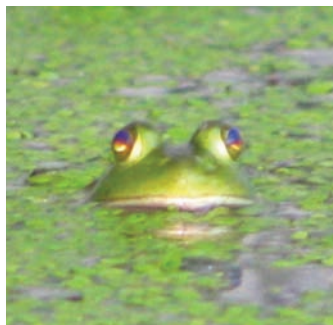




# Sublethal effects of 2,4-D exposure on golf course amphibians

When applied correctly, 2,4-D does not appear to harm amphibians in golf course wetlands.



Pond-breeding amphibians are thought to be more susceptible to waterborne pesticide exposure because, as larvae with limited mobility and relatively thin skin, they must live in an aquatic environment (1).

Although proper application of pesticides on golf courses does not appear to eliminate amphibian communities inhabiting golf course wetlands, episodic contamination may have nonlethal effects on developing amphibian larvae. Such *sublethal effects*, which impair normal biological function without causing direct mortality, are not necessarily less serious than outright mortality. A pesticide's cumulative sublethal effects within the population may make it impossible for the population to survive, for example, by reducing the ability of individuals to produce offspring through reduced survival or reproductive output. Exposure to a toxicant may reduce survival or reproductive success by impairing regular swimming function, altering activity levels or influencing feeding behavior.

Affected populations may become *sinks*, areas that are not able to sustain a viable population without immigration from surrounding source populations (9). Because sink populations may appear healthy and productive, a false sense of security may develop when monitoring fails to recognize subtle sublethal effects.

## Materials

We focused on the effects of a single herbicide, 2,4-D (2,4-dichlorophenoxyacetic acid), which

is commonly applied during golf course maintenance, and a single amphibian species, the southern leopard frog (*Rana sphenoccephala*), in order to better understand the potential range of effects of the herbicide.

### *The herbicide*

Earlier research (6) has shown that 2,4-D may become concentrated in golf course wetlands (11). The potential for aquatic contamination represents an unexplored threat for amphibian larvae and other members of aquatic communities in ponds, marshes or lakes.

No standard toxicological studies of 2,4-D have been performed on embryonic or larval amphibians, which are most likely susceptible to exposure. Because most toxicological studies of 2,4-D have featured mammals, the pesticide may not be ecologically benign (8) in regard to amphibians.

2,4-D is the third-most commonly applied pesticide in the U.S. and is the most widely used herbicide worldwide (8). Thus, our research may have relevance beyond golf course management.

### *The amphibian*

We used the southern leopard frog as the model amphibian for this research because, like many amphibians breeding in golf course wetlands, it has a relatively brief larval period (<90 days), and relatives of *R. sphenoccephala* in the leopard frog (*R. pipiens*) complex are distributed throughout the U.S., giving our study wide geographic applicability.



This study was funded by the USGA.

Travis J. Ryan, Ph.D.



As environmental stewards, superintendents need to understand how pesticide exposure affects the flora and fauna of golf course wetlands. Photo by T. Ryan

Members of the genus *Rana* are commonly used in various stages of development in such diverse research contexts as population biology and metamorphic timing, ecotoxicology and conservation biology, and they are more commonly used in ecotoxicology studies than any other North American genus, giving our results a broad basis for comparison (3).

For all our experiments, we obtained egg masses of *R. sphenoccephala* from Carolina Bays in Aiken County, S.C.

## Experiments

### Chronic exposure study

The goal of our research was to look at the sublethal effects of 2,4-D on *Rana sphenoccephala* in an ecologically realistic manner with regard to golf course wetlands. Because 2,4-D has a fairly short half-life (8) and is most likely to appear in golf course wetlands at high concentrations only under particular conditions (for example, application near wetlands before heavy rainfall), we focused on acute rather than chronic exposure. However, we conducted a chronic exposure study to determine the range of effects on life-history characteristics such as growth, metamorphosis and survival under a worst-case scenario. We also used this study to select concentrations to be used in the locomotor and behavioral studies.

We randomly selected 100 tadpoles for use in an exposure experiment. We used a control (no 2,4-D) and four concentrations of 2,4-D: 285 parts per billion (= 0.285 ppm), 2.85 ppm, 28.5 ppm and 285 ppm. We assigned 20 tadpoles to each treatment; their exposure to 2,4-D remained the same over the course of the experiment.

The mass of the tadpoles was recorded 21 days after the beginning of the experiment and roughly

every 14 days thereafter. The tadpoles were fed ground fish food and they were checked daily throughout the experiment for mortality and signs indicating the beginning of metamorphosis (forelimb eruption) and the completion of metamorphosis (tail resorption).

