response ratio (L) = ln (mean eggs in control) − ln (mean eggs in carbaryl pools)
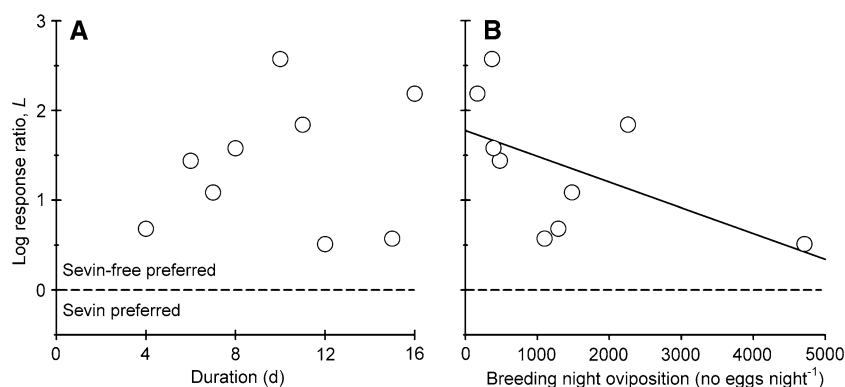
Discussion

Despite increasing interest in how contaminants impact freshwater systems, we still know very little about how contaminants affect colonization of aquatic habitats by organisms with complex life histories (Relyea and Hoverman 2006). This is important because contaminant-induced shifts in habitat selection could magnify or reduce toxic effects by either funneling organisms into or directing them away from contaminated sites. Here we show that an insecticide can alter oviposition site selection by gray treefrogs. Across studies we observed that uncontaminated pools received 2.46 times more eggs than pools treated with Sevin®.

While treefrogs consistently preferred non-carbaryl to carbaryl pools across breeding events and across experiments, there was an eightfold range in the strength of this preference. In our post hoc effort to explain this variation, our initial hypothesis was that it was related to the concentration of carbaryl. Under the conditions in our experimental pools (i.e., pH $\sim$ 8; full sunlight), carbaryl should breakdown rapidly (half-life = 1–2 days). The earliest oviposition in Experiments 1 and 2 occurred five days after carbaryl was added to the pools, and most eggs were laid considerably later than that (>10 days). Assuming a one-day half-life, by days five and ten of the experiment, carbaryl concentrations were likely <0.5 mg/l and <0.01 mg/l, respectively. Thus, we hypothesized that the strength of the preference for noncarbaryl pools would diminish with experimental duration, reflecting the breakdown of carbaryl. To our surprise, there was no evidence that the preference diminished with time. This suggests that either gray treefrogs are capable of detecting low levels of carbaryl or that they are responding to other "inert" ingredients or carbaryl breakdown products that linger in the system.

An alternative explanation for the variation in the strength of the preference is that habitat selection in response to Sevin® is density-dependent (sensu Fretwell and Lucas 1970; Marsh and Borrell 2001). Indeed, Resetarits and Wilbur (1989) showed that gray treefrogs tend to avoid ovipositing in pools with conspecifics, with the possible benefit of reduced intraspecific competition. This explanation is better supported by our results. On nights with high breeding activity, oviposition may spill over into less preferred Sevin® pools, as uncontaminated pools are occupied. Thus, the strength of the preference should diminish on nights with the highest oviposition, which is what we observed.

Neither salamander larvae nor crayfish altered habitat selection. In contrast to our results, Resetarits and Wilbur (1989) showed that gray treefrogs avoid *A. maculatum* larvae during oviposition. This difference could be due to differences in salamander densities between the two studies

(Resetarits and Wilbur: 4.7 *A. maculatum* m^{-2}; this study: 0.32 m^{-2}). Our densities may simply have been too low to elicit a behavioral response of the magnitude observed by Resetarits and Wilbur (1989). The effects of crayfish on treefrog oviposition have not been examined previously.