We stopped recording tadpole mass after day 68 because, after this point, tadpoles began metamorphosis, and growth generally ceases during this process. The experiment was continued until all survivors had completed metamorphosis.

### Locomotor study

To determine how acute exposure to 2,4-D influences locomotor performance, we measured the swimming speed of 45-day-old tadpoles following a 24-hour exposure to 2,4-D.

A total of 18 individual tadpoles were selected randomly, and each one was exposed to one of the experimental treatments: 2,4-D at 150 ppm (high), 2,4-D at 15 ppm (medium), 2,4-D at 1.5 ppm (low) or a control (no 2,4-D). Approximately 24 hours after exposure, we assessed the tadpoles' swimming performance.

We used a digital video camera to record the number of bursts (discrete swimming events required to complete the 100-centimeter track), burst speed (the rate of movement during each burst) and total swimming speed. Appropriate statistical analyses were conducted to determine the effect of acute 2,4-D exposure on swimming speed.

## Tadpole survivorship

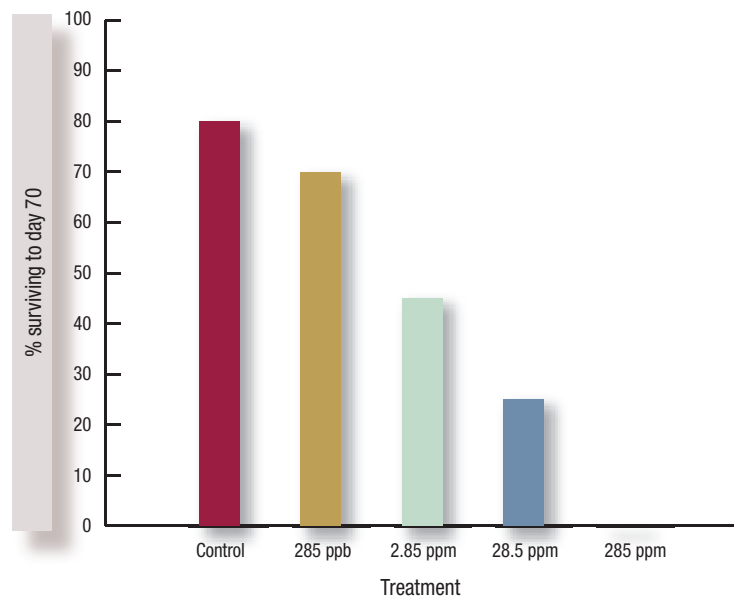


Figure 1. The effect of chronic exposure to 2,4-D on survivorship of southern leopard frog tadpoles.



The southern leopard frog was used as a research model because it has a relatively brief larval period, its relatives are distributed throughout the U.S. and members of its genus are more commonly used in ecotoxicology studies than any other North American genus. Photo courtesy of National Park Service

Behavior studies

We observed the behavior of tadpoles following acute exposure to 2,4-D to determine whether activity, feeding performance or habitat were affected. We selected tadpoles that were approximately 45 days old. Before the behavioral experiments, tadpoles were deprived of food for 72 hours to ensure that tadpoles would be hungry and likely to feed during the trials (5). Exposure levels to 2,4-D were high (150 ppm) and low (1.5 ppm).

Following the 24-hour exposure period, all behavioral observations were made in four 38-liter

aquaria, which were placed on a grid so an unbiased location of the tadpoles could be determined. Each aquarium also contained a refugium located in the center of the tank.

At the start of each observation period, we placed a tadpole into the center of each aquarium. After a two-minute acclimation time, we turned on a digital video recorder above the aquaria and recorded behavior for 25 minutes. No one was present in the room during the recording. The trials were conducted over five days (presence of food experiment) and six days (presence of predator experiment) with three trials of four individuals each recorded each day. Between each trial, we rinsed the aquaria thoroughly to eliminate chemical cues from previous groups.

We placed half the tadpoles exposed to each 2,4-D level (control, low, high) in aquaria containing aged tap water with ground fish food. The remaining tadpoles were placed in aquaria containing aged tap water with no food. Four replicates for each treatment combination were conducted. This test allowed us to determine how acute exposure to 2,4-D affected feeding behavior.

To determine whether the presence of a predator and acute exposure to 2,4-D affected feeding behavior, we placed half of the tadpoles exposed to each 2,4-D level (control, low, high) in aquaria containing aged tap water in which predator chemical cues were present. Tadpoles rely on chemical cues released from the fish as evidence of predator presence (10) and chemical cues released from other tadpoles as a warning signal.

Tadpole growth

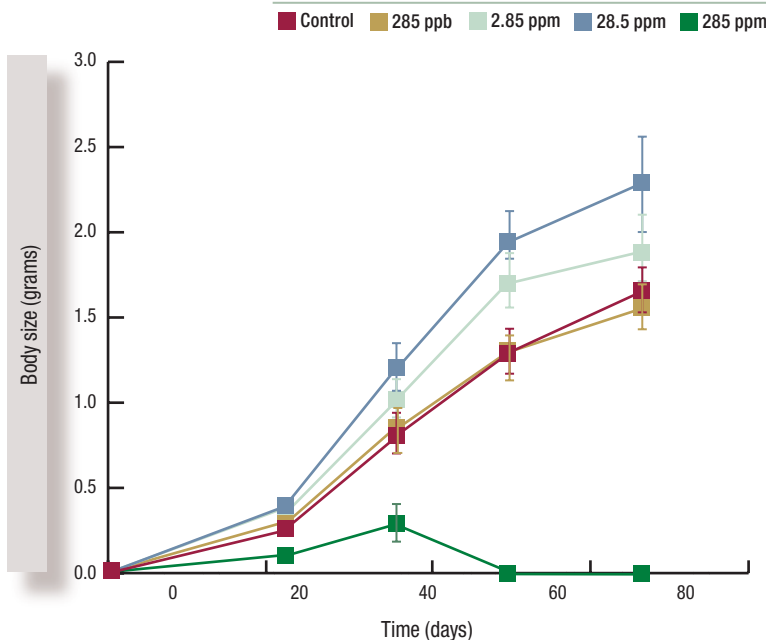


Figure 2. Growth of southern leopard frog tadpoles exposed to 2,4-D for more than 70 days.



The green sunfish releases chemical cues that indicate the presence of a predator to potential prey such as southern leopard frog tadpoles. Illustration by Duane Raver/Image courtesy of U.S. Fish and Wildlife Service

To obtain the “fish water” used as chemical cues in the predator treatment, we fed four green sunfish (*Lepomis cyanellus*) a diet of *R. sphenocéphala* tadpoles not used in the experiment. The fish spent a minimum of 72 hours in a separate container containing aerated aged tap water. This water was used as predator chemical cues.

The remaining half of the tadpoles exposed to



each 2,4-D level were placed in aquaria containing aged tap water. All tadpoles were fed ground fish food during the observation period. Six replicates for each treatment combination were used.

Once all the trials were completed, we reviewed the videotapes and recorded data on time spent active, feeding, and at different locations in the aquaria.

## Results

### Chronic exposure study

Long-term exposure to 2,4-D affected survival to the end of the experiment (Figure 1). However, even at the highest concentration of 2,4-D, mortality was not immediate. Nearly 20 days passed before the survivorship in the 285-ppm treatment was 50% (data not shown). In the 2.85-ppm treatment, survivorship did not dip below 50% of the control group by the end of the study. An analysis of the control treatment and three lower concentrations showed that growth (body size in grams at day 68) was not affected by chronic exposure to 2,4-D (Figure 2). The timing of metamorphosis was also not affected (data not shown), except with the highest concentration, where no individuals survived to metamorphosis.

### Locomotor study

There was no significant effect on the swimming performance of tadpoles that experienced acute exposure to 2,4-D. We analyzed both the number of bursts required to complete the 100-cm track (data not shown) and total sprint speed (Figure 3).

### Behavior study: Effect of food

Exposure levels of 2,4-D did not significantly affect activity, feeding or habitat use (Figure 4). The presence of food influenced time spent in activity, with activity significantly higher in the presence of food, with a significant interaction between exposure levels and the presence of food. For more information about this experiment, see the Discussion section.

### Behavior study: Effect of predator cues

Exposure to 2,4-D did not significantly affect activity when tadpoles were in the presence of predator cues. However, exposure to 2,4-D did affect tadpole feeding behavior (data not shown).

Tadpoles exposed to low levels of 2,4-D fed considerably less than those not exposed to 2,4-D, but not significantly more or less than tadpoles experiencing high exposure. Only a marginally significant difference in time spent feeding was observed between tadpoles with high 2,4-D

exposure and no 2,4-D exposure. As in the first test, exposure to 2,4-D did not significantly affect tadpole location.