While the first two experiments showed a consistent and strong preference for uncontaminated pools, we were concerned that this result might be an artifact of the scale of our experiments. Classic patch selection and optimal foraging models (e.g., marginal value theorem; Charnov 1976) suggest that patch selection decisions involve balancing trade-offs between patch quality and fitness costs associated with moving among patches. In both previous experiments, gray treefrogs had access to uncontaminated sites within a few meters of contaminated pools. Thus, the cost of abandoning a less preferred for a preferred site in terms of travel and search time is artificially low. Natural breeding sites seldom occur in such close proximity as our experimental pools. At what spatial scale is oviposition site selection ecologically relevant? A recent study by Resetarits (2005) showed that habitat selection by gray treefrogs in response to fish predators can function at multiple spatial scales. We were curious about whether treefrogs would exhibit similar patterns of oviposition as in the first experiments, when the costs associated with choosing not to breed in less-preferred (e.g., Sevin®) pools were higher. In our third experiment, pools were arrayed throughout the field station such that all ponds were isolated from other potential breeding sites (both natural and artificial) by at least 100 m. Recruitment to pools in this experiment was low, mostly likely because pools were more difficult to find (i.e., pools in earlier experiments were adjacent to historical breeding sites) and because we conducted this experiment late in the breeding season. However, the pattern of oviposition was consistent with those conducted at smaller spatial scales; Sevin® pools received 51% fewer eggs on average.

Our final experiment allowed us to confirm that carbaryl alone is sufficient to cause treefrogs to shift patterns of habitat selection. Furthermore, it allowed us to demonstrate that the treefrog response is not a generalized response to any volatile compound, because we observed no response to our solvent control treatment, acetone. Both patterns are consistent as we look across taxa colonizing experimental mesocosms; *Culex* mosquitoes, chironomid midges, and diving beetles all respond to Sevin® and carbaryl in a similar way and do not respond to acetone (Vonesh, unpublished data). Interestingly, these other taxa are attracted to, not repelled by, contaminated pools.

Collectively, these experiments show that gray treefrogs strongly prefer pools with no history of contamination with the insecticide Sevin®, and that this preference appears to be a direct response to the insecticide, not mediated via reduced predator survival or via the effects of the insecticide on the

simple aquatic food web in these pools. Furthermore, these results appear to hold over a broad range of spatial scales and occur in response to either Sevin® or technical-grade carbaryl. By altering the distribution of organisms among contaminated and uncontaminated habitats, this behavioral response to contaminants may have consequences for amphibian populations at local and regional scales.

At the local scale of a single breeding site, our results suggest that contaminated aquatic habitats could receive fewer eggs and thus have lower tadpole densities. Thus, contaminants could reduce local recruitment of adults both by reducing survival (i.e., the toxic effect) but also by reducing colonization. This suggests that even in the absence of toxic effects, chemical contaminants could have important effects on local recruitment by modifying habitat selection. Furthermore, simply altering initial densities can alter the toxic effects of contaminants (i.e., density × insecticide interaction; e.g., Boone and Semlitsch 2001; Rohr et al. 2006a, 2006b). The degree to which this local reduction in recruitment reflects the pesticide's net effects at the landscape or metapopulation scale depends on the proportion of sites that have been contaminated and the effects of pesticides on local recruitment in contaminated sites. It also will depend on whether eggs not laid in contaminated sites are able to be redirected to remaining uncontaminated sites or are lost (Osenberg et al. 2006) and the density-dependent consequences of this redirection for recruitment from those sites (Vonesh and De la Cruz 2002).

While most toxicology studies focus on the effects of contaminants during exposure, several recent studies have highlighted the importance of considering post-exposure processes that could mitigate or exacerbate the effects of acute exposure (e.g., density-mediated compensation and carryover effects; Moe et al. 2001; Rohr et al. 2006b). We argue that the effects of contaminants on the pre-exposure process of habitat selection has also been largely overlooked and may have important implications for how contaminants affect aquatic organisms. While current studies have focused on the effects within contaminated patches, pesticides may have consequences beyond the local contamination site if they alter habitat selection. For example, recruitment from two otherwise equal, uncontaminated ponds may differ if one is adjacent to a contaminated site and the other an uncontaminated site. Such spatial effects of pesticides mediated through habitat selection have not previously been considered (but see Resetarits 2005; Resetarits et al. 2005), and they suggest some counterintuitive hypotheses, such as the hypothesis that even nontoxic contaminants could alter both local and regional recruitment.

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