## Swimming speed

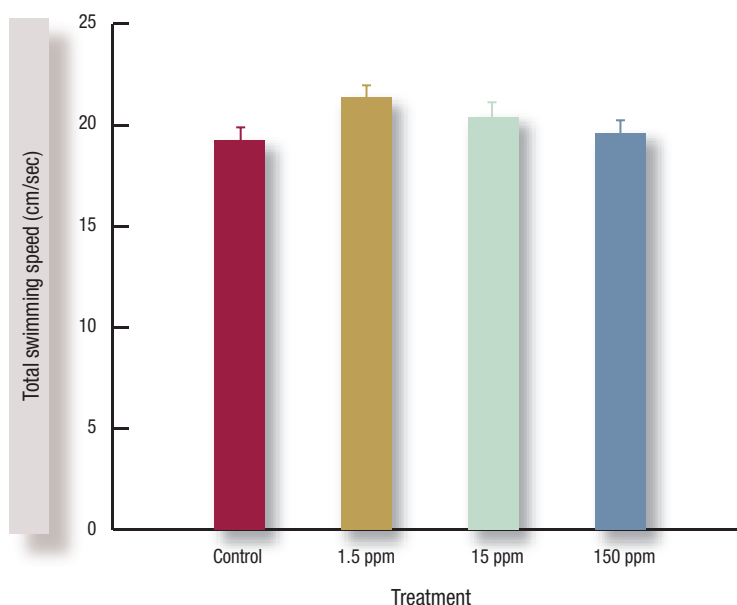


Figure 3. The effect of acute (24-hour) exposure to 2,4-D on the swimming speed of southern leopard frog tadpoles over a distance of 100 centimeters (39.4 inches). Bars are treatment means, and error bars equal one standard error.

## Activity vs. food

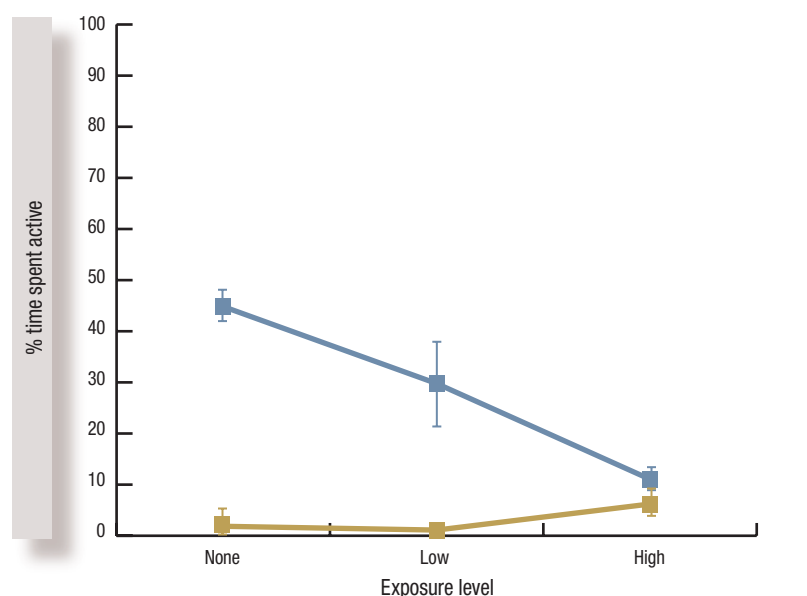


Figure 4. Time southern leopard frog tadpoles spent active with a source of food present and absent at different 2,4-D exposure levels. Points are treatment means with error bars equal to one standard error.



The presence of a predator's chemical cues significantly reduced activity (Figure 5) and feeding compared to when no chemical cues were present, but had no significant effect on tadpole location.

### Discussion

#### Chronic exposure study

Our chronic exposure study demonstrated that long-term exposure to elevated levels of 2,4-D is necessary to elicit significant effects on life-history characteristics of larval *Rana sphenocphala*. By contrast, many other pesticides have been shown to slow growth, increase mortality and impair metamorphosis in a wide array of amphibians after a short period of exposure (1).

Not only is 2,4-D of relative low toxicity to *R. sphenocphala*, but it also breaks down rather quickly in aquatic environments (8). These characteristics taken together suggest that 2,4-D represents a relatively low-level threat in terms of direct mortality to amphibian larvae.

Our results are consistent with a recent large-scale experiment that found no significant effect on amphibian mortality or aquatic community structure when 2,4-D was applied directly to aquatic systems at a concentration of 0.37 fluid ounce/1,000 square feet (0.117 milliliter/square meter; equivalent to a recommended application). A similar application of other pes-

ticides did not elicit such benign responses from tadpoles and other animals in the experimental aquatic communities (7).

#### Locomotor study

The absence of direct mortality and the resulting changes in community organization, however, are only part of the story in regard to the potential detrimental effects of pesticide exposure in amphibian larvae. The sublethal effects we investigated in this study (changes in locomotor performance, activity and feeding behavior) were selected because of their importance to overall biological function. We failed to find any effect of 2,4-D on swimming performance as has been noted when amphibian larvae have been exposed to sublethal doses of other common pesticides, such as carbaryl and cypermethrin (4).

#### Behavior study

We also investigated the effect of sublethal exposure on activity and feeding behavior. In these cases we found that exposure had some effects, but they are subtle and not-at-all straightforward. Activity decreased as the concentration of 2,4-D during the exposure period increased. However, the effect of food presence diminished with increasing concentration. Habitat use within the test chambers was similar among the control and exposure treatments, and there was no effect of the presence of food in this case. Exposed tadpoles responded appropriately to the threat of predation (that is, they decreased activity), but the exposed tadpoles also had decreased levels of feeding in the absence of predators.

Because of its low toxicity, 2,4-D's activity in amphibians is not understood. Decreased movement and feeding levels may be of concern because depression in these activities is likely to decrease growth and thus increase time to metamorphosis and exposure to predators. Indeed, recent research in amphibian ecology highlights the importance of the role of interactions between environmental contaminants and community structure in determining the true ecological effect of chemically intensive practices (2).

When 2,4-D is applied correctly on golf courses, the risk of detrimental effects to larval amphibian populations appears to be very low, given the low toxicity of the compound and its relatively subtle sublethal effects.

### Predator cues

■ Predator present ■ Predator absent

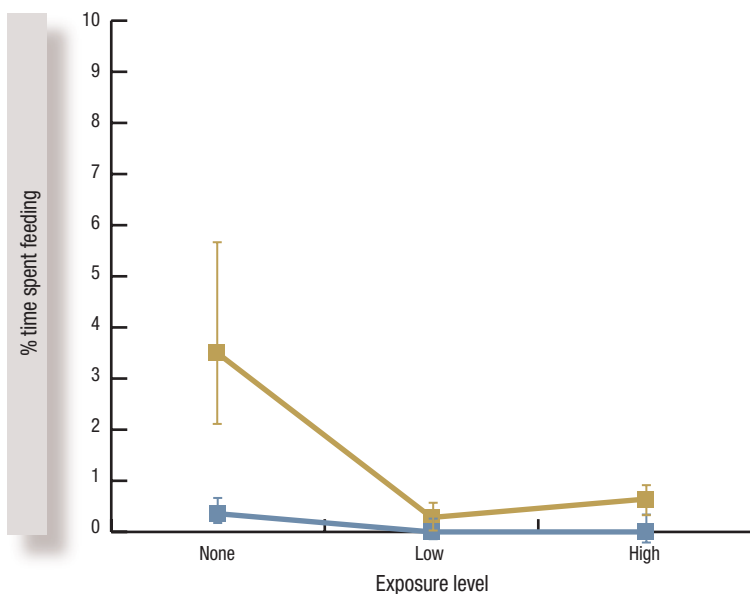


Figure 5. Time southern leopard frog tadpoles spent active with predator cues present and absent at different 2,4-D exposure levels. Points are treatment means with error bars equal to one standard error. The graph shows only 0%-10% time spent feeding so that the exposure-level values can be clearly seen.

### Funding

We thank the USGA's Turfgrass and Environmental Research Program for its support of this project through the Wildlife Links program administered by the National Fish and Wildlife Federation.



Pond-breeding amphibians are thought to be more susceptible to waterborne pesticide exposure because, as larvae with limited mobility and relatively thin skin, they must live in an aquatic environment.  
Photo by T. Ryan



### The research says

→ Amphibians, which are some of the most common vertebrates found on golf courses, frequently inhabit golf course wetlands, where they are likely to be episodically exposed to small quantities of herbicides applied in the course of proper golf course maintenance.

→ Some life-history traits (for example, survival to metamorphosis) may be affected by chronic exposure at high doses, but other traits (growth, timing of metamorphosis and size at metamorphosis) are not.

→ The goal of this study was to investigate whether exposure to sublethal concentrations of 2,4-D would significantly affect growth, swimming speed or feeding ability in tadpoles of the southern leopard.

→ Acute exposure to 2,4-D is unlikely to significantly affect life-history traits. Acute exposure does not alter locomotor ability, but it reduces the activity of tadpoles and feeding activity when predators are present.

→ 2,4-D does not represent a particularly strong threat to amphibian larvae inhabiting golf courses where 2,4-D is applied responsibly.

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### GCM

Travis J. Ryan ([tryan@butler.edu](mailto:tryan@butler.edu)) is an assistant professor in the department of biological sciences at Butler University, Indianapolis.